

Research Article



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Roles of *OLR1* and *IL17A* variants on clinical phenotypes of Turkish patients undergoing coronary artery bypass surgery

<https://doi.org/10.1515/tjb-2021-0214>

Received September 14, 2021; accepted April 11, 2022;

published online May 23, 2022

Abstract

Objectives: Coronary artery disease (CAD) is a pathological condition resulting from atherosclerosis in the coronary arteries. *IL17A* has been shown to recruit and activate macrophages in atherosclerotic lesions, thereby participating in plaque destabilization. Currently, whether *OLR1* and *IL17A* variants are involved in the pathogenesis of CAD is unclear. This case-control study aimed to investigate their roles in CAD etiology and prognosis.

Methods: In this study, 100 severe CAD patients who had undergone the coronary artery bypass graft surgery and 100 healthy controls were genotyped for *OLR1* rs11053646, *IL17A* rs3819025, and rs8193037 variants via RT-PCR.

Results: The patients with *OLR1* rs11053646 CG + GG genotype demonstrated a higher frequency of multi-vessel stenosis (18%) than single- (11.10%) or double-vessel (13.30%) stenosis ($p=0.77$). Additionally, although not statistically significant, this group of patients had 6.280 times more CAD risk than CC genotype carriers ($p=0.089$). Furthermore, logistic regression analysis revealed significant associations between the three variants and the risk factors for CAD development, namely waist circumference ($p=0.002$), body mass index ($p=0.013$), fasting glucose level ($p=0.006$), and triglyceride levels ($p=0.035$).

Conclusions: *OLR1* rs11053646, *IL17A* rs3819025, and rs8193037 variants do not increase the risk for CAD development. However, this conclusion should be confirmed with a larger cohort.

Keywords: coronary artery disease; genetic variant; *IL17A*; *OLR1*; oxidative stress; rs11053646; rs3819025; rs8193037.

Introduction

Coronary artery disease (CAD) is a pathological condition characterized by the accumulation of obstructive or non-obstructive atherosclerotic plaques in the epicardial arteries [1]. The clinical and pathological spectrum of CAD can range from chronic stable angina to myocardial infarction (MI) and even sudden cardiac death. Almost 50% of patients with acute MI have multi-vessel CAD [2]. The formation of atherosclerotic plaques results from the accumulation of lipids within the arterial wall or chronic inflammation in response to vascular injury [3]. There are various common risk factors for CAD, which are categorized either as non-modifiable factors, including advanced age, male gender, race, and family history of heart disease, or as modifiable factors, which you can treat or control, such as smoking, diabetes mellitus, hypertension, dyslipidemia, physical inactivity, overweightness, uncontrolled stress, and unhealthy diet [4]. Lifestyle changes, pharmacological treatments, and invasive interventions can enable disease stabilization or regression. Smoking cessation, avoiding passive smoking, healthy diet, physical activity, and healthy weight $<25 \text{ kg/m}^2$ are among the lifestyle guidance for patients with chronic coronary syndromes [1]. CAD development may also be affected by genetic factors. Studies investigating common variant associations have linked approximately 60 genetic loci to CAD risk [5]. CAD has a complex genetic inheritance pattern, indicating the interplay between genetic and environmental factors [6].

Endothelial injury and accumulation of low-density lipoprotein (LDL)-cholesterol on the inner arterial wall are involved in the initiation step of atherosclerosis. Endothelial

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injury, modification such as oxidation of LDL-cholesterol molecules, and low-grade inflammation generate both innate and adaptive immune responses. Monocytes/macrophages, T and B lymphocytes, and neutrophils all play roles in the progression of atherosclerosis [3]. Moreover T-cell induced inflammation plays an important role in the arteries, kidneys and central nervous system in hypertension which is one of the risk factor of atherosclerosis [7]. Endothelial injury promotes activities of cell-adhesion molecules, thus causing monocytes to adhere onto endothelial cells. Monocyte migration and differentiation into macrophages are crucial for the development of atherosclerosis. Modification of lipoproteins in the arteries leads to lipid peroxidation chain reaction, which forms toxic aldehyde metabolites, such as malondialdehyde. This chain reaction triggers inflammation in vascular cells. Moreover, inflammatory cells expedite cellular uptake of lipoprotein-derived lipids. Altered lipoproteins, such as acetylated and oxidized LDLs (oxLDLs), cannot be recognized by native LDL receptors. Macrophages express families of scavenger receptors that recognize such modified LDLs. Lectin-like oxidized LDL receptor-1 (LOX-1 or OLR1) is one of these scavenger receptors and recognizes oxLDLs. Macrophages ingest oxLDLs and induce lipid accumulation and foam-cell formation. Once formed, oxLDLs promote further oxLDL formation via a positive feedback loop, and production of pro-inflammatory cytokines through an OLR1-mediated cycle. Intracellular levels of reactive oxygen species are elevated by the interaction of oxLDL with its receptor affecting superoxide anions reacting with intracellular nitric oxide, which leads to endothelial dysfunction [3, 8, 9].

Interleukin 17 (IL17) has six isoforms (IL17 A–F), which are secreted by various immune cells, such as a subgroup of T helper (Th) cells, named Th17 cells. Th17 cells have been shown to play roles in the promotion of atherosclerosis in ApoE-deficient mice [10]. These cells synthesize the pro-inflammatory cytokine IL17A, which is found in atherosclerotic plaques in both humans and animals [11]. IL17A is involved in both the innate and adaptive immune responses. Blockade of IL17A function prevents the formation of advanced atherosclerotic lesions and triggers plaque stabilization in the progressive lesions in ApoE-deficient mice [12]. Additionally, it has been reported that oxLDL promotes the imbalance of Th17/Treg in patients with acute coronary syndrome [13]. Another study demonstrated that oxLDL uptake by its receptor induces the dendritic-cell-mediated polarization of Th17 cells by activating IL-6 production. Pro-atherogenic factors advances the polarization and inflammatory function of Th17 cells, critical for atherosclerosis development [14]. Moreover, oxLDL acts as

a dyslipidemic stimulus and thus upregulates IL17 receptors in human primary aortic cells [15].

Rs11053646 (c.501 G>C) variant is caused by a missense mutation in exon 4 of *OLR1*, whereby the lysine (K) at position 167 is converted to an asparagine (N) (p.K167N). This variant has decreased oxLDL binding and internalization capacities [16]. *IL17A* rs3819025 G>A is an intronic variant, and *IL17A* rs8193037 G>A variant results from a mutation that is 2 Kb upstream of *IL17A*. These *IL17A* variants were associated with CAD development in a study [17].

OLR1 and *IL17A* variants may have synergistic roles in CAD development. Exploring the possible relation between *OLR1* and *IL17A* variants may help to determine their molecular roles in CAD development. To date, there has been no investigation exploring this relation with regard to clinical phenotypes. Since *OLR1* and *IL17A* play significant roles in atherogenesis, this study examined the frequencies of *OLR1* rs11053646, *IL17A* rs3819025, and *IL17A* rs8193037 variants in a cohort of Turkish patients with CAD. It was then assessed whether these variants were associated with the clinical phenotypes and severity of CAD.

Materials and methods

Study design and groups

This prospective case-control study included 100 patients (83 men 83.0% and 17 women 17.0%) who had been diagnosed with CAD and had undergone the coronary artery bypass graft (CABG) surgery and 100 healthy controls (80 men 80.0% and 20 women 20.0%). The CAD patients had been diagnosed at Istanbul University-Cerrahpasa, Cerrahpasa Faculty of Medicine, Istanbul, Turkey. This study conformed to the principles of the Declaration of Helsinki and was approved by the Ethical Committee of Cerrahpasa Medical Faculty Hospital (#02-41310). A written informed consent was obtained from each participant before their enrolment for the study.

Each CAD patient had undergone a cardiovascular examination. CAD diagnosis had been performed by clinicians in agreement with the guidelines of the European Society of Cardiology. The major criterion for CAD diagnosis was $\geq 50\%$ stenosis in at least one main coronary artery or multiple significant stenoses in more than one coronary artery due to atherosclerosis detected via coronary angiography. All the patients had undergone the CABG surgery due to significant stenosis. These patients were divided into three groups in terms of vessel stenosis (single-, double-, and multi-vessel disease) to determine their CAD severity. The patients with single- or double-vessel disease had been under beta-blocker, statin, angiotensin converting enzyme (ACE)-inhibitor, and acetylsalicylic-acid therapy. The multivessel CAD patients had been additionally having clopidogrel as the pharmacological therapy. The mean age of the CAD group was 59.41 ± 7.41 years (range 38–73).

The age- and sex-matched healthy control group was composed of individuals who had consulted to Cerrahpasa Medical Faculty Hospital for routine analysis. Anyone with a heart disease, diabetes,

hypertension, auto-immune disease, neurological disease, cancer, severe renal or hepatic disease, or pregnancy was excluded from the study. The mean age of the control group was 59.85 ± 7.62 years (range 40–77).

Blood sampling and total-DNA extraction

Venous blood samples were collected from both the CAD patients and controls into EDTA-containing tubes. Genomic DNA was purified from the whole blood by using a commercial kit (Roche Diagnostics GmbH, Mannheim, Germany) according to the manufacturer's instructions.

Analysis of the participants for the presence of *OLR1* rs11053646, *IL17A* rs3819025, and *IL17A* rs8193037 variants

The participants were analyzed to determine whether they carried any of variants *OLR1* rs11053646, *IL17A* rs3819025, and *IL17A* rs8193037 via real-time polymerase chain reaction (PCR) by using the Light-Cycler 1.5[®] system and 3'-fluorescein-labeled hybridization probes (TIB MOLBIOL GmbH, Berlin, Germany). The thermal cycling steps were as follows: initial denaturation at 95 °C for 10 min; 45 cycles of denaturation at 95 °C for 10 s, annealing at 60 °C for 10 s, and extension at 72 °C for 15 s; followed by a melting reaction at 95 °C for 30 s and incubation at 40 °C for 2 min. The amplicons were specified by the melting temperature of each allele. The PCR reactions were performed in a final volume of 20 µL comprising 2.0 µL of master mix, 1.0 µL of the primer-probe mix, 3.0 mM MgCl₂, and 50 ng of genomic DNA. Randomly selected samples were used as replicates. The results confirmed the initial genotyping results.

Statistical analysis

Two continuous variables were compared using Student's *t*-test. Data are presented as mean $\pm$ SD. Three or more independent groups were analyzed using analysis of variance (ANOVA). The chi-square (χ^2) or Fisher's exact tests were used to assess categorical variables. Categorical data were expressed as observed (n) and/or percentage (%). The genotype frequencies in the cases and controls, and the deviation of the genotype distribution from Hardy–Weinberg equilibrium in both groups were compared using the χ^2 test. The data were further analyzed by using dominant, recessive, and over-dominant genetic models and Fisher's exact test. The odds ratio (OR) and 95% confidence interval (CI) per genotype were calculated using the multiple logistic regression model with adjustment for various CAD risk factors. $p < 0.05$ indicated statistical significance. All the statistical analyses were performed using SPSS version 21.0 (IBM Corp, Armonk, NY, USA).

Results

Demographic data

The clinical and demographic characteristics of the CAD patients and controls are shown in Table 1. No statistically significant difference in age ($p = 0.68$) or gender ($p = 0.72$)

was observed between the CAD patients and controls. Height, weight, and the levels of aspartate aminotransferase (AST), Alanine aminotransferase (ALT), urea, total cholesterol, and HDL- and LDL-cholesterol were not significantly different between the two groups ($p > 0.05$). However, the two groups demonstrated statistically significant differences in waist circumference ($p < 0.001$), body mass index (BMI, $p = 0.04$), and fasting glucose ($p < 0.001$), triglyceride ($p = 0.01$), and creatinine ($p < 0.001$) levels (Table 1).

Next, we assessed whether there was any correlation between the severity of vessel stenosis in the CAD patients and their demographic and clinical characteristics. Fasting glucose and serum creatinine levels were observed to be elevated in the multi-vessel CAD patients, compared with the single- and double-vessel disease patients. However, these results did not reach a statistical significance ($p = 0.19$ and $p = 0.28$, respectively) (Table 2). BMI, waist circumference, and the levels of AST, ALT, triglycerides, urea, and total-, HDL-, and LDL-cholesterol did not differ among the single-, double-, and multi-vessel CAD patients. Additionally, no significant difference in incidence of hypertension, type 2 diabetes, hyperlipidemia, and myocardial infarction was observed among the single-, double-, and multi-vessel CAD patients (Table 2).

Frequencies of *OLR1* rs11053646, *IL17A* rs3819025, and *IL17A* rs8193037 variants

The frequencies of *OLR1* rs11053646, *IL17A* rs3819025, and *IL17A* rs8193037 in the groups of CAD patients and controls are shown in Supplementary Table S1. The observed frequencies were consistent with the expected values based on Hardy–Weinberg equilibrium among the groups of CAD patients ($p = 0.38$, $p = 0.26$, and $p = 0.67$, respectively) and controls ($p = 0.27$, $p = 0.41$, and $p = 0.63$, respectively).

The CAD patients and controls did not significantly differ in the frequency of any of the variants, as assessed using the dominant genetic model ($p = 0.29$, $p = 0.46$, and $p = 1.00$) (Supp. Table S1).

The associations between any of the variants and CAD risk that are based on various genetic models are shown in Supplementary Table S2. *OLR1* rs11053646 variant did not confer a statistically significant risk of CAD in co-dominant (OR=1.71, 95% CI:0.74–3.98, $p = 0.21$), recessive (OR=1.00, 95% CI:0.02–50.89, $p = 1.00$), and over-dominant (OR=1.71, 95% CI:0.73–3.98, $p = 0.21$) genetic models. *IL17A* rs3819025 variant did not have a significant effect on CAD risk in co-dominant (OR=1.41, 95% CI:0.68–2.96, $p = 0.35$), recessive (OR=1.00, 95% CI:0.02–50.89, $p = 1.00$), and over-dominant

Table 1: Clinical and demographic characteristics of CAD patients and controls.

Parameter	CAD (n=100)	Controls (n=100)	p-Value
Age, years (mean $\pm$ SD)	59.41 $\pm$ 7.41	59.85 $\pm$ 7.62	0.68
Sex (M/F), n (%)	83/17 (83.00/17.00)	80/20 (80.00/20.00)	0.72
Height, m	1.68 $\pm$ 0.09	1.67 $\pm$ 0.09	0.63
Weight, kg	79.27 $\pm$ 12.44	82.93 $\pm$ 14.63	0.12
BMI, kg/m ²	28.05 $\pm$ 4.10	29.59 $\pm$ 4.72	0.04 ^a
Waist circumference, cm	100.430 $\pm$ 13.715	91.540 $\pm$ 10.066	<0.001 ^a
Fasting glucose, mg/dL	131.490 $\pm$ 52.947	95.674 $\pm$ 33.173	<0.001 ^a
AST, U/L	22.27 $\pm$ 11.53	19.38 $\pm$ 7.17	0.06
ALT, U/L	20.66 $\pm$ 11.47	21.35 $\pm$ 9.30	0.66
T-cholesterol, mg/dL	186.42 $\pm$ 51.92	192.07 $\pm$ 32.89	0.39
HDL-cholesterol, mg/dL	42.18 $\pm$ 11.31	46.19 $\pm$ 16.57	0.07
LDL-cholesterol, mg/dL	127.28 $\pm$ 43.79	130.28 $\pm$ 32.52	0.61
Triglycerides, mg/dL	145.60 $\pm$ 57.51	171.23 $\pm$ 63.18	0.01 ^a
Urea, mg/dL	38.32 $\pm$ 19.57	33.91 $\pm$ 10.35	0.07
Creatinine, mg/dL	0.956 $\pm$ 0.299	0.809 $\pm$ 0.220	<0.001 ^a

Student's t-test, and χ^2 tests were performed. Data are shown as mean $\pm$ standard deviation; ALT, alanine aminotransferase; AST, aspartate aminotransferase; T-cholesterol, total cholesterol; HDL, high-density lipoprotein; LDL, low-density lipoprotein. ^aStatistically significant.

Table 2: Comparison of the severity of vessel stenosis in terms of demographic and clinical characteristics of coronary artery disease patients.

Parameter	Single-vessel stenosis (n=9)	Double-vessel stenosis (n=30)	Multi-vessel stenosis (n=61)	p-Value
Age, years (mean $\pm$ SD)	58.88 $\pm$ 7.62	59.56 $\pm$ 7.35	59.41 $\pm$ 7.53	0.97
Sex (M/F), n (%)	5/4 (55.60/44.40)	26/4 (86.70/13.30)	52/9 (85.20/14.80)	0.07
Height, m	1.65 $\pm$ 0.09	1.68 $\pm$ 0.09	1.68 $\pm$ 0.08	0.59
Weight, kg	80.88 $\pm$ 9.59	75.76 $\pm$ 11.64	80.76 $\pm$ 12.99	0.18
BMI, kg/m ²	29.70 $\pm$ 4.25	26.82 $\pm$ 3.33	28.41 $\pm$ 4.32	0.10
Waist circumference, cm	104.33 $\pm$ 12.62	98.37 $\pm$ 14.79	100.87 $\pm$ 13.36	0.48
Fasting glucose, mg/dL	121.11 $\pm$ 21.16	119.00 $\pm$ 46.68	139.16 $\pm$ 57.95	0.19
AST, U/L	21.44 $\pm$ 6.22	22.43 $\pm$ 15.36	22.31 $\pm$ 10.00	0.26
ALT, U/L	15.91 $\pm$ 7.64	22.88 $\pm$ 13.11	20.28 $\pm$ 10.96	0.26
T-cholesterol, mg/dL	205.44 $\pm$ 53.12	181.0 $\pm$ 47.06	186.27 $\pm$ 54.15	0.47
HDL-cholesterol, mg/dL	44.55 $\pm$ 7.31	44.03 $\pm$ 12.48	40.91 $\pm$ 11.15	0.38
LDL-cholesterol, mg/dL	144.00 $\pm$ 51.18	120.73 $\pm$ 42.97	128.03 $\pm$ 43.11	0.37
Triglycerides, mg/dL	158.11 $\pm$ 63.55	128.70 $\pm$ 52.47	152.06 $\pm$ 58.13	0.15
Urea, mg/dL	30.55 $\pm$ 9.34	39.80 $\pm$ 20.81	38.73 $\pm$ 20.01	0.45
Creatinine, mg/dL	0.83 $\pm$ 0.17	0.93 $\pm$ 0.30	0.99 $\pm$ 0.31	0.28
HT $\pm$ n (%)	6/3 (66.70/33.30)	20/9 (69.00/31.00)	41/18 (69.50/30.50)	0.98
T2D $\pm$ n (%)	5/4 (55.60/44.40)	8/21 (27.60/72.40)	24/35 (40.70/59.30)	0.26
HL $\pm$ n (%)	1/8 (11.10/88.90)	0/30 (0.00/100.00)	3/58 (4.90/95.10)	0.27
MI $\pm$ n (%)	2/7 (22.20/77.80)	7/23 (23.30/76.70)	25/36 (41.00/59.00)	0.18

Data are shown as mean $\pm$ standard deviation (SD); ALT, alanine aminotransferase; AST, aspartate aminotransferase; T-cholesterol, total cholesterol; HDL, high-density lipoprotein; LDL, low-density lipoprotein; HT, hypertension; T2D type 2 diabetes; HL, hyperlipidemia; MI, myocardial infarction.

(OR=1.41, 95% CI:0.68–2.96, p=0.35) genetic models. Likewise, analysis of variant *IL17A* rs8193037 did not reveal a significant relationship with CAD risk in co-dominant (OR=0.88, 95% CI:0.32–2.38, p=0.80), recessive (OR=1.00, 95% CI:0.02–50.89, p=1.00), and over-dominant (OR=0.87, 95% CI:0.32–2.38, p=0.79) genetic models (Supplementary Table S2).

CAD symptom severity in patients with *OLR1* rs11053646, *IL17A* rs3819025, or *IL17A* rs8193037 variant

We also assessed for any association between the variants and severity of vascular stenosis in CAD patients. We observed that, although not statistically significant, the

patients with the CG + GG genotype of *OLR1* rs11053646 variant demonstrated a higher frequency of multi-vessel stenosis (18%) than single-vessel (11.10%) or double-vessel (13.30%) stenosis ($p=0.77$). Likewise, the patients with *IL17A* rs3819025 GA + AA genotype had a higher frequency of multi-vessel stenosis (23%) than single-vessel (11.10%) or double-vessel (16.70%) stenosis. However, this result did not reach statistical significance ($p=0.61$). In addition, no significant difference in severity of vascular stenosis was observed among the patients carrying *IL17A* rs8193037 variant ($p=0.18$) (Supplementary Table S3).

Assessment for any correlation between CAD risk and the *OLR1* and *IL17A* variants

We investigated the potential selective impact of the variants on CAD patients by testing *OLR1* rs11053646, *IL17A* rs3819025, and *IL17A* rs8193037 SNPs through a multivariate logistic regression model with adjustment for CAD risk factors. The backward stepwise method was applied. It was observed that, although not statistically significant, having *OLR1* rs11053646 CG + GG genotype posed 6.280 times more CAD risk compared with having CC genotype ($p=0.089$). Additionally, in the logistic regression model analysis, waist circumference ($p=0.002$, OR=1.141; 95% CI:1.049–1.240), BMI ($p=0.013$, OR=0.783; 95% CI:0.646–0.949), fasting glucose ($p=0.006$, OR=1.035; 95% CI:1.010–1.061) and triglyceride ($p=0.035$, OR=0.988; 95% CI:0.977–0.999) levels were found to be significant risk factors for CAD development in individuals carrying the investigated SNPs (Table 3).

Table 3: Investigation of CAD risk factors with logistic regression model.

Parameter	p-Value	Exp (B)	95.0% CI	
			Lower	Upper
Sex female	Ref.	Ref.		
Sex male	0.078	0.310	0.083	1.141
<i>OLR1</i> rs11053646				
CC	Ref.	Ref.		
CG + GG	0.089	6.280	0.755	52.193
Waist circumference, cm	0.002	1.141	1.049	1.240
Body mass index, BMI	0.013	0.783	0.646	0.949
Fasting glucose, mg/dL	0.006	1.035	1.010	1.061
Triglycerides, mg/dL	0.035	0.988	0.977	0.999
Constant	0.090	0.004		

Adjustments for gender, waist circumference, body mass index, fasting glucose, and triglycerides were performed. Exp (B); exponentiation of the B coefficient, 95.0% CI; difference of means at 95% confidence interval.

Combined genotype analysis of *IL17A* SNPs rs3819025 and rs8193037

To analyze the additive effect of *IL17A* SNPs rs3819025 and rs8193037 on the risk of CAD development, combined genotypes for the two loci were calculated. Our data indicated no significant relation between the combined genotype effect and disease formation ($p>0.05$) (Supplementary Table S4).

Discussion

CAD is a multifactorial and polygenic vascular disease, and its complex pathogenesis arises from both genetic interactions and lifestyle factors. In this study, we assessed for any association of *OLR1* and *IL17A* variants with CAD pathogenesis and CAD-related clinical variants. Our results demonstrated that, although there was no significant difference in the frequencies of *OLR1* and *IL17A* gene variants between the patient and control groups, the patients with CG + GG genotype of *OLR1* rs11053646 displayed a higher frequency of multi-vessel stenosis. Moreover, individuals with this genotype were estimated to be 6.28 times more likely to develop CAD compared with CC genotype carriers. Likewise, the patients with *IL17A* rs3819025 GA + AA genotype had a higher frequency of multi-vessel stenosis than single-vessel or double-vessel stenosis. Additionally, serum creatinine and fasting glucose levels were elevated in the multi-vessel CAD patients, compared with the levels in the single- or double-vessel CAD patients. In addition, multivariate logistic regression analysis revealed that waist circumference, BMI, and fasting glucose and triglyceride levels are significant risk factors for CAD development in individuals with the investigated variants.

To date, several studies have reported a relationship between cardiovascular phenotypes and *OLR1* and *IL17A* variants in various populations. Tatsuguchi et al. investigated for a relationship between *OLR1* G501C (rs11053646) variants and myocardial infarction (MI) in a Japanese cohort and reported that the MI group had a significantly higher frequency of GC + CC (38.2%) variants than the healthy controls (17.6%). The authors concluded that *OLR1* or a neighboring gene linked to G501C SNP is important for the MI incidence in the Japanese population [18]. Likewise, Ohmori et al. evaluated the involvement of *OLR1* G501C polymorphism in 586 patients who had undergone coronary angiography. The patients were classified as those with normal/minimal, mild, or significant stenosis. *OLR1* CC or CG variants were less frequent in the patients with

significant stenosis than in those with normal/minimal stenosis. Additionally, the frequencies of the *OLR1* variants were not different between the patients with MI and those without. Nevertheless, the authors observed a gradual decline in the frequencies of these variants by CAD severity; 49, 41, 39, 35, and 32% in normal/minimal, mild, single-vessel, double-vessel, and triple-vessel CAD, respectively. In addition, the *OLR1* variants were found to be inversely associated with the presence of significant stenosis. The authors concluded that *OLR1* variants at nucleotide location 501 were inversely correlated with CAD severity [19]. Likewise, Knowles et al. investigated for a correlation between four *OLR1* SNPs and CAD risk and reported that the C allele of *OLR1* rs11053646 was correlated with a reduced risk of CAD [20].

Kurnaz et al. investigated whether *OLR1* K167N (rs11053646) polymorphism was protective against CAD. They found that the frequencies of KK (GG) genotype and G-allele were significantly higher in the CAD group than in the controls. Additionally, NN (CC) genotype was more frequent in the control group than in the CAD group. The authors found that individuals with C allele had decreased risk of CAD compared with G allele carriers and it was concluded that male sex and smoking diminished the protective effects of C allele. Moreover, the adverse effects of G allele on CAD risk seemed to be independent of other cardiovascular risk factors [21]. We also assessed for the involvement of *OLR1* rs11053646 variants in severe CAD development. Our CAD group presented with 34% MI. Additionally, 22.2, 23.3, and 41.0% of the single-, double-, and multi-vessel CAD patients in our study displayed MI. In addition, the patients and controls demonstrated statistically significant differences in waist circumference, BMI, and fasting glucose, triglyceride, and creatinine levels. Moreover, serum creatinine and fasting glucose levels were elevated in the multi-vessel CAD patients, compared with the levels in the single- or double-vessel CAD patients. We observed a higher frequency of *OLR1* rs11053646 GC + GG (16.0%) variants in our patient group than in the healthy controls (10%). Nevertheless, this result did not reach statistical significance.

Our study results are not consistent with the findings of Tatsuguchi et al., and this discrepancy may be due to the ethnic differences between the two study populations or due to the fact that not all of the patients in our study had MI. Although our results were not statistically significant, CG + GG variants were more frequent in the patients with significant stenosis (Multi-vessel) (18%) than in those with less severe stenosis (11.10% and 13.30% in patients with single- and double-vessel stenosis, respectively). Our study results agree with the findings of Ohmori et al. [19]

and Knowles et al. [20] in terms of the roles of *OLR1* variants in vascular-stenosis severity since we found that, although not significant, C allele frequency was lower in the patients with multiple-vessel occlusion. As in the study of Kurnaz et al., G allele was more frequent in our patient group (0.08) than in the controls (0.05), and C allele was more frequent in the control group (0.95) than the patients (0.92), although this result was not statistically significant.

Paquette et al. explored whether there was any correlation between *OLR1* rs11053646 and CAD risk in a cohort of adult patients with heterozygous familial hypercholesterolemia. The authors observed that C variant was associated with CAD risk in these patients, and this risk was greater in smokers and young patients [22]. Association between *OLR1* rs11053646 3'-UTR-C188T polymorphisms and CAD was investigated in a meta-analysis. The study concluded that *OLR1* 3'-UTR-188T increases CAD susceptibility; nonetheless, G501C was not associated with CAD [23]. Another meta-analysis investigated the relationship between 3'-UTR C188T G501C polymorphisms and CAD risk. The authors reported that the variant C allele of G501C polymorphism is a low penetrant risk factor for CAD development [24]. In our study, although not statistically significant, being an *OLR1* rs11053646 CG + GG genotype carrier posed 6.28 times more CAD risk than being a CC genotype carrier. Additionally, waist circumference, BMI, and fasting glucose and triglyceride levels were also found to be significant risk factors for CAD development.

Various studies have investigated the association between *IL17A* and atherosclerosis [12, 17, 25–27]. A study investigated the involvement of the IL17/IL17RA axis in atherogenesis in *Il17a*^{-/-}Apoe^{-/-} or *Il17ra*^{-/-}Apoe^{-/-} mice, and it was concluded that this axis increased the aortic-arch inflammation during atherogenesis via inducing the secretion of aortic chemokines and recruiting monocytes and neutrophils [25]. Erbel et al. investigated the effects of IL17A on progressive atherosclerosis in mice and humans. Their *in vitro* experiments demonstrated that IL17A plays a role in chemoattractance, monocyte adhesion, and sensitization of atherosclerotic plaque characteristics. They also demonstrated that IL17A confers a particular transcriptome pattern specifically to monocyte-derived macrophages. The same authors also observed that IL17A induced a pro-inflammatory environment in human carotid plaques *ex vivo* [12].

Zhang et al. reported that the frequencies of *IL17A* rs8193037 GG homozygote and G allele are significantly higher in Chinese Han CAD patients than in controls. They also observed that *IL17A* rs8193037 G allele significantly increased CAD risk only among male individuals. After adjustment for common risk factors, the G-allele carriers

(GG + AG) were found to have a significantly increased CAD risk compared with AA homozygotes in logistic regression analysis. Additionally, rs8193037 G allele was found to be associated with increased *IL17A* expression in acute myocardial infarction patients and a likely independent predictive factor for CAD [17]. A study investigated whether *IL17A* polymorphisms (rs8193036, rs3819024, rs2275913, and rs8193037) are susceptibility markers for premature CAD in the Mexican population. No significant relationship was reported between the distribution of the investigated polymorphisms and premature CAD in any inheritance model. For rs8193037, the genotype frequencies were as follows: GG 0.85, GA 0.14, and AA 0.01 [26]. In addition, another study demonstrated the association between three SNPs (*IL17A* rs2275913, rs3819025, and rs3748067) and CAD development in the Chinese population. Although the authors reported a significant association between rs2275913 and CAD, they did not find such an association between rs3819025 and CAD development. Their genotype frequencies were as follows: for rs3819025, 43.13% AA, 42.65% AG, 14.22% GG, and 56.87% AG + GG [28]. Another study conducted on the Chinese population investigated 5 *IL17A* SNPs (rs8193037, rs8193036, rs3819024, rs2275913, and rs3748067) in CAD patients. The allele and genotype frequencies of the SNPs in the promoter region (rs8193037 and rs8193036) were significantly different from those of the healthy controls. A allele of rs8193037 was significantly less frequent among the CAD patients [29].

We also explored the relationship between CAD risk and *IL17A* rs3819025 and rs8193037 variants. Our patient group demonstrated 80% GG and 20% GA + AA genotype frequencies for rs3819025. Although we did not find any correlation between these SNPs and CAD risk in any inheritance model, we observed that the *IL17A* rs3819025 GA + AA genotype carriers had a higher frequency of multi-vessel stenosis (23%), than single- (11.10%) or double- (16.70%) vessel stenosis. However, this result did not reach statistical significance. Secondly, no significant correlation was observed between the *IL17A* rs8193037 genotype carriers and the severity of vascular stenosis. Our genotype frequencies for rs3819025 were not similar to those of Shuang et al., and this discrepancy can be because of the ethnic differences between the two study groups. We also did not find any relationship between SNP rs3819025 and CAD. For rs8193037, our results agree with those of Vargas-Alarcón et al. However, our results regarding rs8193037 are not in agreement with those of Zhang et al. or Su et al. All these differences may result from the ethnic differences or the polygenic and multifactorial nature of the disease.

Sandip et al. investigated the relationship between frequent variants in the IL-17A/IL-17RA axis and predisposition to congestive heart failure. They reported that *IL17A* rs8193037 is correlated with the risk of congestive heart failure after adjustment for various cardiovascular risk factors, including age, sex, smoking status, diabetes, hypertension, and dyslipidemia, and this connection was noticeable in both ischemic and non-ischemic heart failure [30]. In a meta-analysis, it was shown that *IL17A* rs3748067 TT genotype is associated with a lower risk of CAD in Asians. However, no significant relation was reported for rs2275913, rs3819024, rs3819025, rs8193037, rs4711998, or rs8193036 with CAD susceptibility. The same study also made a subgroup analysis and reported an elevated risk of CAD in Asians with GG genotype and G allele of rs8193037 in a heterozygous, dominant, or allelic model. The authors concluded that *IL17A* rs3748067 and rs8193037 polymorphisms may be a predictor for susceptibility to CAD in Asians [31]. Our results are in line with the results of this meta-analysis study. However, our subgroup analysis did not reveal a significant difference with rs8193037 variants. This discrepancy may be due to our small sample size.

Finally, our previous study demonstrated the relationship of *OLR1* and *IL17A* with femoropopliteal artery occlusive disease. *OLR1* and *IL17A* mRNA levels were significantly increased in these patients compared with the levels in the controls. However, no significant difference in the genotypic frequency of *OLR1* rs11053646, *IL17A* rs3819025, and *IL17A* rs8193037 polymorphisms was observed between the patients and controls. *OLR1* expression was also positively correlated with triglyceride, LDL-cholesterol, and total cholesterol levels in these patients [27].

Nevertheless, the study presented here has some limitations, namely its limited sample size and the lack of plasma *OLR1* and *IL17* levels as well as the mRNA levels in coronary tissues. Further research is required for elucidating the molecular roles of *OLR1* and *IL17A* in CAD pathogenesis.

In conclusion, this report is the first to investigate the roles of *OLR1* rs11053646, *IL17A* rs3819025, and *IL17A* rs8193037 variants by using all inheritance models and also in CAD subgroups in a cohort of Turkish CAD patients. Our study commends that *OLR1* rs11053646, *IL17A* rs3819025, and *IL17A* rs8193037 variants do not confer an increased risk for CAD development; however, this conclusion should be confirmed with a larger cohort of CAD patients.

Conclusions

- *OLR1* rs11053646 CG + GG genotype carriers demonstrated higher frequency of multi-vessel stenosis CAD.
- *OLR1* rs11053646 CG + GG genotype carriers had 6.28 times CAD risk.
- *IL17A* rs3819025 and rs8193037 genotype frequencies were not associated with CAD risk.

Acknowledgments: The preliminary findings of this study were presented in the VI. International Congress of Molecular Medicine, Istanbul, Turkey, May 22-25, 2017.

Research funding: This work was supported by the Scientific Research Projects Coordination Unit of Istanbul University-Cerrahpasa. Project #50188.

Author contributions: All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Competing interests: The authors declare that they have no conflict of interest.

Informed consent: Informed consent was obtained from all individuals included in this study.

Ethical approval: This study conformed to the principles of the Declaration of Helsinki and was approved by the Ethical Committee of Cerrahpasa Medical Faculty Hospital (#02-41310).

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- Supplementary Material:** The online version of this article offers supplementary material (<https://doi.org/10.1515/tjb-2021-0214>).