The association between the chromosome 9p21 *CDKN2B-AS1* gene variants and the lipid metabolism: A pre-diagnostic biomarker for coronary artery disease

Şehime Gülsün Temel, **, Mahmut Çerkez Ergören[1]*

Departments of *Medical Genetics, and **Histology&Embryology, Faculty of Medicine, Uludağ University; Bursa-*Turkey*
[1]Department of Medical Biology, Faculty of Medicine, Near East University; Nicosia-*Turkish Republic of Northern Cyprus*

Abstract

Objective: Recent genome-wide association studies have established that polymorphisms within *CDKN2B-AS1* of chr9p21.3 locus increased susceptibility to coronary artery disease (CAD) or myocardial infarction. Common variants of *CDKN2B*-AS1 (including rs4977574 A>G and rs1333040 C>T) are determined to be directly associated with CADs in many populations worldwide and suggested biomarkers for the early detection of CAD. There is a lack of investigation for the association between *CDKN2B-AS1* rs4977574 A>G and rs1333040 C>T genetic modifiers and CAD in a Turkish Cypriot population. The aim of the present study was to investigate the potential effects of these variants on susceptibility to developing CAD in a Turkish Cypriot population and their contribution to lipid metabolism.

Methods: Seventy-one patients with angiography-confirmed CAD were recruited to the CAD group, whereas 153 voluntary subjects without CAD symptoms were enrolled to the control group. Genotyping for the *CDKN2B-AS1* gene polymorphisms was performed by polymerase chain reaction, followed by restriction fragment length polymorphism analysis.

Results: There is no statistical significant association observed between rs4977574 and rs1333040 single-nucleotide polymorphisms and two studied groups [odds ratio (OR): 0.763, p=0.185, 95% confidence interval (CI): 0.511–1.139 and OR: 1.060, p=0.802, 95% CI: 0.672–1.671, respectively]. However, rs2977574 G and rs1333040 T alleles–the risk alleles–were found to be associated with higher level of serum total cholesterol and lower level of high-density lipoprotein-cholesterol in the CAD group (p=0.019, p=0.006 and p=0.022, p=0.031, respectively). To our knowledge, this is the first study that establishes the effect of rs1333040 on lipid metabolism.

Conclusion: The presence of rs4977574 G and rs1333040 T alleles and interaction may exist as environmental factors associated with lipid metabolism and might be responsible for the development of CAD in a Turkish Cypriot population. *(Anatol J Cardiol 2019; 21: 31-8)*

Keywords: chr9p21, rs4977574, rs1333040, biomarkers, coronary artery disease

Introduction

Coronary artery disease (CAD) belongs to the cardiovascular disease (CVD) group, which includes the heart and blood vessels, resulting from the build-up of plaques in the coronary arteries and the ruptured plaques that may induce thrombosis in coronary atherosclerosis (1). CAD is the leading cause of death including both morbidity and mortality globally, especially in developing countries (2, 3). Despite the genetic basis for CAD remains relatively unknown, previous studies suggested that several independent risk factors including smoking, hypercholesterolemia, hypertension, obesity, and diabetes have a strong association for the developing CAD pathology (4). Recently, genome-wide association studies (GWAS) have reported the locus codes for an antisense RNA (*CDKN2B-AS1* or ANRIL), which is located nearby the *CDKN2A–CDKN2B* gene cluster with an increased susceptibility to CAD or myocardial infraction in carriers of the particular single-nucleotide polymorphisms (SNPs) within the chromosome 9p21.3 locus (5–12). Although these SNPs are located within the intronic region, their functional link still remains suppositional (13). The risk alleles of the CAD-associated variants were shown to be strongly associated with an increased of CAD pathogenesis 20% to 30% (14). The *CDKN2B-AS1* gene encodes a functional RNA molecule that interacts with polycomb repressive complex 1 (PRC1) and 2 (PRC2), suggesting epigenetic silencing of other genes in the *CDKN2A–CDKN2B* gene cluster (15). SNPs within this region that influenced the *CDKN2B* expression are involved in the pathogenesis of atherosclerosis, whereas *CDKN2B*

Address for correspondence: Dr. Şehime Gülsün Temel, Uludağ Üniversitesi Tıp Fakültesi, Tıbbi Genetik Anabilim Dalı, 16059, Bursa-*Türkiye*
Phone: +90 532 236 16 46 E-mail: sehime@uludag.edu.tr
Accepted Date: 01.10.2018 **Available Online Date:** 07.12.2018

DOI:10.14744/AnatolJCardiol.2018.90907

Anatol J Cardiol 2019; 21: 31-8
DOI:10.14744/AnatolJCardiol.2018.90907

is a downstream target for transforming growth factor (TGF)-β that suggested its role in the TGF-β-induced growth inhibition (16, 17). Even though its molecular mechanism is still not clear, it is known that TGF-β plays an important role in maintaining normal vessel wall structure, and a lack of this function affects the development of atherosclerosis (18). *MTAP* is a protein-coding gene that encodes the ubiquitously expressed enzyme methylthioadenosine phosphorylase close to the chr9p21.3 region (17). *MTAP* belongs to the polyamine metabolism and plays an important role in releasing adenine and methionine (19).

Common variants of *CDKN2B-AS1* (including rs4977574 A>G/T and rs1333040 C>T) are determined to be directly associated with CADs in many populations worldwide and suggested biomarkers for the early detection of CAD (20–26). However, there is a lack of investigation for the association between *CDKN2B-AS1* rs4977574 A>G/T and rs1333040 C>T genetic modifiers and CAD in a Turkish Cypriot population. Turkish Cypriots are a developing society and have a relatively high ratio of CVD, which could be due to lifestyle, unhealthy diet, and restrictive Island-spesific gene pool (27). The aim of the present study was to investigate the potential effects of *CDKN2B-AS1* rs4977574 A>G/T and rs1333040 C>T polymorphisms on susceptibility to CAD in a Turkish Cypriot population.

Methods

A total of 224 unrelated volunteers who belong to the Turkish Cypriot population were included in the study. The study protocol was approved by the Institutional Review Board (NEU/2016/36/382). Informed consent was obtained from all study participants. Each subject was provided with a questionnaire to determine personal characteristics, including age, ethnicity, and general health status. The Turkish Cypriot ethnicity was defined as residing in North Cyprus as well as being born to parents who have been living in the island of Cyprus for at least three generations. Additionally, considering the small size of the island population and high number of relatives in North Cyprus, subjects who are relatively related were excluded from the study. One hundred fifty-three healthy subjects with no clinical evidence of type 2 diabetes, hypertension, obesity, hypercholesterolemia, family/history of stroke, or transient ischemic attacks and 71 patients with angiography-confirmed CAD who were diagnosed by a cardiologist constituted two study groups (control group and CAD group, respectively). Antecubital venous blood from the subjects was collected in tubes containing Ethylenediaminetetraacetic acid (EDTA) and centrifuged within 2 h of collection. The fasting levels of plasma glucose, serum total cholesterol, high-density lipoprotein-cholesterol (HDL-C), low-density lipoprotein-cholesterol (LDL-C), and triglyceride (TG) were measured using an automatic biochemical analyzer [Clinical Biochemistry Analyzer (CA); JEOL, Japan] in the Medical Biochemistry Laboratory of the Near East University Hospital.

Genotyping

DNA was extracted using PureLink Genomic DNA Mini Kit (Thermo Fisher Scientific, USA). Two *CDKN2B-AS1* (rs4977574 A>G/T and rs1333040 C>T) polymorphisms were analyzed by polymerase chain reaction (PCR) restriction fragment length polymorphism according to previous studies (28, 29). PCR was performed in a total reaction volume of 25 µl in 200 µl tubes on an Applied Biosystems Veriti Thermal Cycler. The reaction mixture consisted of 10 ng genomic DNA, 0.5 µM forward and reverse primers (Table 1), 1× Taq polymerase buffer with KCL (Thermo Scientific, EP0402), 1.5 mM MgCl2 (Thermo Scientific), 200 mM dNTP mix (Thermo Scientific, R0191), and 1.5 U Taq polymerase (Thermo Scientific, EP0402). A class II laminar flow hood, designated pipettes, PCR clean plasticware and reagents, and ultraviolet-treated solutions were used to minimize the risk of contamination during DNA extraction and PCR preparation. The digested fragments were separated in 2% agarose gels and visualized by ethidium bromide staining. Genotypes were determined according to the presence and absence of restriction sites, and alleles were designated with respect to actual base change according to the dbSNP (https://www.ncbi.nlm.nih.gov/SNP/) and Ensembl (http://www.ensembl.org/) websites (Table 1).

Statistical analysis

Data were expressed as mean±standard deviation for normally distributed continuous variables. Intergroup differences in continuous variables were assessed by the Student's unpaired t-test. Genotype distributions and allele frequencies were calculated by the gene-counting method, and their compliance to the Hardy–Weinberg equilibrium (HWE) was evaluated by the Pearson's goodness-of-fit chi-square, log likelihood ratio chi-square,

Table 1. The details of PCR primers and restriction enzymes for the *CDKN2B-AS1* gene SNPs rs4977574 A>G and rs1333040 C>T.

SNP	Primers	Restriction enzyme	Reference
rs4977574	F 5′-ATAGGGGTTATGGGAAATGC – 3′	*HhaI*	29
	R 5′- AAACCTAAAAGGGCTTGCTGA – 3′		
rs1333040	F 5′ - TCTGGAAGCACTGGGAAGGATG – 3′	*BsmI*	30
	R 5′- TTG ATT TGG GAG CCA CTG TTG - 3′		

SNP - single-nucleotide polymorphism

and Fisher's exact test. The association between the case–control status and each polymorphism was assessed by the odds ratio and its corresponding 95% confidence interval. The influence of the assigned genotypes on biochemical parameters was evaluated using a one-way analysis of variance (ANOVA) for each polymorphism. The aforementioned single-locus data analyses were performed using the commercial GraphPad Prism software (GraphPad Software Inc., San Diego, CA, USA). The HaploBlock software was used for haplotype and linkage disequilibrium analyses. A p value <0.05 was considered statistically significant.

Results

Demographic, clinical, and laboratory characteristics of the studied group

The personal characteristics and biochemical parameters of the subjects, from whom blood samples were obtained, are shown in Table 2. The subjects comprised 224 Turkish Cypriot individuals, including 71 patients with CAD and 153 Turkish Cypriots as the control group. The CAD group showed no statistically significant difference from the control group with respect to age, fasting plasma glucose levels, and serum concentrations of total cholesterol, HDL-C, and LDL-C, whereas the serum concentrations of TG were significantly higher in the CAD group than in the control group (p=0.001). It should be noted, however, that the serum concentrations of TG in the CAD group (153.2±66.0 mg dL−1) are within the slightly higher than normal range of >150 mg dL−1 (National Cholesterol Education Program Expert Panel, 2002), which should not be a major risk factor for heart disease nor considered protective against heart disease.

Table 2. Basic characteristics of all studied subjects

Variable	Control n=153	Two-tailed n=71	*P* value
Age (years)	41.4±11.5	44.9±15.0	0.092
Sex	64.7% F	63.4% F	
	35.3% M	36.6% M	0.847
Glucose (mg dL−1)	92.5±24.2	96.1±24.2	0.421
Cholesterol (mg dL−1)	196.3±51.9	202.8±42.6	0.432
	54.6±13.1	50.9±10.9	0.092
	129.4±41.2	130.1±30.9	0.920
Triglyceride (mg dL−1)	113.1±43.0	153.2±66.0	0.001

Data are represented as mean±standard deviation.
M - male, F - female
Patients with abnormal lipid levels were identified by cut-off points of >90 mg dL−1 for glucose, >200 mg dL−1 for total cholesterol, >130 mg dL−1 for LDL-cholesterol, >40 mg dL−1 for HDL-cholesterol, and >150 mg dL−1 for triglycerides

Distribution of the *CDKN2B-AS1* gene polymorphisms in the studied population

The genotype distributions and allele frequencies of *CDKN2B-AS1* rs4977574 A>G and rs1333040 C>T among the 71

Table 3. Genotype and allele frequencies for the two *CDKN2B-AS1* gene polymorphisms, rs4977574 A>G/T and rs1333040 C>T, in the two groups

	Genotype/allele	CAD n[a] (%)	Control n[b] (%)	OR	95% CI	*P**
rs4977574	AA	24 (33.9)	39 (23.5)			
	AG	33 (46.4)	76 (49.6)			
	GG	14 (19.7)	38 (24.9)			
	A	81 (57.0)	154 (50.3)			
	G	61 (43.0)	152 (49.7)	1.310	0.877-1.956	0.185
HWE *P*- value		0.935	0.663			
rs1333040	CC	4 (5.6)	14 (9.2)			
	CT	28 (39.4)	53 (34.6)			
	TT	39 (55.0)	86 (56.2)			
	C	36 (25.3)	81 (26.5)			
	T	106 (74.7)	225 (73.5)	1.06	0.672-1.671	0.06
HWE *P*-value		0.173	0.723			

[a]n=71
[b]n=153
*Pearson chi-square test.
Hardy–Weinberg equilibrium test was performed to compare the observed and expected genotypes and to compute the allele frequencies as well as *P* values for each single-nucleotide polymorphism.
HWE - Hardy–Weinberg equilibrium; CI - confidence interval

Anatol J Cardiol 2019; 21: 31-8
DOI:10.14744/AnatolJCardiol.2018.90907

Table 4. The tests for association and for deviation from the HWE are adapted from Sasieni (31)

SNPs	Tests for deviation from HWE		Tests for association (95% CI)				
	Controls	Cases	Allele freq. difference	Heterozygous	Homozygous	Allele positivity	Armitage's trend test
			Risk allele G				
			[A]<->[G]	[A]<->[G]	[A]<->[G]	[A]<->[G]	[A]<->[G]
			OR=0.763	OR=0.763	OR=0.763	OR=0.763	OR=0.763
	AA=39	AA=24	CI=0.511-1.139	CI=0.511-1.139	CI=0.511-1.139	CI=0.511-1.139	CI=0.511-1.139
	AG=76	AG=33	X^2=1.75	X^2=1.75	X^2=1.75	X^2=1.75	X^2=1.75
	GG=38	GG=14	*P*=0.185	*P*=0.185	*P*=0.185	*P*=0.185	*P*=0.185
rs4977574	f_a1=0.50 ±0.029	f_a1=0.57 ±0.043	Risk allele A				
	F=0.006	F=0.051	[G]<->[A]	[GG]<->[AG]	[AG+GG]<->[AA]	[AA+AG]<->GG]	Common OD's
	p=0.935	p=0.663	OR=1.311	OR=1.179	OR=1.670	OR=1.345	OR=1.297
			CI=0.878-1.957	CI=0.564-2.462	CI=0.753-3.704	CI=0.675-2.683	X^2=1.71
			X^2=1.75	X^2=0.19	X^2=1.61	X^2=0.71	*P*=0.190
			P=0.185	*P*=0.661	*P*=0.204	*P*=0.398	
			Risk allele T				
			[C]<->[T]	[CC]<->[CT]	[CC+CT]<->[TT]	[CC]<->[CT+TT]	Common OD's
			OR=1.060	OR =1.849	OR=1.587	OR=1.687	OR=1.128
	CC=14	CC=4	CI=0.672-1.672	CI=0.556-6.150	CI=0.491-5.134	CI=0.535-5.322	X^2=0.06
	CT=53	CT=28	X^2=0.06	X^2=1.03	X^2=0.60	X^2=0.81	*P*=0.807
	TT=86	TT=39	*P*=0.802	*P*=0.311	*P*=0.437	*P*=0.367	
rs1333040	f_a1=0.26 +/-0.027	f_a1=0.25 +/-0.036	Risk allele C				
	F=0.110	F=-0.041	[T]<->[C]	[TT]<->[CT]	[TT]<->[CC]	[CC+CT]<->[TT]	Common OD's
	p=0.173	p=0.723	OR=0.943	OR=1.165	OR=0.630	OR=1.053	OR=0.898
	-		CI=0.598-1.488	CI=0.643-2.110	CI=0.195-2.038	CI=0.598-1.855	X^2=0.06
			X^2=0.06	X^2=0.25	X^2=0.60	X^2=0.03	*P*=0.807
			P=0.802	*P*=0.614	*P*=0.437	*P*=0.857	

The evaluation of genotype comparison did not show any statistical significance between the CAD and control groups.
f_al: frequency of allele 1±standard deviation, F: inbreeding coefficient.
HWE - Hardy–Weinberg equilibrium; CI - confidence interval; OR - odd ratio; SNP - single-nucleotide polymorphism

cases and 153 controls are shown in Table 3. The distributions of the *CDKN2B-AS1* rs4977574 A>G and rs1333040 genotypes were in compliance with the HWE (p>0.050). The frequencies of the minor alleles *CDKN2B-AS1* rs4977574 G and rs1333040 C among the case group were 0.43 and 0.25, respectively. The minor allele frequency for *CDKN2B-AS1* rs1333040 C in the control group was similar with the case group (0.26), whereas *CDKN2B-AS1* rs4977574 had equal allele frequency for both G and T alleles (0.50/0.50). To test the genetic association using the case–control study design, data for a single biallelic marker calculation were adapted from Sasieni (30). The comparison test for association and for deviation from the HWE did not present any statistical difference between the two studied groups and analyzed SNP genotypes (Table 4).

Comparison of the two *CDKN2B-AS1* rs4977574 A>G/T and rs1333040 C>T gene polymorphisms with clinical parameters within the case–control subjects

The distribution of all biochemical parameters according to the *CDKN2B-AS1* genotypes in the case–control populations is presented in Table 5. ANOVA standard weighted-means analysis for independent samples (df: 2) was made to determine the association studies for the other two APOA5 SNPs and biochemical parameters. No association between the two studied *CDKN2B-AS1* polymorphisms (rs4977574 and rs1333040) and the biochemical components of glucose, serum LDL-C, and TG was observed in both the case and control groups. On the other hand, a strong statistically significant association between serum total cholesterol clinical parameter and *CDKN2B-AS1*

Table 5. Comparison of the *CDKN2B-AS1* gene rs4977574 A>G and rs1333040 C>T polymorphisms with clinical parameters within both studied groups

Clinical parameters		**rs4977574 A>G**		**ANOVA *P* value**
Control	**GG**	**AG**	**AA**	
Glucose (mg dL-1)	90.6±09.0	94.6±33.3	90.3±7.9	0.720
Cholestrol (mg dL-1)	197.2±49.8	196.1±60.6	195.8±38.4	1.000
HDL-C (mg dL-1)	55.5±13.1	55.2±14.5	52.9±10.9	0.746
LDL-C (mg dL-1)	122.7±39.9	135.0±44.4	124.7±36.7	0.480
Triglyceride (mg dL-1)	123.6±46.4	115.6±39.5	101.0±45.5	0.236
Clinical parameters				**ANOVA *P* value**
CAD	**GG**	**AG**	**AA**	
Glucose (mg dL-1)	100.5±18.0	101.6±21.1	95.5±10.5	0.756
Cholestrol (mg dL-1)	240.8±70.2	202.1±43.7	213.0±40.9	0.019
HDL-C (mg dL-1)	44.0±08.4	53.1±09.9	56.0±12.9	0.006
LDL-C (mg dL-1)	130.2±43.8	128.5±31.8	102.1±20.3	0.109
Triglyceride (mg dL-1)	188.2±44.9	103.6±40.3	90.5±19.2	0.305
Clinical parameters		**rs1333040 C>T**		**ANOVA *P* value**
Control	**CC**	**CT**	**TT**	
Glucose (mg dL-1)	91.2±10.8	89.9±10.2	95.3±32.3	0.608
Cholestrol (mg dL-1)	195.9±57.6	197.1±44.6	203.4±53.2	0.951
HDL-C (mg dL-1)	51.4±07.5	52.2±11.5	44.9±27.9	0.361
LDL-C (mg dL-1)	135.8±53.4	127.0±37.9	131.1±43.1	0.869
Triglyceride (mg dL-1)	109.0±37.5	111.6±47.6	115.6±41.3	0.904
Clinical parameters				**ANOVA *P* value**
CAD	**CC**	**CT**	**TT**	
Glucose (mg dL-1)	95.4±11.5	101.9±21.1	101.1±19.1	0.795
Cholestrol (mg dL-1)	199.9±47.0	201.1±38.1	246.3±71.4	0.022
HDL-C (mg dL-1)	55.9±11.7	47.7±4.5	46.1±13.7	0.031
LDL-C (mg dL-1)	108.7±11.9	126.4±31.6	140.6±23.8	0.762
Triglycerid (mg dL-1)	104.0±29.5	113.8±77.2	183.6±96.9	0.028

CAD - coronary artery disease; HDL-C - high-density lipoprotein-cholesterol; LDL-C - low-density lipoprotein-cholesterol

rs4977574 GG and rs1333040 TT genotypes was found (p=0.019 and p=0.022, respectively) in the CAD group. Moreover, the same strong statistical significant association has been observed between HDL-C and *CDKN2B-AS1* rs4977574 GG and rs1333040 TT genotypes (p=0.006 and p=0.031, respectively). Individuals who are homozygous for either *CDKN2B-AS1* rs4977574 GG or rs1333040 TT genotype have an increased number of serum total cholesterol and decrease HDL-C levels (240.8±70.2 and 246.3±71.4 for total cholesterol and 44.0±08.4 and 46.1±13.7 for HDL-C, respectively).

Haplotype analysis

Linkage equilibrium analysis was made to determine and better understand the -cis regulation effect of both rs4977574 and rs1333040 intronic variants in patients with CAD. All observed haplotypes have been compared with each other The comparison analysis of -cis configuration analysis showed no observed difference between both *CDKN2B-AS1* SNP (rs4977574 and rs1333040) genotypes in a Turkish Cypriot population with CAD (data not shown).

Discussion

For the last decade, GWAS mostly examined the molecular factors involved in the pathological development of CAD (31). GWAS meta-analysis investigations revealed that the chr9p21.3 region contains several SNPs that are directly associated with CAD risk, especially with a younger age of onset (32). Previously, two *CDKN2B-AS1* gene variants (rs4977574 A>G and rs1333040

Anatol J Cardiol 2019; 21: 31-8
DOI:10.14744/AnatolJCardiol.2018.90907

C>T) within the chr9p21.3 locus were found to be associated with CAD risk (20, 32).

Thus, in the present study, we attempted to investigate the association of the *CDKN2B-AS1* rs4977574 A>G and rs1333040 C>T polymorphisms with the risk of CAD in an islandic population of White Caucasian of Turkish Cypriot origin. To our knowledge, this is the first study in the relevant scientific literature to examine the *CDKN2B-AS1* gene polymorphisms in this population and better understand the genetic predisposition of Turkish Cypriots to CAD, in addition to the expected Mediterranean diet.

Several GWAS and replication studies have shown a consistent association with the non-protein-coding SNP rs4977574 A>G and the risk of CAD in populations of European or Eastern Asian descent (33–36). GWAS also showed that individuals with the rs4977574 AA genotype have higher risk of coronary heart diseases after controlling for potential confounders including age, sex, body mass index, cigarette smoking, hypertension, diabetes, and hyperlipidemia (37). Recently, Lu et al. (38) found that the rs4977574 G allele is potentially associated with non-cardioembolic cerebral infarction and carotid plaque in a Chinese Han population. Controversially, Cheng et al. (39) presented that there is no association between the rs4977574 variant and stroke subtypes. Moreover, Hindy et al. (40) suggested that rs4977574 interacts with vegetable and wine intake–main sources of Mediterranean diet–to affect the incidence of CAD. Previously, an independent SNP in the *CDKN2A/B* locus near the 9p21 53-kb LD block has been robustly associated with type 2 diabetes due to the rs4977574 risk allele associated with elevated glycated hemoglobin levels among individuals with a lower vegetable intake (40-42). In the same study, they observed manipulations of the association between rs4977574 and HDL-C levels by smoking, providing evidence that pathological risk may increase with environmental factors, leading to derangements at the level of glucose and lipid metabolism (40). In the present study, there is no statistically significant association observed between rs4977574 SNP and two studied groups (p=0.185). However, the rs4977574 G allele was found to be associated with higher level of serum total cholesterol and lower level of HDL-C in the CAD group (p=0.019 and p=0.006, respectively).

Various studies previously have indicated that the rs1333040 C<T polymorphism was significantly associated with the risk of development of CAD in North Indian (43), German (31), and Chinese Taiwanese (44) populations, but not in Iranian (45) and Chinese Han (37). Beckie et al. (46) have suggested that the risk allele for rs1333040 among Black women is diagnosed for coronary heat diseases 6.5 years earlier compared with those with the good allele, whereas this effect was absent in White women. In the present study, there is no association shown between rs1333040 SNP and two studied groups (p=0.802). On the other hand, the rs1333040 T allele–risk variant–was found to be associated with higher level of serum total cholesterol and lower level of HDL-C in the Turkish Cypriot CAD group (p=0.022 and p=0.031, respectively). HDL-C is believed to reflect the ability of HDL particles to remove excess cholesterol molecules from peripheral cells for return to the liver (46, 47). Therefore, lower level of HDL-C will not be able to protect cholesterol hierarchy as increase levels result in atherosclerotic plaques that cause CADs. To our knowledge, this is the first study that establishes the effect of rs1333040 on lipid metabolism.

Study limitations

As with many other genetic association studies, the present study also has several limitations. First, the number of subjects included in our study is relatively small, and this lowers the statistical power. Second, the rs4977574 A>G and rs1333040 C>T polymorphisms are located within the intronic regions of the *CDKN2A/B* gene of chr9p21.3, and this confronts us with the challenge of precisely describing their functional relevance. Third, the epistatic interactions between the *CDKN2A/B* polymorphism and other genes and also *CDKN2A/B*–environment interactions remain to be thoroughly characterized, and this makes it difficult to draw definite conclusions about the causal connections between the *CDKN2A/B* variants and risk of CAD.

Conclusion

In conclusion, the results from our study suggest the homozygous wild-type genotypes of rs4977574 GG and rs1333040 TT at the *CDKN2A/B* as a genetic risk factor with elevated serum total cholesterol and lower HDL-C effects in a Turkish Cypriot population with CAD. However, allele A for rs4977574 was found to be statistically higher in the CAD groups (p=0.014). With interaction with dominant lifestyles, minimal physical activity, and meat heavy fast food culture in the population, these risk alleles may affect lipid metabolism. Thus, these SNPs could have clinical importance as predisposition biomarkers. The relatively small number of inhabitants in North Cyprus calls for GWAS of CAD and other CVDs in a Turkish Cypriot population. Further study is required to determine the functional effects of these SNPs and validate these findings in larger populations.

Conflict of interest: None declared.

Peer-review: Externally peer-reviewed.

Authorship contributions: Concept – Ş.G.T., M.Ç.E.; Design – Ş.G.T., M.Ç.E.; Supervision – Ş.G.T., M.Ç.E.; Fundings – Ş.G.T., M.Ç.E.; Materials – Ş.G.T., M.Ç.E.; Data collection &/or processing – Ş.G.T., M.Ç.E.; Analysis &/or interpretation – Ş.G.T., M.Ç.E.; Literature search – Ş.G.T., M.Ç.E.; Writing – Ş.G.T., M.Ç.E.; Critical review – Ş.G.T., M.Ç.E.

References

1. Sathyamurthy I, Jayanthi K. Dual antiplatelet therapy in acute coronary syndromes and coronary artery interventions. J Assoc Physicians India 2014; 62: 596-601.

2. Bonow RO, Smaha LA, Smith SC Jr, Mensah GA, Lenfant C. World Heart Day 2002: the international burden of cardiovascular disease: responding to the emerging global epidemic. Circulation 2002; 106: 1602-5.
3. Levenson JW, Skerrett PJ, Gaziano JM. Reducing the global burden of cardiovascular disease: the role of risk factors. Prev Cardiol 2002; 5: 188–99.
4. AbdulAzeez S, Al-Nafie AN, Al-Shehri A, Borgio JF, Baranova EV, Al-Madan MS, et al. Intronic Polymorphisms in the CDKN2B-AS1 Gene Are Strongly Associated with the Risk of Myocardial Infarction and Coronary Artery Disease in the Saudi Population. Int J Mol Sci 2016; 17: 395.
5. Wellcome Trust Case Control Consortium. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature 2007; 447: 661-78.
6. Samani NJ, Erdmann J, Hall AS, Hengstenberg C, Mangino M, Mayer B, et al. Genomewide association analysis of coronary artery disease. N Engl J Med 2007; 357: 443-53.
7. Abdullah KG, Li L, Shen GQ, Hu Y, Yang Y, MacKinlay KG, et al. Four SNPS on chromosome 9p21 confer risk to premature, familial CAD and MI in an American Caucasian population (GeneQuest). Ann Hum Genet 2008; 72(Pt 5): 654-7.
8. Durda P, Sabourin J, Lange EM, Nalls MA, Mychaleckyj JC, Jenny NS, et al. Plasma levels of soluble interleukin-2 receptor α associations with clinical cardiovascular events and genome-wide association scan. Arterioscler Thromb Vasc Biol 2015; 35: 2246-53.
9. Roberts R, Stewart AF. 9p21 and the genetic revolution for coronary artery disease. Clin Chem 2012; 58: 104-12.
10. McPherson R, Pertsemlidis A, Kavaslar N, Stewart A, Roberts R, Cox DR, et al. A common allele on chromosome 9 associated with coronary heart disease. Science 2007; 16: 1488-91.
11. Broadbent HM, Peden JF, Lorkowski S, Goel A, Ongen H, Green F, et al. Susceptibility to coronary artery disease and diabetes is encoded by distinct, tightly linked SNPs in the ANRIL locus on chromosome 9p. Hum Mol Genet 2008; 17: 806-14.
12. Szpakowicz A, Pepinski W, Waszkiewicz E, Maciorkowska D, Skawronska M, Niemcunowicz-Janica A, et al. Polymorphism of 9p21.3 locus is associated with 5-year survival in high-risk patients with myocardial infarction. PLoS One 2013; 8: e72333.
13. Helgadottir A, Thorleifsson G, Manolescu A, Gretarsdottir S, Blondal T, Jonasdottir A, et al. A common variant on chromosome 9p21 affects the risk of myocardial infarction. Science 2007; 316: 1491-3.
14. Samani NJ, Schunkert H. Chromosome 9p21 and cardiovascular disease: the story unfolds. Circ Cardiovasc Genet 2008; 1: 81-4.
15. Burd CE, Jeck WR, Liu Y, Sanoff HK, Wang Z, Sharpless NE. Expression of linear and novel circular forms of an INK4/ARF-associated non-coding RNA correlates with atherosclerosis risk. PLoS Genet 2010; 6: e1001233.
16. Kalinina N, Agrotis A, Antropova Y, Ilyinskaya O, Smirnov V, Tararak E, et al. Smad expression in human atherosclerotic lesions: evidence for impaired TGF-b/Smad signaling in smooth muscle cells of fibrofatty lesions. Arterioscler Thromb Vasc Biol 2004; 24: 1391-6.
17. Grainger DJ. Transforming growth factor beta and atherosclerosis: so far, so good for the protective cytokine hypothesis. Arterioscler Thromb Vasc Biol 2004; 24: 399-404.
18. Schmid M, Sen M, Rosenbach MD, Carrera CJ, Friedman H, Carson DA. A methylthioadenosine phosphorylase (MTAP) fusion transcript identifies a new gene on chromosome 9p21 that is frequently deleted in cancer. Oncogene 2000; 19: 5747-54.
19. Ellis KL, Pilbrow AP, Frampton CM, Doughty RN, Whalley GA, Ellis CJ, et al. A common variant at chromosome 9P21.3 is associated with age of onset of coronary disease but not subsequent mortality. Circ Cardiovasc Genet 2010; 3: 286-93.
20. Tang O, Lv J, Cheng Y, Qin F. The Correlation Between 9p21 Chromosome rs4977574 Polymorphism Genotypes and the Development of Coronary Artery Heart Disease. Cardiovasc Toxicol 2017; 17: 185-9.
21. Roberts R. Genetics of coronary artery disease. Circ Res 2014; 114: 1890-903.
22. Munir MS, Wang Z, Alahdab F, Steffen MW, Erwin PJ, Kullo IJ, et al. The association of 9p21-3 locus with coronary atherosclerosis: A systematic review and meta-analysis. BMC Med Genet 2014; 15: 66.
23. Rivera NV, Carreras-Torres R, Roncarati R, Viviani-Anselmi C, De Micco F, Mezzelani A, et al. Assessment of the 9p21.3 locus in severity of coronary artery disease in the presence and absence of type 2 diabetes. BMC Med Genet 2013; 14: 11.
24. Mendonça I, dos Reis RP, Pereira A, Café H, Serrão M, Sousa AC, et al. Independent association of the variant rs1333049 at the 9p21 locus and coronary heart disease. Rev Port Cardiol 2011; 30: 575-91.
25. Osmak GJ, Titov BV, Matveeva NA, Bashinskaya VV, Shakhnovich RM, Sukhinina TS, et al. Impact of 9p21.3 region and atherosclerosis-related genes' variants on long-term recurrent hard cardiac events after a myocardial infarction. Gene 2018; 647: 283-8.
26. Pignataro P, Pezone L, Di Gioia G, Franco D, Iaccarino G, Iolascon A, et al. Association Study Between Coronary Artery Disease and rs1333049 Polymorphism at 9p21.3 Locus in Italian Population. J Cardiovasc Transl Res 2017; 10: 455-8.
27. Fahrioğlu U, Ergören MÇ. The Association Between APOA5 Gene Polymorphisms and Plasma Lipids in the Turkish Cypriot Population: A Possible Biomarker for Preventing Cardiovascular Diseases. Biochem Genet 2018; 56: 176-87.
28. Sakalar C, Gurbuz E, Kalay N, Kaya MG. Higher frequency of rs4977574 (the G Allele) on chromosome 9p21.3 in patients with myocardial infarction as revealed by PCR-RFLP analysis. Tohoku J Exp Med 2013; 230: 171-6.
29. Sturiale CL, Gatto I, Puca A, D'Arrigo S, Giarretta I, Albanese A, et al. Association between the rs1333040 polymorphism on the chromosomal 9p21 locus and sporadic brain arteriovenous malformations. J Neurol Neurosurg Psychiatry 2013; 84: 1059-62.
30. Sasieni PD. From genotypes to genes: doubling the sample size. Biometrics 1997; 53: 1253-61.
31. Scheffold T, Kullmann S, Huge A, Binner P, Ochs HR, Schöls W, et al.; Forschungsverbund Herz-Kreislauf in NRW (Research Consortium Heart and Circulation in North Rhine-Westphalia). Six sequence variants on chromosome 9p21.3 are associated with a positive family history of myocardial infarction: a multicenter registry. BMC Cardiovasc Disord 2011; 11: 9.
32. Huang Y, Ye H, Hong Q, Xu X, Jiang D, Xu L, et al. Association of CDKN2BAS polymorphism rs4977574 with coronary heart disease: a case-control study and a meta-analysis. Int J Mol Sci 2014; 15: 17478-92.
33. Qi L, Ma J, Qi Q, Hartiala J, Allayee H, Campos H. Genetic risk score and risk of myocardial infarction in Hispanics. Circulation 2011; 123: 374-80.
34. Lee JY, Lee BS, Shin DJ, Woo Park K, Shin YA, Joong Kim K, et al. A genome-wide association study of a coronary artery disease risk variant. J Hum Genet 2013; 58: 120-6.
35. Wang Y, Wang L, Liu X, Zhang Y, Yu L, Zhang F, et al. Genetic variants associated with myocardial infarction and the risk factors in Chinese population. PLoS One 2014; 9: e86332.

36. Matsuoka R, Abe S, Tokoro F, Arai M, Noda T, Watanabe S, et al. Association of six genetic variants with myocardial infarction. Int J Mol Med 2015; 35: 1451-9.
37. Cao XL, Yin RX, Huang F, Wu JZ, Chen WX. Chromosome 9p21 and ABCA1 Genetic Variants and Their Interactions on Coronary Heart Disease and Ischemic Stroke in a Chinese Han Population. Int J Mol Sci 2016; 17: 586.
38. Lu Z, Zhang Y, Maimaiti Y, Feng Y, Sun J, Zhuang J, et al. Variants on chromosome 9p21 confer risks of noncardioembolic cerebral infarction and carotid plaque in the Chinese Han population. J Atheroscler Thromb 2015; 22: 1061-70.
39. Cheng YC, Anderson CD, Bione S, Keene K, Maguire JM, Nalls M, et al.; GENEVA Consortium; International Stroke Genetics Consortium. Are myocardial infarction-associated single-nucleotide polymorphisms associated with ischemic stroke? Stroke 2012; 43: 980-6.
40. Hindy G, Ericson U, Hamrefors V, Drake I, Wirfält E, Melander O, et al. The chromosome 9p21 variant interacts with vegetable and wine intake to influence the risk of cardiovascular disease: a population based cohort study. BMC Med Genet 2014; 15: 1220.
41. Diabetes Genetics Initiative of Broad Institute of Harvard and MIT, Lund University, and Novartis Institutes of BioMedical Research, Saxena R, Voight BF, Lyssenko V, Burtt NP, de Bakker PI, et al. Genome-wide association analysis identifies loci for type 2 diabetes and triglyceride levels. Science 2007; 316: 1331-6.
42. Scott LJ, Mohlke KL, Bonnycastle LL, Willer CJ, Li Y, Duren WL, et al. A genome-wide association study of type 2 diabetes in Finns detects multiple susceptibility variants. Science 2007; 316: 1341-5.
43. Kumar J, Yumnam S, Basu T, Ghosh A, Garg G, Karthikeyan G, et al. Association of polymorphisms in 9p21 region with CAD in North Indian population: Replication of SNPs identified through GWAS. Clin Genet 2011; 79: 588-93.
44. Lin HF, Tsai PC, Liao YC, Lin TH, Tai CT, Juo SH, et al. Chromosome 9p21 genetic variants are associated with myocardial infarction but not with ischemic stroke in a Taiwanese population. J Investig Med 2011; 59: 926-30.
45. Golabgir Khademi K, Foroughmand AM, Galehdari H, Yazdankhah S, Pourmahdi Borujeni M, Shahbazi Z, et al. Association study of rs1333040 and rs1004638 polymorphisms in the 9p21 locus with coronary artery disease in Southwest of Iran. Iran Biomed J 2016; 20: 122-7.
46. Beckie TM, Groër MW, Beckstead JW. The relationship between polymorphisms on chromosome 9p21 and age of onset of coronary heart disease in black and white women. Genet Test Mol Biomarkers 2011; 15: 435-42.
47. Hewing B, Moore KJ, Fisher EA. HDL and cardiovascular risk: time to call the plumber? Circ Res 2012; 111: 1117-20.