


## ORIGINAL ARTICLE

# Evaluation of rs1748195 ANGPTL3 gene polymorphism in patients with angiographic coronary artery disease compared to healthy individuals

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## Abstract

**Subject:** The Angiopoietin-like 3 (ANGPTL3) gene has been reported to be associated with cardiovascular risk. This study is designed to compare the genetic variant (rs1748195) of the ANGPTL3 gene and the presence of a coronary artery occlusion of >50% in Iranian nation.

**Method:** In this study, 184 patients underwent angiography and 317 healthy individuals were evaluated for polymorphism of rs1748195 the ANGPTL3 gene using Tetra-ARMS PCR. Coronary patients who experience angiography were categorized into two groups: 54 patients who had an angiography indication for the first time and coronary occlusion was <50% (Angio-) and 134 patients who formerly underwent coronary stent implanting at least 1 month before with coronary occlusion of ≥50% that again have an angiography indication (Angio+).

In addition, individuals with angio+ are categorized in two groups: (1) non-in-stent restenosis (NISR); patient with a patent stent ( $N = 92$ ). (2) in-stent restenosis (ISR); in-stent stenosis >50% ( $N = 42$ ).

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**Result:** The fundamental of characteristics of our study design population was categorized based on undergoing angiography or not. In the present study, we investigated that the CC genotype, and also the A allele corresponding to rs1748195 at the ANGPTL3 gene loci, was associated with negative angiogram and directly related to the risk of coronary occlusion >50%. In contrast, this result was not significant in genotypes of ANGPTL3 between non-ISR and ISR groups.

**Conclusion:** The outcomes of this study showed that rs1748195 polymorphism at the ANGPTL3 gene loci is associated with an elevated risk for the existence of a coronary occlusion of >50%.

#### KEYWORDS

angiography, angiotensin-like3 (ANGPTL3), atherosclerosis, cardiovascular diseases (CVD)

## 1 | INTRODUCTION

Cardiovascular disease (CVD), consisting of ischemic heart disease, heart failure, arterial disease and other heart problems, causes high mortality in the world (De Hert et al., 2018). Based on the research studies, CVD is one of the main reasons of reducing quality and duration of life (Mensah et al., 2019). Given to the World Health Organization (WHO) report in 2020, about 37% of premature non-communicable deaths and approximately 50% of total annual deaths in Iran are caused by CVD (Roth et al., 2017).

Atherosclerosis is a major cause of heart attack and stroke (Parsamanesh et al., 2021). Observations showed that cholesterol is an important component of arterial plaque that leads to atherosclerosis (Samadi et al., 2019). LDL cholesterol and Apo lipoprotein B (ApoB100) are directly related to the risk of atherosclerotic cardiovascular events (ASCVE) (Aghasizadeh, Bizhaem, et al., 2021; Levin, 2014). Angiopoietin 3 is a secreted glycoprotein expressed in the liver (Estey et al., 2013) and contained 460 amino acid polypeptides containing a peptide signal, an N-terminal domain, and a fibrinogen-like C-terminal domain (Zierk et al., 2013). This protein inhibits lipoprotein lipase, which has been one of the important enzymes associated with CVD risk. Besides controlling the activity of this enzyme is very important in preventing diseases. Studies show that this gene is a pathway for a therapeutic target for lipoprotein metabolism (Gomaschi et al., 2011). Coronary angiography is a medical procedure that uses a special dye and x-rays to visualize the lumen of blood vessel and the heart chambers (Parsamanesh et al., 2021). It has known as a serious diagnostic method of detecting atherosclerotic plaque. This medical technology can be used as a detection method of coronary heart and peripheral arterial diseases (PAD). Coronary angiography

is often done along with catheterization (Aghasizadeh, Samadi, et al., 2021; Saberi-Karimian et al., 2021). In recent dedicate, coronary angioplasty has made a considerable decrease in bypass operation in patients with atherosclerotic plaque, but however in 15%–40% of patients restenosis of arteries veins happen (Gimbrone Jr et al., 2000; Roth et al., 2017; Rubin et al., 2014).

Restenosis is defined as stenosis in the coronary arteries >50% after percutaneous coronary intervention (PCI). This technique is the most important method of revascularization in the coronary heart disease therapy (Kivelä & Hartikainen, 2006; Oguri et al., 2007; Sadeghi et al., 2017). Repeated treatment of coronary angioplasty are cost high. Hence, to diminish this cost, the other method of treatment such as coronary bypass surgery, and implantation of drug-eluting stents, is very critical to diminish the cost (Htay & Liu, 2005; Reiner et al., 2011; Teslovich et al., 2010). Although drug-eluting stents implantation is one way to regenerate and prevent restenosis of the coronary arteries, stent thrombosis and in-stent restenosis (ISR) demonstrate inflammatory response by increasing the diffusion of reactive oxygen species (ROS), and individual susceptibility play a critical role in their onset (Montone et al., 2013; Niccoli et al., 2010). This adverse effect has been shown in 15%–27% of implanted patients (Kathiresan et al., 2008; The 1000 Genomes Project Consortium, 2010; Willer et al., 2008).

Base on genome wide association studies (GWAS), single nucleotide polymorphism (SNP) is one of the major risk factor for cardiovascular disease (Kathiresan & Srivastava, 2012; Teslovich et al., 2010). Angiotensin-like 3 (ANGPTL3) gene has critical role in regulation of lipid profile and lipoprotein concentration therefore (Kharazmi-Khorassani et al., 2019), this gene has been shown to be associated with CVD risk (Bea et al., 2021; Kathiresan & Srivastava, 2012).

The ANGPTL3 gene encodes Angiopoietin-Like3 protein as a secretory protein that contributed in angiogenesis (Kersten, 2017; Willer et al., 2008). This gene is a liver derived circulating factor with a C-terminal fibrinogen chain and a N-terminal coiled-coil domain that prohibits the enzyme lipoprotein lipase (LPL) (Kersten, 2017; Mattijssen & Kersten, 2012). LPL is a hydrolysis enzyme that break down triglycerides into free fatty acids (FFA) and monoacylglycerols and then this product transported by very low density lipoproteins (VLDL) and chylomicrons from different organs to the bloodstream (Goldberg, 1996; Kobayashi et al., 2002; Zechner et al., 2000). Hence, LPL plays a critical role in lipid metabolism. Lipoprotein lipase deficiency is an infrequent inherited disease define by chylomicronaemia and severe hypertriglyceridemia, recurrent acute pancreatitis and increased risk of atherosclerosis or other complications (Gaudet et al., 2012; Nordestgaard et al., 1997). Higher level of LPL decrease bloodstream TG level and thus protecting individual from CVD risk (Rip et al., 2006). In 2017, genetic research in CVD in GWAS showed that single nucleotide polymorphisms (SNPs) can affect blood lipid levels and contributing factors that cause CVD (Li et al., 2018). Base on the GWAS study this **rs1748195** is located at the enhancer histone signature site of the ANGPTL3 that effects gene transcription (Oldoni et al., 2016). This SNP is located on the forward (F) strand of the chromosome 1 that is encoding a protein involved in angiogenesis (Li et al., 2018).

The purpose of this study was to compare the genetic variant (rs1748195) of ANGPTL3 gene in angiographic patients with healthy ones.

## 2 | METHODS

### 2.1 | Study population

In this case-control study, 501 subjects were recruited that 317 were healthy individuals and 184 underwent angiography. The following patients were categorized into two group:

1. Having a symptom to undergo angiography that defines as a subject who has an angiography indication for the first time and coronary occlusion was <50% ( $N = 54$ ).

2. Having a symptom to underwent angiography that defines as angio+ group who formerly experienced coronary stent implanting and at least one month ago with coronary occlusion of  $\geq 50\%$  that again have an angiography indication ( $N = 134$ ).

Also, classify individuals with angio+ in two groups include patients in-stent restenosis (ISR); stent stenosis >50% ( $N = 42$ ), and non-in-stent restenosis (NISR); patient with patent stent ( $N = 92$ ).

### 2.2 | Genotyping

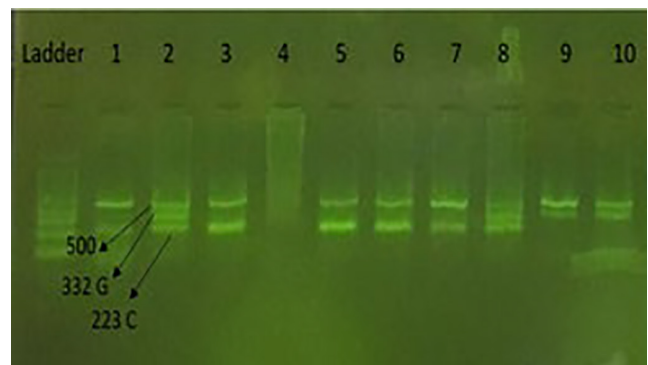
DNA extracted from the whole blood of these individuals by salting out method was done, detailed in our pervious study (Valizadeh et al., 2021) and DNA samples was stored at  $-20^{\circ}\text{C}$ . In this study, Tetra amplification-refractory mutation system (ARMS) PCR reaction was used to determine the genotypes of rs1748195 polymorphism. Tetra ARMS PCR reaction was performed in a total volume of 20 ml containing DNA template-dNTP-primers and Mgcl2 buffer. To determine the rs1748195 genotype, a sample containing 10  $\mu\text{l}$  of Master Mix (Pars Tous company), 0.5  $\mu\text{l}$  of FO, RO and 1  $\mu\text{l}$  of primer FI, RI and 2  $\mu\text{l}$  of sample DNA and 5  $\mu\text{l}$  values deionized is used. Primer sequences was “ACCTACTATACAAATTGTTGGCTTCA” for FO, “AGTTGTTTTACCAGTTGTGTTGATG” for RO, and “ACTTCTGGAGTAATAACAGGAATACTCTC” for FI, and “TATCTAATCAGCTGCTCTGATTATCC” for RI. After the reaction, the product was electrophoresed in agarose powder 2%. To end (Figure 1), the genotypes were established by sanger sequencing as shown in Figure 2 and all sequenced examples were analyzed by Finch TV (version 1.4.0).

### 2.3 | Biochemical measurements

HDL-C, TG and TC concentration was determined by Pishtaz Teb kit and auto-analyzer. Furthermore, Biosystem company kit was used to determined level of Hs-CRP by autoanalyzer model BT3000.

### 2.4 | Statistical analysis

All analyses were performed by SPSS software. To determine variables with normal distribution performed t-test.



**FIGURE 1** Agarose gel for rs1748195 polymorphism (C > G). Number 1, CC genotype. Number 2, CG genotype. Number 9, GG genotype.

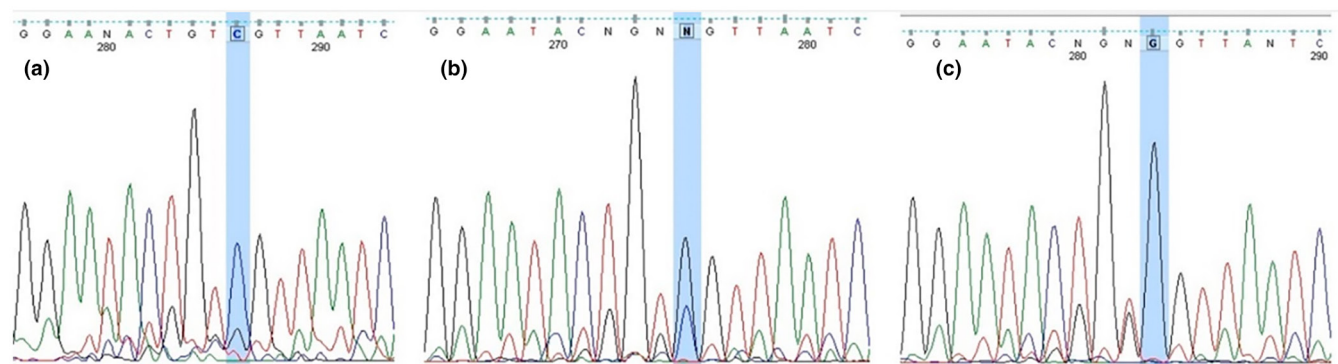


FIGURE 2 The results of DNA sequencing of rs1748195, ANGPTL3 gene; (a) CC genotype, (b) CG genotype, (c) GG genotype.

	Healthy	Angio–	Angio+	<i>p</i>
Age	48.73 ± 7.69	54.68 ± 9.22	59.95 ± 8.65	<0.001*
Female	185 (59.1%)	29 (58%)	35 (26.7%)	<0.001*
HDL	44.74 ± 12.95	36.21 ± 8.79	35.35 ± 8.87	<0.001*
LDL	114.65 ± 37.90	95.48 ± 32.20	93.80 ± 33.46	<0.001*
TG	136.11 ± 92.55	138.72 ± 84.53	130.15 ± 62.13	0.762
TC	192.13 ± 40.50	160.77 ± 42.62	154.30 ± 38.91	<0.001*
SBP	121.43 ± 19.64	121.16 ± 12.99	124.66 ± 14.20	0.257
DBP	79.39 ± 11.80	78.86 ± 7.84	78.59 ± 9.81	0.801
BMI	27.75 ± 4.77	27.33 ± 4.04	28.77 ± 20.96	0.654
CRPnew	5.37 ± 10.87	4.43 ± 4.22	3.55 ± 3.36	0.181
PAB	93.37 ± 46.30	1.04 ± 0.29	1.08 ± 0.30	<0.001*
Hypertention	96 (30.7%)	23 (46%)	76 (58%)	<0.001*
Diabetes	51 (16.6%)	20 (40%)	50 (38.5%)	<0.001*
Dyslipidemia	256 (81.8%)	28 (56%)	71 (54.2%)	<0.001*

TABLE 1 Baseline characteristics of healthy individuals, Angio– and Angio+

Note: Values are expressed as mean ± standard deviation or median (interquartile range 25th–75th) for normal and non-normal distribution data respectively. \* $p \leq 0.05$  is indicates as significant.

Used square-Chi test to determine variables without a normal. Estimated the risk ratio at a 95% confidence interval to determined logistic regression of the correlation between genotype and clinical information/patient pathology. The level of statistical significance was considered <0.05.

### 3 | RESULTS

#### 3.1 | Study population characteristics

The clinical characteristics of the three healthy, Angio– and Angio+ groups studied are summarized in Table 1. The mean age of healthy, Angio– and Angio+ individuals was  $48.73 \pm 7.69$  and  $54.68 \pm 9.22$  and  $59.95 \pm 8.65$ , respectively ( $p < 0.001$ ). Based on the results, there was a significant difference in terms of gender between the study groups ( $p < 0.001$ ). According to the analyses, the mean

levels of HDL, LDL and TC in the healthy group were significantly higher compared to Angio– and Angio+ individuals ( $p < 0.001$ ). Also, in comparison between the two groups of Angio– and Angio+, it was found that individuals with a history of hypertension, diabetes and dyslipidemia were more present in the Angio+ group ( $p < 0.001$ ). The mean of other factors studied was different between the study groups, but these differences were not statistically significant ( $p > 0.05$ ).

#### 3.2 | Association of rs1748195 genotypes of ANGPTL3 gene with angiographic events

The frequency of AA, AC and CC, polymorphism rs1748195 genotypes in healthy, Angio– and Angio+ groups is shown in Table 2. According to univariate regression analysis, it was found that among the three

TABLE 2 Distribution of genotypes and allele frequencies and their association with Angio− and Angio+ groups

	Frequency		p	Univariate regression (odds ratio 95% CI) p-value			Multivariate regression (odds ratio 95% CI)					
	Healthy	Angio−		Angio+	Healthy	Angio−	Angio+	Healthy	Angio−	Angio+		
Rs1748195			0.101									
AA	102 (32.6)	25 (50)	42 (33.9)	Ref	Ref	Ref	Ref	Ref	Ref	Ref		
AC	146 (46.6)	19 (38)	63 (50.8)	Ref	0.53 (0.28, 1.01)	0.055	1.05 (0.66, 1.67)	0.844	0.50 (0.17, 1.43)	0.196	0.68 (0.28, 1.61)	0.378
CC	65 (20.8)	6 (12)	19 (15.3)	Ref	0.38 (0.15, 0.97)	0.043*	0.71 (0.38, 1.33)	0.282	0.49 (0.22, 1.11)	0.089	0.92 (0.47, 1.79)	0.808
Dominant			0.054									
AA	102 (32.6)	25 (50)	42 (33.9)	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	
AC + CC	211 (67.4)	25 (50)	82 (66.1)	Ref	0.48 (0.26, 0.88)	0.018*	0.94 (0.61, 1.47)	0.797	0.49 (0.23, 1.04)	0.064	0.85 (0.45, 1.59)	0.603
Recessive			0.192									
AA + AC	248 (79.2)	44 (88)	105 (84.7)	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	
CC	65 (20.8)	6 (12)	19 (15.3)	Ref	0.52 (0.21, 1.27)	0.153	0.69 (0.39, 1.21)	0.195	0.73 (0.27, 1.94)	0.529	0.71 (0.33, 1.54)	0.384
Additive			0.088									
AA	102 (61.1)	25 (80.6)	42 (68.9)	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	
CC	65 (38.9)	6 (19.4)	19 (31.1)	Ref	0.38 (0.15, 0.97)	0.043*	0.71 (0.38, 1.33)	0.282	0.43 (0.14, 1.33)	0.144	0.78 (0.31, 1.93)	0.587
Codominant			0.308									
AA + CC	167 (53.4)	31 (62)	61 (49.2)	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	
AC	146 (46.6)	19 (38)	63 (50.8)	Ref	0.70 (0.38, 1.29)	0.256	1.18 (0.78, 1.79)	0.433	0.63 (0.3, 1.32)	0.222	1.07 (0.59, 1.93)	0.824
Allele			0.044*									
A	350 (55.9)	69 (69)	147 (59.3)	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	
C	276 (44.1)	31 (31)	101 (40.7)	Ref	0.57 (0.36, 0.89)	0.015*	0.87 (0.65, 1.17)	0.366	0.62 (0.39, 1.004)	0.052	0.91 (0.62, 1.34)	0.641

Note: Multivariate regression model adjusted with age, sex, BMI, Diabetes, HDL, TC, LD. Values are expressed as mean ± standard deviation or median (interquartile range 25th–75th) for normal and non-normal distribution data respectively. \* $p \leq 0.05$  is indicates as significant.

TABLE 3 Genotype distribution and frequency of alleles and its relationship with non-ISR and ISR

	Non-ISR N (%)	ISR N (%)	p	Univariate regression (odds ratio 95% CI)			Multivariate regression (odds ratio 95% CI)		
				Non-ISR	ISR	p	Non-ISR	ISR	p
Rs1748195			0.829						
AA	29 (33.3)	15 (37.5)		Ref	Ref		Ref	Ref	
AC	44 (50.6)	20 (50)		Ref	0.88 (0.39, 1.99)	0.757	Ref	0.56 (0.20, 1.54)	0.262
CC	14 (16.1)	5 (12.5)		Ref	0.69 (0.21, 2.28)	0.544	Ref	0.42 (0.09, 1.97)	0.272
Dominant			0.647						
AA	29 (33.3)	15 (37.5)		Ref	Ref		Ref	Ref	
AC + CC	58 (66.7)	25 (62.5)		Ref	0.83 (0.38, 1.82)	0.647	Ref	0.53 (0.20, 1.39)	0.195
Recessive			0.598						
AA + AC	73 (83.9)	35 (87.5)		Ref	Ref		Ref	Ref	
CC	14 (16.1)	5 (12.5)		Ref	0.74 (0.25, 2.23)	0.599	Ref	0.59 (0.14, 2.44)	0.466
Additive			0.543						
AA	29 (67.4)	15 (75)		Ref	Ref		Ref	Ref	
CC	14 (32.6)	5 (25)		Ref	0.69 (0.21, 2.28)	0.544	Ref	0.40 (0.08, 2.02)	0.270
Codominant			0.952						
AA + CC	43 (49.4)	20 (50)		Ref	Ref		Ref	Ref	
CC	44 (50.6)	20 (50)		Ref	0.98 (0.46, 2.07)	0.952	Ref	0.71 (0.28, 1.79)	0.464

studied genotypes, only the CC genotype of rs1748195 polymorphism has a significant relationship with the risk of negative angiogram in individuals (OR = 0.38, 95%CI = 0.15–0.97,  $p=0.043$ ). Also, based on dominant and additive genetic models, after univariate regression analysis, it was found that AC+CC and CC genotypes, respectively, increase the risk of negative angiogram compared to other genotypes ( $p < 0.05$ ), while after adjusting the parameters of age, sex, BMI, Diabetes, HDL, LDL and TC in the multiple regression model this relationship did not remain significant ( $p > 0.05$ ). The data showed that allele A of rs1748195 polymorphism was directly related to the risk of coronary occlusion <50% in individuals. In addition, the C allele frequency of this genetic variant was higher in the control group ( $p = 0.44$ ).

### 3.3 | Association of polymorphism rs1748195 genotypes with non-ISR and ISR

The frequencies of AA, AC and CC genotypes associated rs1748195 in the non-ISR group were 33.3%, 50.6% and 16.1%, and in the ISR group were 37.5%, 50% and 12.5%, respectively. According to the obtain results, no significant differences were observed between rs1748195 polymorphism genotypes and non-in-stent restenosis and in-stent restenosis>50% in dominant, recessive, additive and codominant genetic models ( $p > 0.05$ ) (Table 3).

### 3.4 | Association between genotypes of genetic variants rs1748195 and lipid profile

According to the data in Table 4 which was analyzed with Pearson  $\chi^2$  tests, there was a significant difference between lipid profiles (HDL and TC) and rs1748195 in ANGPTL3 gene with AA, AC and CC genotypes in all groups ( $p < 0.05$ ). Analysis have revealed that the level of PAB in the group of healthy individuals in all studied genotypes is higher than the other two groups ( $p < 0.05$ ). Evaluation of some disorders related to CVD events such as hypertension, diabetes and dyslipidemia showed that these disorders are more common in individuals with negative angiogram and carriers of AC genotype compared to Angio+ group ( $p < 0.001$ ). In contrast, the investigation of TG, SBP, DBP, BMI and CRP factors in three groups of healthy individuals, Angio– and Angio+ did not show a significant relationship in any of the rs1748195 polymorphism genotypes ( $p > 0.05$ ).

## 4 | DISCUSSION

Statistical analyzes in the present study demonstrated that lipid profiles (including LDL, HDL, and TC), history of hypertension, diabetes, and dyslipidemia were associated with angiography. According to the results, we found that the CC genotype of the rs1748195 polymorphism at the ANGPTL3 gene loci has a significant association with the

TABLE 4 Distribution of genotypes and clinical characteristics of individuals and their relationship with angio

	AA			AC			CC					
	Healthy	Angio-	Angio+	Healthy	Angio-	Angio+	Healthy	Angio-	Angio+			
Age	48.04 ± 7.77	56.80 ± 8.60	60.98 ± 8.09	<0.001*	49.08 ± 7.74	54.79 ± 8.83	59.48 ± 9.37	<0.001*	49 ± 7.5	45.50 ± 8.55	60.26 ± 8.01	<0.001*
Female	52 (51)	17 (68)	15 (35.7)	0.035*	94 (64.4)	9 (47.4)	13 (20.6)	<0.001*	39 (60)	3 (50)	6 (31.6)	0.091
HDL	44.09 ± 12.38	38.21 ± 9.07	35.12 ± 7.62	<0.001*	44.98 ± 13.37	35.47 ± 6.90	36.34 ± 9.12	<0.001*	45.23 ± 13.04	30.33 ± 10.86	34.12 ± 11.48	0.001*
LDL	109.82 ± 34.41	93.97 ± 26	88.97 ± 33.58	0.002*	112.73 ± 35.99	92.99 ± 36.25	96.86 ± 31.03	0.003*	126.45 ± 44.81	108.60 ± 44.60	96.92 ± 45.20	0.50
TG	130.71 ± 79.28	130.64 ± 74.98	138.02 ± 67	0.868	143.36 ± 102.33	129.76 ± 75.68	124.91 ± 60.39	0.398	128.28 ± 88.80	197.83 ± 130.64	134.41 ± 58.19	0.179
TC	187.01 ± 40.24	160.84 ± 37.90	151.70 ± 40.34	<0.001*	192.88 ± 36.70	154.41 ± 44.53	157.07 ± 35.92	<0.001*	198.51 ± 47.99	178.5 ± 57.62	157.29 ± 50.83	0.010*
SBP	122.24 ± 19.05	118.75 ± 10.86	125.29 ± 15.07	0.364	120.89 ± 18.98	122.14 ± 16.61	124.33 ± 14.02	0.475	121.38 ± 22.18	130 ± 7.91	124.06 ± 14.52	0.622
DBP	79.14 ± 10.96	76.46 ± 6.67	79.29 ± 8.01	0.461	78.95 ± 11.60	81.07 ± 9.44	78.87 ± 11.42	0.794	80.78 ± 13.52	83.33 ± 5.16	76.25 ± 8.47	0.346
BMI	27.81 ± 4.98	28.18 ± 4.92	26.24 ± 3.38	0.156	27.64 ± 4.80	26.56 ± 3.14	30.03 ± 27.39	0.516	27.93 ± 4.41	26.57 ± 2.61	31.75 ± 24.71	0.429
CRPnew	6.78 ± 11.68	4.09 ± 3.59	4.12 ± 3.85	0.227	4.78 ± 10.19	5.54 ± 5.30	3.30 ± 3.03	0.468	4.47 ± 10.98	2.73 ± 2.66	2.82 ± 2.09	0.815
PAB	77.79 ± 34.37	1.06 ± 0.31	1.05 ± 0.25	<0.001*	100.70 ± 46.05	1.04 ± 0.29	1.13 ± 0.35	<0.001*	80.72 ± 56.52	0.98 ± 0.13	0.99 ± 0.11	0.005*
Hypertention	27 (26.5)	9 (36)	19 (45.2)	0.085	49 (33.6)	10 (52.6)	39 (61.9)	<0.001*	20 (30.8)	4 (66.7)	14 (73.7)	0.002*
Diabetes	10 (10.1)	12 (48)	11 (26.8)	<0.001*	26 (17.9)	6 (31.6)	28 (44.4)	<0.001*	15 (23.8)	2 (33.3)	7 (36.8)	0.504
Hyperlipidemia	76 (74.5)	16 (64)	26 (61.9)	0.257	124 (84.9)	8 (42.1)	33 (52.4)	<0.001*	56 (86.2)	4 (66.7)	8 (42.1)	

Note: Values are expressed as mean ± standard deviation or median (interquartile range 25th–75th) for normal and non-normal distribution data respectively. \* $p \leq 0.05$  is indicates as significant.

negative angiogram. Whereas, the frequency of C allele of this SNP was higher in the group of healthy individuals. In contrast, we did not find any significant differences between the genotypes of this genetic variant and the NISR and in-stent restenosis >50% groups in any of the studied genetic models. Also, the clinical characteristics of individuals, such as PAB and some disorders related to CVD events such as blood pressure, showed a significant and effective difference in the types of genotypes.

In the reviews, we found that, Cardiovascular disease is the leading cause of death worldwide and involves a range of circulatory disorders (Francula-Zaninovic & Nola, 2018). One of the most common causes of CVD is an inflammatory disease called atherosclerosis, in which the accumulation of LDL particles containing cholesterol blocks the coronary arteries of the heart (Lnsis, 2000). ANGPTL3 belongs to the family of ANGPTLs and is expressed almost exclusively in stages of embryonic development and in adults in the liver (Lupo & Ferri, 2018). ANGPTLs are regulators of plasma lipids in animals and humans (Su & Peng, 2018). This protein inhibits LPL and EL and thus affects the level of plasma TGs (Olkonen et al., 2018). Dewey et al. reported that one type of loss function in ANGPTL3 was associated with a 41% reduction in CAD risk. It was also found that the anti-ANGPTL3 monoclonal antibody, called evinacumab can reduce plasma TG and LDL-C levels by up to 76% and 23%, respectively (Dewey et al., 2017). Several GWAS have shown that many single nucleotide polymorphisms, including the genetic variant rs1748195 of the ANGPTL3 gene, have a significant relationship with serum TGA levels. In this way, it plays an effective role in cardiovascular disease. (Jeemon et al., 2011). Willer et al. (2008) in the study of 18,243 samples stated that this polymorphism with  $p = 1.7 \times 10^{-10}$  is related to plasma triglyceride levels. Also, by studying Murray et al. on 1155 eligible Italians, after determining the effect of this SNP on the TG level, it was determined that the C allele of this polymorphism with frequency = 0.675 is the TG risk-increasing allele (Murray et al., 2009). In addition, the study of Chung et al. which was performed on 3060 staff of Korean medical clinics (Chung et al., 2014) and 5537 individuals from the cohort study, confirmed the association of serum TG levels with genetic variants rs10889353 and rs1748195 of ANGPTL3 gene (Cho et al., 2009). In 2021, a study by Aghasizadeh et al. on 1002 samples reported a strong association between CVD events and different genotypes of ANGPTL3 gene polymorphisms, including rs1748195 polymorphism. In contrast, no significant association was found between this SNP and HTN, dyslipidemia and metabolic syndrome (MetS) (Aghasizadeh, Zare-Feyzabadi, et al., 2021). Based on the results of a study by Li et al. in 2018 on 568 patients with CAD and 539 patients with ischemic stroke, it was

found that the genetic variant rs1748195 would increase the risk of atherosclerosis as well as CAD (Li et al., 2018).

In recent years, genome-related studies have provided new insights into genetics and its association with cardiovascular disease. Although many studies have been performed to find an association between the ANGPTL3 gene and CVD on this gene and its polymorphisms, there are still many unknowns. Therefore, it is suggested that more studies be done in this regard so that the resulting information can have more effective clinical applications for us.

## 5 | CONCLUSION

In the present study, we investigated that the CC genotype, and also the A allele corresponding to rs1748195 at the ANGPTL3 gene loci was associated with negative angiogram and directly related to the risk of coronary occlusion >50%. In contrast, this result was not significant between ISR and non-ISR groups. Furthermore, statistical analysis demonstrates that the fundamental characteristics of this study population were related to the existence of a coronary occlusion of >50%. Therefore, it is suggested that more studies should be done to have more effective clinical applications for us.

### AUTHOR CONTRIBUTIONS

We declare that we contributed significantly towards the research study; MA, NA, NS, and SGH designed the experiments and revised the manuscript. MA and NA performed the experiments. MA, SS, MR and GF wrote the manuscript. ARE, KK and HE carried out the data analysis. RAD and MGM were supervisor of the study. All authors reviewed, considered, and approved the manuscript.

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### AVAILABILITY OF DATA AND MATERIALS

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


### CONFLICT OF INTEREST

The authors declare no conflict of interest.

### ETHICS 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 Science.

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