

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

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## 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 an-

tisense RNA (*CDKN2B-AS1* or ANRIL), which is located nearby the *CDKN2A-CDKN2B* gene cluster with an increased susceptibility to CAD or myocardial infarction 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*

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**Accepted Date:** 01.10.2018 **Available Online Date:** 07.12.2018

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 DOI:10.14744/AnatolJCardiol.2018.90907



is a downstream target for transforming growth factor (TGF)- $\beta$  that suggested its role in the TGF- $\beta$ -induced growth inhibition (16, 17). Even though its molecular mechanism is still not clear, it is known that TGF- $\beta$  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-specific 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  $\mu$ l in 200  $\mu$ l tubes on an Applied Biosystems Veriti Thermal Cycler. The reaction mixture consisted of 10 ng genomic DNA, 0.5  $\mu$ M forward and reverse primers (Table 1), 1 $\times$  Taq polymerase buffer with KCL (Thermo Scientific, EP0402), 1.5 mM MgCl<sub>2</sub> (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 $\pm$ 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' R 5'- AAACCTAAAAGGGCTTGCTGA – 3'	<i>HhaI</i>	29
rs1333040	F 5' - TCTGGAAGCACTGGGAAGGATG – 3' R 5'- TTG ATT TGG GAG CCA CTG TTG - 3'	<i>BsmI</i>	30

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<sup>-1</sup>) are within the slightly higher than normal

**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 35.3% M	63.4% F 36.6% M	0.847
Glucose (mg dL <sup>-1</sup> )	92.5±24.2	96.1±24.2	0.421
Cholesterol (mg dL <sup>-1</sup> )	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 <sup>-1</sup> )	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<sup>-1</sup> for glucose, >200 mg dL<sup>-1</sup> for total cholesterol, >130 mg dL<sup>-1</sup> for LDL-cholesterol, >40 mg dL<sup>-1</sup> for HDL-cholesterol, and >150 mg dL<sup>-1</sup> for triglycerides

range of >150 mg dL<sup>-1</sup> (National Cholesterol Education Program Expert Panel, 2002), which should not be a major risk factor for heart disease nor considered protective against heart disease.

### 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 <sup>a</sup> (%)	Control n <sup>b</sup> (%)	OR	95% CI	P*
rs4977574	AA	24 (33.9)	39 (23.5)	1.310	0.877-1.956	0.185
	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)			
HWE P- value		0.935	0.663			
rs1333040	CC	4 (5.6)	14 (9.2)	1.06	0.672-1.671	0.06
	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)			
HWE P-value		0.173	0.723			

<sup>a</sup>n=71

<sup>b</sup>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

**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
rs4977574			Risk allele G [A]<->[G] OR=0.763 CI=0.511-1.139	[A]<->[G] OR=0.763 CI=0.511-1.139	[A]<->[G] OR=0.763 CI=0.511-1.139	[A]<->[G] OR=0.763 CI=0.511-1.139	[A]<->[G] OR=0.763 CI=0.511-1.139
	AA=39	AA=24					
	AG=76	AG=33	X <sup>2</sup> =1.75 P=0.185	X <sup>2</sup> =1.75 P=0.185	X <sup>2</sup> =1.75 P=0.185	X <sup>2</sup> =1.75 P=0.185	X <sup>2</sup> =1.75 P=0.185
	GG=38	GG=14					
	f <sub>a1</sub> =0.50 ±0.029	f <sub>a1</sub> =0.57 ±0.043	Risk allele A [G]<->[A] OR=1.311 CI=0.878-1.957	[GG]<->[AG] OR=1.179 CI=0.564-2.462	[AG+GG]<->[AA] OR=1.670 CI=0.753-3.704	[AA+AG]<->[GG] OR=1.345 CI=0.675-2.683	Common OD's OR=1.297 X <sup>2</sup> =1.71 P=0.190
	F=0.006	F=0.051					
	p=0.935	p=0.663					
			Risk allele T [C]<->[T] OR=1.060 CI=0.672-1.672	[CC]<->[CT] OR=1.849 CI=0.556-6.150	[CC+CT]<->[TT] OR=1.587 CI=0.491-5.134	[CC]<->[CT+TT] OR=1.687 CI=0.535-5.322	Common OD's OR=1.128 X <sup>2</sup> =0.06 P=0.807
	CC=14	CC=4					
	CT=53	CT=28	X <sup>2</sup> =0.06 P=0.802	X <sup>2</sup> =1.03 P=0.311	X <sup>2</sup> =0.60 P=0.437	X <sup>2</sup> =0.81 P=0.367	
TT=86	TT=39						
rs1333040	f <sub>a1</sub> =0.26 +/-0.027	f <sub>a1</sub> =0.25 +/-0.036	Risk allele C [T]<->[C] OR=0.943 CI=0.598-1.488	[TT]<->[CT] OR=1.165 CI=0.643-2.110	[TT]<->[CC] OR=0.630 CI=0.195-2.038	[CC+CT]<->[TT] OR=1.053 CI=0.598-1.855	Common OD's OR=0.898 X <sup>2</sup> =0.06 P=0.807
	F=0.110	F=-0.041					
	p=0.173	p=0.723					
	-						

The evaluation of genotype comparison did not show any statistical significance between the CAD and control groups.

f<sub>a1</sub>: 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
Cholesterol (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	rs4977574 A>G			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
Cholesterol (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
Cholesterol (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	rs1333040 C>T			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
Cholesterol (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



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

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 heart 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 par-

ticles 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.

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