Article

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Dysregulated Vitamin D, *CYP2R1*, *TCF7L2*, and *CCR5* △32 Gene Variations are Associated with Coronary Artery Disease

Jamsheed Javid[1], Rashid Mir[1,*], Imadeldin Elfaki[2,*], Reema Almotairi[1], Jameel Barnawi[1], Naseh A. Algehainy[1], Mohammed M. Jalal[1], Malik A. Altayar[1], Mohammad A Alanazi[1], Salem Owaid Albalawi[3], Tanzeela Bhat[1], Eram Hussain[1], Faisel M AbuDuhier[1]

[1]Department of Medical Lab Technology, Prince Fahad Bin Sultan Chair for Biomedical Research, Faculty of Applied Medical Sciences, University of Tabuk, 71491 Tabuk, Saudi Arabia
[2]Department of Biochemistry, Faculty of Science, University of Tabuk, 71491 Tabuk, Saudi Arabia
[3]Department of Cardiology, King Fahd Specialist Hospital, 71491 Tabuk, Saudi Arabia
*Correspondence: rashidmirut@gmail.com (Rashid Mir); elfakiimadeldin@gmail.com (Imadeldin Elfaki)
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Background: Insufficient vitamin D (vit D) levels are associated with various chronic conditions such as cancers, autoimmune diseases, diabetes, and cardiovascular diseases, notably coronary artery disease (CAD). The enzyme 25-hydroxylase, cytochrome P450 2R1 (CYP2R1), catalyzes the hydroxylation of vitamin D in the liver, producing the 25-hydroxyvitamin D, which is then activated in the kidney by cytochrome P450 27B1 (CYP27B1) to form 1,25-dihydroxyvitamin D. Mutations in the *CYP2R1* gene can impair vitamin D production. The C-C chemokine receptor type 5 (CCR5) supports endothelial repair and angiogenesis, with its mutation (*CCR5* 59029 G to A) being linked to insulin resistance and type 2 diabetes (T2D). Additionally, the transcription factor 7-like 2 (TCF7L2), part of the Wnt signaling pathway, regulates glucose homeostasis and the development of tissues, brain, liver and muscles and has been linked to obesity, insulin insensitivity, and elevated blood sugar levels.

Materials and Methods: We evaluated the association of reduced serum vitamin D levels with CAD using enzyme-linked immunosorbent assay (ELISA). Genotyping of the *CYP2R1* rs1562902 C > T, *TCF7L2* rs12255372 G > T, and *CCR5* △32 bp deletion mutation were performed using amplification-refractory mutation system polymerase chain reaction (PCR) and allele-specific PCR to evaluate their association with CAD risk.

Results: The *CYP2R1* rs1562902 C > T single nucleotide polymorphism (SNP) genotypes CT and TT were significantly associated with CAD, with odds ratios (ORs) of 4.1 and 7.6 and *p*-values of 0.0001 and 0.0008, respectively. The +/△ genotype of the *CCR5* △32 bp (ins/del) mutation was also associated with CAD (OR = 2.51, $p = 0.006$). Additionally, the T allele of the *TCF7L2* rs12255372 G > T SNP was linked to an increased risk of CAD (OR = 1.89, $p = 0.006$).

Conclusion: The *CYP2R1* rs1562902 C > T, *CCR5* △32 (rs333), and *TCF7L2* rs12255372 G > T polymorphisms are potential genetic loci associated with increased CAD risk. Furthermore, *CYP2R1* variants are associated with vitamin D deficiency, predisposing carriers of *CYP2R1* to associated pathologies. These findings warrant further validation through larger case-control studies and functional protein analysis.

Keywords: coronary artery disease; vitamin D; C-C chemokine receptor type 5; cytochrome P450 2R1; transcription factor 7-like 2; amplification-refractory mutation system-PCR

Introduction

Coronary artery disease (CAD) is a leading cause of mortality and morbidity globally and in the Kingdom of Saudi Arabia (KSA) [1]. In recent years, KSA has experienced significant economic growth, urbanization, and lifestyle changes, all of which may contribute to the increasing prevalence of cardiovascular disease (CVD) [2]. CVD encompasses conditions such as CAD, stroke, peripheral arterial disease, congenital heart disease (congenital heart anomaly), deep vein thrombosis, and pulmonary embolism [2]. Key risk factors for CVD include hyperlipidemia, hypertension, diabetes mellitus, obesity, smoking, physical inactivity, unhealthy diets, genetic predisposition, age, and gender [3,4]. Inflammation also plays a pivotal role in atherosclerotic CVD, accelerating the progression of atherosclerosis, a key factor in CAD pathogenesis [5,6]. Biomarkers of inflammation, such as C-reactive protein, interleukins, and tumor necrosis factor-alpha, contribute to the development of atherosclerosis [7–9]. Atherosclerosis, the primary risk factor for CVD, begins with endothelial cell activation, followed by lipid accumulation, fibro-

sis, calcification, and ultimately vascular inflammation and narrowing [10].

Vitamin D deficiency has also been associated with an increased risk of CAD [11]. The deficiency impairs endothelial function, leading to inflammation, oxidative stress, and atherosclerosis [12]. Vitamin D influences blood pressure regulation via the renin-angiotensin-aldosterone system and is thought to modulate endothelial function and arterial thrombogenesis [12]. Furthermore, vitamin D deficiency is associated with insulin resistance, resulting in hyperglycemia, dyslipidemia, and inflammation [13]. Vitamin D is essential to cardiovascular homeostasis due to its anti-atherogenic, anti-inflammatory, and protective properties [13,14]. Reduced vitamin D levels have been implicated in numerous pathologies, including asthma, diabetes mellitus, CVD, and various cancers [15].

Cytochrome P450 2R1 (CYP2R1, 25-hydroxylase) is a key enzyme in the vitamin D metabolic pathway [15]. Active vitamin D (vit D) (1,25-dihydroxyvitamin D) is produced through a series of enzyme-catalyzed reactions [15]. Vitamin D3 (cholecalciferol) is synthesized in the skin from 7-dehydrocholesterol upon exposure to sunlight, while vitamin D2 (ergocalciferol) is obtained from plant sources [16]. In the live, inactive vitamin D is converted to 25-hydroxyvitamin D by CYP2R1 (25-hydroxylase) [15], followed by conversion to the active form, 1,25-dihydroxyvitamin D, in renal tissues by CYP27B1 (25-hydroxyvitamin D (25(OH) D)-1α-hydroxylase) [15]. Then enzyme CYP24A1 (24-hydroxylase) subsequently degrades 1,25-dihydroxyvitamin D in target tissues (Fig. 1) [17].

The C-C chemokine receptor type 5 (CCR5) is a transmembrane receptor coupled to G proteins that play an essential role in immune responses as chemokine receptors [18,19]. A 32-base-pair deletion in the *CCR5* gene (*CCR5* Δ32) leads to a truncated, nonfunctional receptor [20]. The *CCR5* Δ32 mutation leads to a frameshift that produces a truncated CCR5 protein, preventing its localization to the cell surface [21]. Furthermore, CCR5 and its ligands have been implicated in insulin resistance, obesity, and diabetes mellitus [18].

Transcription factor 7-like 2 (TCF7L2) is a member of the T cell factor/lymphoid enhancer factor family [22]. It plays pivotal roles in various physiological processes, especially in hepatic tissues, pancreatic islets, and adipose tissues [22]. TCF7L2 suppresses hepatic gluconeogenesis and promotes fat accumulation and adipose tissue development [22]. Additionally, it regulates insulin secretion from pancreatic beta cells. Dysregulated TCF7L2 expression is associated with metabolic syndrome, dyslipidemia, and type 2 diabetes [22,23]. As part of the Wnt signaling pathway, TCF7L2 is crucial for cardiac development, and defects in this pathway are linked to vascular diseases and atherosclerosis [24]. TCF7L2 regulates the genes involved in lipid metabolism, with nonfunctional TCF7L2 leading to fat accumulation and adipocyte hypertrophy [25]. In contrast, increased TCF7L2 expression enhances glucose metabolism [25]. Genome-wide association studies (GWAs) have identified several loci associated with diseases such as CVD, diabetes mellitus, and cancer [26–29].

This study aimed to investigate the potential association between serum vitamin D levels, *CYP2R1* rs1562902 C > T SNP, and CAD risk in the Saudi population. Additionally, we examined the relationship between *CCR5* Δ32 (rs333) mutation, the *TCF7L2* rs12255372 G > T single nucleotide polymorphism (SNP), and CAD in this population.

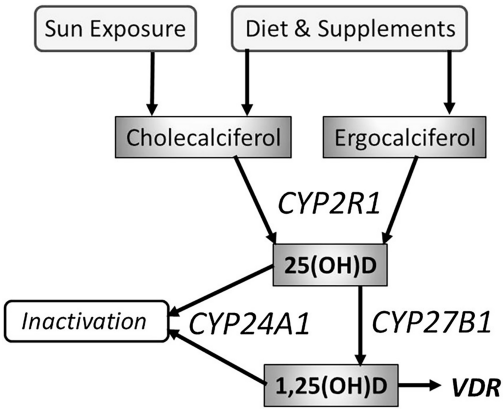

Fig. 1. Role of cytochrome P450 2R1 (CYP2R1) in vitamin D metabolism. Cholecalciferol is synthesized in the skin under sunlight exposure, while plants produce ergocalciferol. Vitamin D is converted to 25-hydroxyvitamin D (25(OH) D) by CYP2R1. Subsequently, 25(OH) D is converted to 1,25-dihydroxyvitamin D (1,25(OH) D) in the kidneys, catalyzed by CYP27B1. The active 1,25(OH) D binds to the vitamin D receptor (VDR), while CYP24A1 catalyzes its degradation in the target tissues. This figure was created using Microsoft Office 2021 (Microsoft Corporation, Redmond, WA, USA).

Materials and Methods

Subjects

This study was conducted in accordance with the guidelines outlined in the Declaration of Helsinki and was ethically approved by the University of Tabuk Research Ethics Committee (Registration number UT-91-23-2020). A total of 100 confirmed CAD patients and 100 age- and gender-matched healthy controls were enrolled. All participants provided informed written consent in this study. The CAD patients were recruited from King Fahad Specialist Hospital, Tabuk, Saudi Arabia, where they had undergone elective angiography for the diagnosis of stable angina. Diagnostic tests included X-rays, exercise stress tests, myocardial perfusion imaging, ambulatory electrocardiography, Holter monitoring, chest echocardiogram, computed tomography coronary angiography, and multigated acqui-

Table 1. General characteristics of the study population.

Characteristics		CAD cases (%)	Healthy controls (%)
Study population		100 (100%)	100 (100%)
Gender	Males	80 (80%)	75 (75%)
	Females	20 (20%)	25 (25%)
Age	≤55 years	58 (58%)	65 (65%)
	>55 years	42 (42%)	35 (35%)
Smoking status	Non-smoker	56 (56%)	80 (80%)
	Smoker	44 (44%)	20 (20%)
Hypertension	No	59 (59%)	99 (99%)
	Yes	41 (41%)	1 (1%)
Hyperlipidemia	No	73 (73%)	98 (97%)
	Yes	27 (27%)	2 (3%)
Obesity	No	59 (59%)	
	Yes	41 (41%)	
Diabetes mellitus	No	66 (66%)	
	Yes	34 (34%)	
Angina	Stable	88 (88%)	
	Unstable	12 (12%)	
Myocardial infarction (MI)	No	70 (70%)	
	Stemi	18 (18%)	
	Nstemi	12 (12%)	
Familial history	No	76 (76%)	
	Yes	24 (24%)	

CAD, coronary artery disease.

sition (MUGA) scans. Participants were divided into two groups: the first group comprised patients with significant CAD, defined as those with ischemic heart disease (stenosis ≥50%). The second group consisted of healthy individuals who served as control; these participants had no history of CVD, diabetes, or cancer (Table 1). Patients with neoplasms or chronic diseases were excluded from the study.

Serum Vitamin D Level

Blood samples were collected in plain or serum separator tubes and allowed to clot at room temperature for 10–15 minutes. The serum was separated by centrifugation at 3500 rpm for 10 minutes, after which it was carefully transferred to new tubes and stored at –20 °C. Serum vitamin D levels were estimated using a highly sensitive enzyme-linked immunosorbent assay (ELISA) with the human vitamin D ELISA kit (Cat. No. VD220B, Calbiotech, El Cajon, CA, USA). Standards and samples were analyzed in duplicate, and the average absorbance values were calculated for each sample set. Vit D concentrations were determined by plotting the optical density (OD) values against standard vitamin D concentrations using a standard curve. The final vitamin D concentrations for each sample were calculated by applying the appropriate dilution factors used during sample preparation.

Genotype Analysis

Genomic DNA was extracted from blood samples collected in ethylenediaminetetraacetic acid (EDTA) vials using a DNA extraction kit (Qiagen, Germantown, MD, USA; Cat. No. 56304). Genotyping of the *CYP2R1* rs1562902 C > T and *TCF7L2* rs12255372G > T SNPs and the C-C chemokine receptor 5 Δ32 (*CCR5* Δ32) mutation was performed using Amplification refractory mutation system-polymerase chain reaction (ARMS-PCR) and allele-specific PCR, respectively using the primers listed in Table 2.

Preparation of PCR Mix

The genotyping of *CYP2R1* (rs1562902 C > T) and *TCF7L2* (rs12255372G > T) SNPs was conducted using tetra-primer ARMS-PCR. The genotyping of *CCR5* Δ32 bp (ins/del, rs333) mutation was performed using allele-specific PCR. For all reactions, we used the Green PCR Master Mix (2×) (Promega, Madison, WI, USA, Cat. No. M7122). The final reaction volume of 25 µL was achieved by adding nuclease-free ddH_2O, with 50 ng of template DNA added last.

CYP2R1 (rs1562902 C > T) SNP Genotyping

The outer region of CYP2R1 was amplified using outer primers FO/RO, yielding a 416 bp band that served as a DNA quality check. The C allele was identified by a 273 bp band amplified by primers FO/RI, while the T allele was indicated by a 198 bp band amplified by primers FI/RO (Table 2, Fig. 2).

Table 2. PCR primers for the analysis of *CYP2R1* rs1562902 C/T and *CCR5* Δ32 bp (ins/del, rs333).

Primers		Sequence (5′ to 3′)	Annealing temperature (°C)	Product size (bp)
CYP2R1 (rs1562902 C/T)				
CYP2R1-FO		CCCTGTGTTCCTGGGGTTTACATTCTAT	64 °C	416 bp
CYP2R1-RO		TGAAAATCATTTGCAAAGCAGAAGAAGG		
CYP2R1-FI	T	CTTATATCCAGGGACTCCCCCATGCT		198 bp
CYP2R1-RI	C	GAATTGTAGTAACATCTTCCATGAACCCG		273 bp
TCF7L2 rs12255372G > T *SNP*				
TCF7L2-FO		GGGCAATAGATACATTTTAAGA	59 °C	760 bp
TCF7L2-RO		GAGATAGATGATAGGCTGTT		
TCF7L2-FI	G	GGAATATCCAGGCAAGAATG		494 bp
TCF7L2-RI	T	CCTGAGTAATTATCAGAATATGGTA		310 bp
CCR5 Δ32 bp (ins/del, rs333)				
F-*CCR5* Δ32	(wild type)	TGTTTGCGTCTCTCCCAG	53 °C	193 bp
R-*CCR5* Δ32	(deletion)	CACAGCCCTGTGCCTCTT		161 bp

CCR5, C-C chemokine receptor type 5; *TCF7L2*, transcription factor 7-like 2; *SNP*, single nucleotide polymorphism.

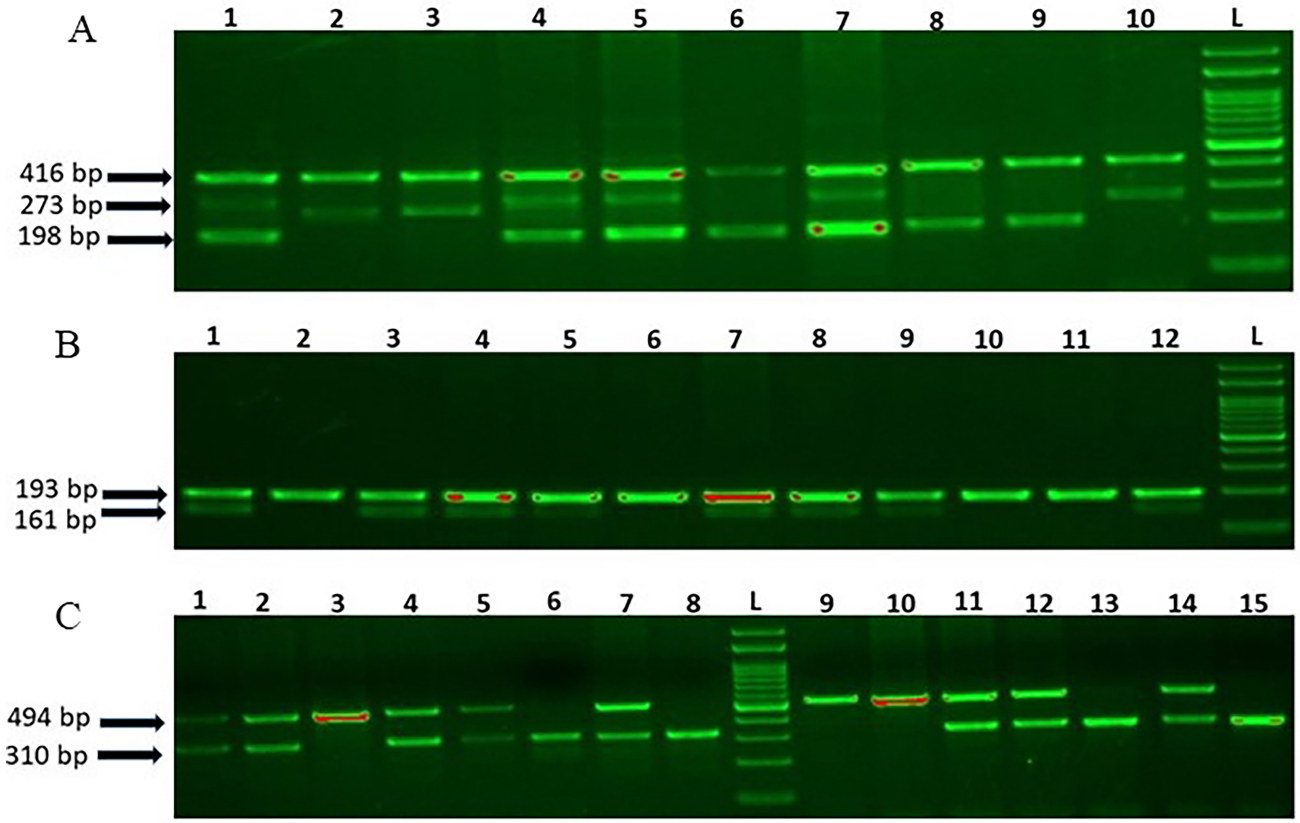

Fig. 2. Agarose gel electrophoresis of genotyping results for *CYP2R1* (rs1562902 C > T), *TCF7L2* (rs12255372 G > T), and *CCR5* Δ32 mutations in CAD patients. (A) *CYP2R1* genotypes CC (P2, P3, and P10); CT (P1, P4, P5 and P7); TT (P6, P8, and P9). (B) *CCR5* Δ32 mutation wild type (P1, P3, P4, P5, P7, P8, P9, and P12); heterozygous mutation (P2, P6, P10, and P11). (C) *TCF7L2* genotypes GG (P3, P9, and P10); GT (P1, P2, P4, P7, P7, P11, P12, and P14); TT (P6, P8, P13, and P15). "L" represents the lane of the marker.

CCR5 Δ32 bp (Ins/del, rs333) Mutation Genotyping

Allele-specific PCR was performed to genotype the *CCR5* gene using the primers F-*CCR5* Δ32/R-*CCR5* Δ32 [30]. The PCR cycling conditions were initial denaturation at 95 °C for 5 minutes, followed by 35 cycles of 95 °C for 30 seconds, 59 °C for 45 seconds, and 72 °C for 1 minute, with a final extension at 72 °C for 5 minutes. The reactions were carried out using a thermal cycler (Table 2, Fig. 2).

TCF7L2 (rs12255372 G > T) SNP Genotyping

A 760 bp band amplified by the outer primers FO/RO was used as a control for DNA quality and quantity evalu-

Table 3. Vitamin D levels in relation to the clinical characteristics of CAD patients.

Clinical features	Variables	Number of patients (%)	Median vitamin D ng/mL (min–max)	*p*-value
Gender	Male	80 (80%)	8.4 (4.5–29.5)	0.410
	Female	20 (20%)	8.1 (5.0–32.4)	
Age	≤50	58 (58%)	8.4 (4.5–32.4)	0.156
	>50	42 (42%)	8.2 (5.0–15.6)	
Smoking status	Non-smoker	56 (56%)	8.3 (4.5–29.5)	0.975
	Smoker	44 (44%)	8.3 (4.5–32.4)	
Hypertension	No	59 (59%)	7.8 (4.5–29.5)	0.116
	Yes	41 (41%)	8.8 (4.5–32.4)	
Hyperlipidemia	No	73 (73%)	8.2 (4.5–29.5)	0.053
	Yes	27 (27%)	8.5 (4.5–32.4)	
Obesity	No	59 (59%)	7.8 (4.5–32.4)	0.442
	Yes	41 (41%)	9.3 (4.5–29.5)	
Diabetes mellitus	No	66 (66%)	8.2 (4.5–29.5)	0.318
	Yes	34 (34%)	8.4 (4.5–32.4)	
Angina	Stable	88 (88%)	8.2 (4.5–32.4)	0.656
	Unstable	12 (12%)	9.6 (5.0–20.2)	
Myocardial infarction (MI)	No	70 (70%)	8.2 (4.5–29.5)	
	Stemi	18 (18%)	8.3 (5.0–32.4)	0.93
	Nstemi	12 (12%)	9.3 (4.5–25.5)	0.124
Familial history	No	76 (76%)	8.4 (4.5–32.4)	0.394
	Yes	24 (24%)	8.3 (4.5–17.6)	

ation. A 310 bp band indicated the presence of the T allele, amplified by primers FO/RI, while a 494 bp band indicated the G allele, amplified by primers RO/FI (Table 2, Fig. 2).

Statistical Analysis

Serum vitamin D levels were expressed as median (ng/mL) values and group comparisons were performed using the Mann–Whitney U and Kruskal-Wallis tests. Genotype distribution and allele frequencies were evaluated using Fisher's exact or chi-square tests, and Hardy Weinberg equilibrium was tested for each SNP. The association between *CYP2R1* rs1562902 C > T, *TCF7L2* rs12255372 G > T SNPs, and the *CCR5* Δ32 mutation with CAD risk was estimated by calculating odds ratios (ORs). Statistical analyses were performed using GraphPad Prism (version 8.0, GraphPad Software, Inc., San Diego, CA, USA) and SPSS (version 25, IBM Corp., Armonk, NY, USA), with a p-value < 0.05 considered statistically significant.

Results

Demographic Characteristics

The clinical characteristics of the study population are presented in Table 1. The majority of CAD patients were male (80%) compared to female (20%). The average age of the CAD patients was approximately 50 years. Among the patients, 56% were non-smokers, while 44% were smokers. The clinical complications observed among CAD patients included hypertension (41%), hyperlipidemia (27%), obesity (41%), diabetes (34%), angina (12%), and myocardial infarction (30%).

Serum Vitamin D and CAD

The median serum vitamin D level among CAD patients was 8.3 ng/mL, which was significantly lower ($p < 0.0001$) than the median vitamin D level in healthy controls (16 ng/mL). CAD patients exhibited approximately half the vitamin D levels of healthy controls (Fig. 3). When comparing vitamin D serum levels across different clinical subgroups of CAD patients, no significant differences were observed ($p > 0.05$) (Table 3).

CYP2R1 (rs1562902 C > T) and CAD

The genotype distribution of *CYP2R1* rs1562902 C > T in the study population is shown in Table 4. Among CAD patients, the CC, CT, and TT genotype frequencies were 15%, 72%, and 13%, respectively, compared to 44%, 51%, and 5% in healthy controls. The C and T allele frequencies were 0.51 and 0.49 in CAD patients and 0.70 and 0.30 in healthy controls, respectively ($p < 0.001$). Compared to the *CYP2R1* CC genotype, the odds ratios (ORs) for developing CAD were 4.1 for the CT genotype ($p = 0.0001$) and 7.6 for the TT genotypes ($p = 0.0008$) (Table 5).

Additionally, the distribution of *CYP2R1* (rs1562902 C > T) genotypes was significantly different between CAD cases aged ≤50 and those aged >50 years ($p = 0.004$). A significant association was also observed between *CYP2R1* (rs1562902 C > T) genotypes and hyperlipidemia ($p = 0.02$) (Table 6).

Table 4. Genotypic frequency of *CYP2R1* (rs1562902 C > T), *TCF7L2* (rs12255372 G > T), and *CCR5* Δ32 mutation in controls and CAD patients.

Frequency of *CYP2R1* (rs1562902 C > T genotypes in CAD patients and controls									
Subjects	n	CC (%)	CT (%)	TT (%)	C	T	DF	χ^2	*p*-value
Cases	100	15 (15%)	72 (72%)	13 (13%)	0.51	0.49	2	21.40	0.0001*
Controls	100	44 (44%)	51 (51%)	5 (5%)	0.70	0.30			
Frequency of *TCF7L2* (rs12255372 G > T) genotypes in CAD patients and controls									
Subjects	n	GG (%)	GT (%)	TT (%)	G	T	DF	χ^2	*p*-value
Cases	100	49 (49%)	39 (39%)	12 (12%)	0.69	0.31	2	6.87	0.032*
Controls	100	66 (66%)	29 (29%)	5 (5%)	0.81	0.19			
Frequency of CCR5 Δ32 mutation genotypes in CAD patients and controls									
CCR5 Δ32 bp	n	+/+ (%)	+/Δ (%)	Δ /Δ (%)					*p*-value
Cases	100	66 (66%)	34 (34%)	0 (0%)			2	7.606	0.006*
Controls	100	83 (83%)	17 (17%)	0 (0%)					

DF, Degree of Freedom; χ^2, Chi-Square value. *Statistically significant difference ($p < 0.05$).

Table 5. Risk of developing CAD in relation to *CYP2R1* rs1562902 C > T genotypes and *CCR5* Δ32 bp ins/del mutation among the population of Tabuk, KSA.

Genotype	Controls (n = 100)	CAD patients (n = 100)	OR	*p*-value
CYP2R1 rs1562902 C/T				
CC (reference)	44	15	1.00	
CT	51	72	4.1 (2.08 to 8.23)	0.0001*
TT	5	13	7.6 (2.32 to 24.97)	0.0008*
CCR5 Δ32 bp ins/del (rs333)				
+/+ (reference)	83	66	1.00	
+/Δ	17	34	2.51 (1.29 to 4.89)	0.006*

* Statistically significant difference. KSA, Kingdom of Saudi Arabia; OR, odds ratio.

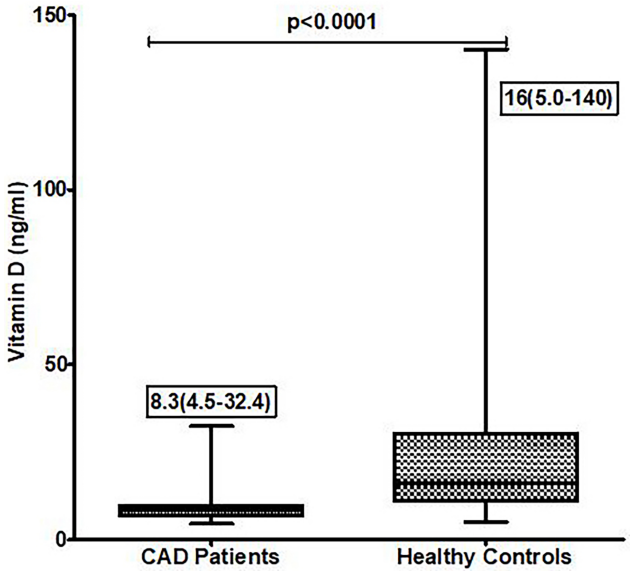

Fig. 3. Serum vitamin D levels in CAD patients compared to healthy controls. Data is presented using a box plot, the lines represents the median (min-max). A *p*-value < 0.05 was considered statistically significant. *p*-value < 0.05 statistically significant.

CCR5 Δ32 Mutation and CAD

The *CCR5* Δ32 heterozygous mutation was observed in 34% of CAD patients (Table 4). There was a statistically significant difference in *CCR5* Δ32 mutation genotype distribution between CAD patients and healthy controls ($p = 0.006$) (Table 4). However, no *CCR5* Δ32 homozygous mutations were observed in the CAD patients or the control group (Table 4). The *CCR5* Δ32 heterozygous mutant (+/Δ) was associated with an increased risk of CAD with an OR of 2.51 ($p = 0.006$) (Table 5). Additionally, the *CCR5* Δ32 heterozygous mutation (+/Δ) was significantly associated with gender, obesity, and angina ($p < 0.05$) (Table 7).

Serum Vitamin D and CYP2R1 rs1562902 C > T/CCR5 Δ32 Mutation

The relationship between *CYP2R1* rs1562902 C > T genotypes and serum vitamin D levels in CAD patients was examined (Fig. 4). The median vitamin D levels among CAD patients with *CYP2R1* rs1562902 CC, CT, and TT genotypes were 9.3, 8.5, and 6.5 ng/mL, respectively, with a significant difference ($p = 0.001$). Additionally, serum vitamin D levels were compared between CAD patients with the *CCR5* wild type and *CCR5* Δ32 heterozygous mutation genotypes. No significant difference was observed in vita-

Table 6. Distribution of *CYP2R1* rs1562902 C > T genotypes in relation to clinical characteristics of CAD patients.

Clinical features	Variables	CC (%)	CT (%)	TT (%)	*p*-value
Gender	Male	11 (13.8%)	60 (75.0%)	9 (11.3%)	0.39
	Female	4 (20.0%)	12 (60.0%)	4 (20.0%)	
Age	≤50	9 (15.5%)	47 (81.0%)	2 (3.4%)	0.004*
	>50	6 (14.3%)	25 (59.5%)	11 (26.2%)	
Smoking status	Non-smoker	8 (14.3%)	39 (69.6%)	9 (16.1%)	0.58
	Smoker	7 (15.9%)	33 (75.0%)	4 (9.1%)	
Hypertension	No	8 (13.6%)	46 (78.0%)	5 (8.5%)	0.20
	Yes	7 (17.1%)	26 (63.4%)	8 (19.5%)	
Hyperlipidemia	No	8 (11.0%)	58 (79.5%)	7 (9.6%)	0.02*
	Yes	7 (25.9%)	14 (51.9%)	6 (22.2%)	
Obesity	No	7 (11.9%)	47 (79.7%)	5 (8.5%)	0.11
	Yes	8 (19.5%)	25 (61.0%)	8 (19.5%)	
Diabetes mellitus	No	9 (13.6%)	51 (77.3%)	6 (9.1%)	0.19
	Yes	6 (17.6%)	21 (61.8%)	7 (20.6%)	
Angina	Stable	12 (13.6%)	65 (73.9%)	11 (12.5%)	0.49
	Unstable	3 (25.0%)	7 (58.3%)	2 (16.7%)	
Myocardial infarction (MI)	No	12 (17.1%)	49 (70.0%)	9 (12.9%)	
	Stemi	3 (16.7%)	13 (72.2%)	2 (11.1%)	0.99
	Nstemi	0 (0.0%)	10 (83.3%)	2 (16.7%)	0.33
Familial history	No	11 (14.5%)	54 (71.1%)	11 (14.5%)	0.73
	Yes	4 (16.7%)	18 (75.0%)	2 (8.3%)	

* Statistically significant difference.

min D levels between these groups, with median levels of 8.2 ng/mL in the wild type and 9.2 ng/mL in the heterozygous group ($p = 0.43$) (Fig. 5).

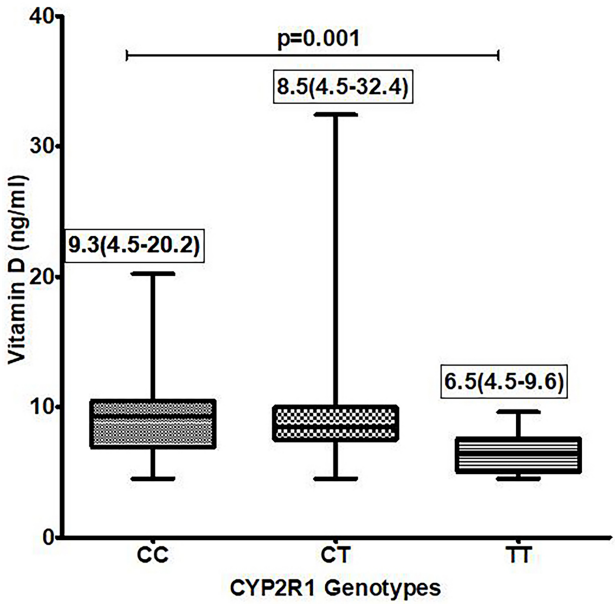

Fig. 4. Association of serum vitamin D levels with *CYPR1* genotypes in CAD patients. A *p*-value < 0.05 was considered statistically significant.

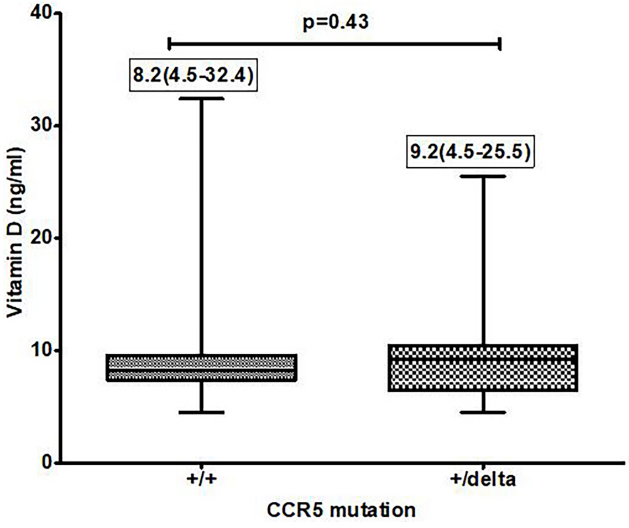

Fig. 5. Association of serum vitamin D levels with *CCR5* Δ32 bp mutation in CAD patients.

Distribution of TCF7L2 rs12255372 G > T Genotype among the Study Population

The genotype *TCF7L2* rs12255372 G > T among CAD patients was as follows: GG (49%), GT (39%), and TT (12%). In the healthy controls, the genotype distribution was GG (66%), GT (29%), and TT (5%) (Table 4). The allele frequencies of the G and T alleles were 0.69 and 0.31, respectively, in CAD patients, compared to 0.81 and 0.19 in healthy controls, showing a significant difference ($p = 0.032$) (Table 4).

Table 7. Distribution of the *CCR5* Δ32 bp ins/del mutation in relation to clinical characteristics of CAD patients.

Clinical features	Variables	+/+ (%)	+/Δ (%)	*p*-value
Gender	Male	49 (61.3%)	31 (38.8%)	0.04*
	Female	17 (85.0%)	3 (15.0%)	
Age	≤50	35 (60.3%)	23 (39.7%)	0.16
	>50	31 (73.8%)	11 (26.2%)	
Smoking status	Non-smoker	38 (67.9%)	18 (32.1%)	0.65
	Smoker	28 (63.6%)	16 (36.4%)	
Hypertension	No	37 (62.7%)	22 (37.3%)	0.40
	Yes	29 (70.7%)	12 (29.3%)	
Hyperlipidemia	No	50 (68.5%)	23 (31.5%)	0.38
	Yes	16 (59.3%)	11 (40.7%)	
Obesity	No	34 (57.6%)	25 (42.4%)	0.03*
	Yes	32 (78.0%)	9 (22.0%)	
Diabetes mellitus	No	47 (71.2%)	19 (28.8%)	0.12
	Yes	19 (55.9%)	15 (44.1%)	
Angina	Stable	55 (62.5%)	33 (37.5%)	0.04*
	Unstable	11 (91.7%)	1 (8.3%)	
Myocardial infarction (MI)	No	47 (67.1%)	23 (32.9%)	0.15
	Stemi	9 (50.0%)	9 (50.0%)	
	Nstemi	10 (83.3%)	2 (16.7%)	
Familial history	No	53 (69.7%)	23 (30.3%)	0.16
	Yes	13 (54.2%)	11 (45.8%)	

* Statistically significant difference.

In the codominant model, the *TCF7L2* rs12255372 TT genotype was associated with increased susceptibility to CAD, with OR of 3.23, and the association was statistically significant ($p = 0.037$) (Table 8). In the dominant model, the combined *TCF7L2* rs12255372 (GT + TT) genotypes were also linked with increased CAD susceptibility, with an OR of 2.20, $p = 0.015$ (Table 8). Additionally, the allelic comparison showed a significant correlation between the T allele and CAD susceptibility, with an OR of 1.89, $p = 0.006$ (Table 8).

TCF7L2 rs12255372 G > T and CAD

Significant differences ($p < 0.05$) were observed between the gender and age of CAD patients and their *TCF7L2* rs12255372 G > T genotypes (Table 9). Male CAD patients exhibited a higher incidence of the heterozygous genotype (GT) compared to female CAD patients. Moreover, significant associations ($p < 0.05$) were found between *TCF7L2* rs12255372 G > T genotypes and clinical features such as hypertension, hyperlipidemia, obesity, diabetes mellitus, angina, and myocardial infarction in CAD cases (Table 9).

Discussion

CAD is a prevalent life-threatening condition and has emerged as one of the leading causes of death in Saudi Arabia and globally [1,31]. CAD is induced by the interaction of genetic and environmental risk factors [32]. Reducing modifiable risk factors and maintaining a healthy lifestyle (e.g., smoking cessation, weight management, regular exercise, a healthy diet, and controlling total cholesterol, blood pressure, and fasting blood sugar levels), can help delay or prevent cardiovascular disease [33,34]. Identifying genetic risk factors (risk loci) through genome-wide association studies [28,35,36] assists in detecting susceptible individuals or populations through genetic testing [27].

This study aimed to evaluate the association between serum vitamin D dysregulation and the *CYP2R1* rs1562902 C > T SNP in relation to CAD development. Additionally, we examined the link between the *CCR5* Δ32 mutation and the *TCF7L2* rs12255372 G > T SNP with the risk of developing CAD.

A case-control study involving CAD patients and matched healthy controls was conducted. Our results demonstrated that serum vitamin D levels were significantly reduced in CAD cases compared to healthy controls (Table 3, Fig. 3). In CAD patients, vitamin D was also associated with angina and myocardial infarction (MI) (Table 3). These findings align with previous research showing an association between low vitamin D levels and major cardiovascular diseases such as CAD, heart failure (HF), and atrial fibrillation [37]. Moreover, our findings are consistent with studies suggesting that vitamin D supplementation may decrease the incidence of CVD [38,39] and that serum vitamin D levels are significantly lower in patients with stable angina or acute myocardial infarction (AMI)

Table 8. Association between *TCF7L2* rs12255372 G > T genotypes and CAD risk.

Genotypes			OR	Risk ratio (RR)	*p*-value
	100	100			
Codominant model					
TCF7L2-GG	66	49	1 (ref.)	1 (ref.)	
TCF7L2-GT	29	39	1.81 (0.99 to 3.32)	1.34 (0.98 to 1.84)	0.066
TCF7L2-TT	5	12	3.23 (1.07 to 9.78)	1.95 (0.92 to 4.14)	0.037*
Dominant model					
TCF7L2-GG	66	49	1 (ref.)	1 (ref.)	
TCF7L2 (GT+TT)	34	51	2.20 (1.14 to 3.57)	1.43 (1.06 to 1.95)	0.015*
Recessive model					
TCF7L2 (GG+GT)	95	88	1 (ref.)	1 (ref.)	
TCF7L2-TT	5	12	2.59 (0.88 to 7.65)	1.76 (0.83 to 3.73)	0.84
Allele analysis					
TCF7L2-G	161	137	1 (ref.)	1 (ref.)	
TCF7L2-T	39	63	1.89 (1.20 to 3.01)	1.41 (1.08 to 1.85)	0.006*
Over dominant model					
TCF7L2-GG+TT	71	61	1 (ref.)	1 (ref.)	
TCF7L2-GT	29	39	1.56 (0.87 to 2.82)	1.26 (0.92 to 1.73)	0.13

* Statistically significant difference.

than in healthy controls [37]. Nevertheless, others studies reported that supplementation with vitamin D did not lower the incidence of CVD [40,41].

Vitamin D regulates microRNA-145, the most abundant in vascular smooth muscle cells [37]. Reduced levels of microRNA-145 have been linked to adverse vascular changes, including vascular stenosis and calcification [42, 43]. Additionally, vitamin D exhibits anti-atherosclerotic and anti-inflammatory properties [44], potentially affecting the pathophysiology of atherosclerosis by modulating the inflammatory response, reducing the expression of tumor necrosis factor-alpha (TNF-α), and inhibiting nuclear factor kappa B signaling [44,45]. Vitamin D also reduces cholesterol accumulation in macrophage-derived foam cells and promotes low-density lipoprotein cholesterol (LDL-C) efflux [44,46]. Foam cells ingest LDL-C and play a crucial role in initiating and advancing atherosclerosis [47]. Moreover, vitamin D has been suggested to enhance the high-density lipoprotein cholesterol (HDL-C) formation in liver cells [46].

Our findings demonstrated that the CT genotype and T allele of the *CYP2R1* rs1562902 C > T SNP were associated with CAD (Tables 4,5, Fig. 3). The distribution of this SNP also differed between CAD patients with hyperlipidemia and those with normal lipid profiles (Table 6). CAD patients carrying the TT genotype of *CYP2R1* rs1562902 C > T had significantly lower vitamin D levels compared to those with CC or CT genotypes (Fig. 4). We observed no significant association between the *CCR5* Δ32 bp genotype and vitamin D levels in CAD patients (Fig. 5). CYP2R1 catalyzes the 25-hydroxylation of pre-vitamin D in the liver, converting it to 25-hydroxyvitamin D, which is further hydroxylated by CYP27B1 (1α-hydroxylase) in the kidney to form the active hormone 1,25-hydroxyvitamin D [15]. Variants in CYP2R1 have been previously linked to serum vitamin D levels [48,49], with CYP2R1 functioning as the main hepatic enzyme for vitamin D activation (1,25-dihydroxyvitamin D) [50]. This is one of the first studies to demonstrate a potential association between the *CYP2R1* rs1562902 C > T variant, vitamin D levels, and CAD risk, consistent with findings from a study showing *CYP2R1* gene variation (rs12794714) is associated with hypertension risk in a Chinese population [51].

CCR5 has been described as a double-edged sword in metabolic disorders like CAD. CCR5 increases inflammatory responses in adipose tissue by regulating macrophage recruitment and altering M1/M2 phenotypes, leading to insulin resistance and obesity [18]. However, targeting CCR5 is not always beneficial, as evidence suggests that CCR5 can enhance progenitor cell recruitment and support vascular endothelial repair [52]. Our results showed that 34% of CAD patients had *CCR5* Δ32 heterozygous mutation (+/Δ), associated with a 2.2-fold increased risk of CAD compared to wild type *CCR5* (Tables 4,5). The heterozygous genotype reduces *CCR5* expression on the cell surface [21]. We also observed significant associations between the *CCR5* Δ32 mutation and gender, obesity, and angina (Table 7), consistent with studies showing males are more susceptible to CAD than females [53] and that *CCR5* Δ32 is linked to angina, an important clinical symptom of CAD [54].

Our findings align with studies suggesting the *CCR5* Δ32 mutation is associated with atherosclerosis in Asian populations [55], though the relationship between *CCR5* variants and atherosclerosis remains controversial [18]. Our results differ from those indicating that the *CCR5* Δ32

Table 9. Correlation of the *TCF7L2* rs12255372 G > T genotypes with clinical characteristics of CAD patients.

Clinical features	Variables	100	GG	GT	TT	DF	χ^2	*p*-value
			49	39	12			
Association of *TCF7L2* rs12255372 G > T genotypes with gender								
Gender	Male	80	41	34	5	2	12.69	0.0018*
	Female	20	8	5	7			
Association of *TCF7L2* rs12255372 G > T genotypes with age								
Age	≤55	58	34	16	8	2	7.59	0.022*
	>55	42	15	23	4			
Association of *TCF7L2* rs12255372 G > T genotypes with smoking								
Smoking status	Non-smoker	56	33	19	4	2	5.9	0.0523
	Smoker	44	16	20	8			
Association of *TCF7L2* rs12255372 G > T genotypes with hypertension of CAD patients								
Hypertension	No	59	34	22	3	2	8.03	0.018*
	Yes	41	15	17	9			
Association of *TCF7L2* rs12255372 G > T genotypes with obesity of CAD patients								
Hyperlipidemia	No	73	41	26	6	2	6.85	0.032*
	Yes	27	8	13	6			
Association of *TCF7L2* rs12255372 G > T genotypes with hypertension of CAD patients								
Obesity	No	59	35	19	5	2	6.32	0.042*
	Yes	41	14	20	7			
Association of *TCF7L2* rs12255372 G > T genotypes with CAD patients								
Diabetes mellitus	No	66	41	21	4	2	15.9	0.0005*
	Yes	34	8	18	8			
Association of *TCF7L2* rs12255372 G > T genotypes with stable and non-stable angina of CAD patients								
Angina	Stable	88	46	34	8	2	6.8	0.033*
	Unstable	12	3	5	4			
Association of *TCF7L2* rs12255372 G > T genotypes with myocardial infarction (MI) patients								
Myocardial infarction (MI)	No	70	35	30	5	2	9.89	0.042*
	Stemi	18	7	5	6			
	Nstemi	12	7	4	1			
Association of *TCF7L2* rs12255372 G > T genotypes with Familial history of CAD patients								
Familial history	No	76	43	26	7	2	7.63	0.022*
	Yes	24	6	13	5			

* Statistically significant difference.

mutation is beneficial for cardiovascular health by increasing HDL-C and reducing triglyceride levels [18]. However, our results align with studies linking *CCR5* Δ32 to increased susceptibility to atherosclerosis, including CAD, in Asian and Bruneck populations [55,56] and with findings from a Danish population study suggesting that *CCR5* Δ32 carriers are at a higher risk for cardiovascular disease due to elevated levels of C-reactive protein [57]. This study provides further evidence for the association between *CCR5* Δ32 mutation and atherosclerotic CVD.

TCF7L2, encoded by the *TCF7L2* gene, is a transcription factor involved in the Wnt signaling pathway [58], which plays a key role in cell proliferation, differentiation, and development, with limited activity in the cardiovascular system [59]. However, Wnt signaling is associated with metabolic syndrome [59,60], characterized by cardiovascular risk factors such as insulin resistance, hypertension, abdominal obesity, impaired sugar metabolism, dyslipidemia, genetic predisposition, and inflammation [61,62]. Metabolic syndrome is significantly associated with an elevated risk of diabetes mellitus and cardiovascular disease [63]. TCF7L2 plays pivotal a role in physiological processes across various organs and tissues, such as the liver, islet of the pancreas, and adipose tissues [22]. Variants in *TCF7L2* have been linked to obesity, cancer, metabolic syndrome, and CVD [22]. Our results showed that the *TCF7L2* rs12255372 GT genotype and T allele were associated with CAD (Tables 4,8). Our findings also indicated that the distribution of *TCF7L2* rs12255372 G > T genotypes differed significantly by gender and age (Table 9). These findings are in line with previous studies showing variations in CAD susceptibility based on gender and age [53,64].

Furthermore, we observed associations between *TCF7L2* rs12255372 G > T genotypes and hypertension, hyperlipidemia, angina, and family history (Table 9). These are well-established risk factors for CVD [65]. Angina is

a hallmark symptom of CAD [54], and family history is a known risk factor for CAD [66]. *TCF7L2* has been reported to have anti-atherosclerotic properties [67], and promoting TCF7L2 expression has been suggested as a potential therapeutic target for atherosclerotic CVD [67]. The TCF7L2 rs7903146 SNP, in particular, has been associated with type 2 diabetes (T2D) and CVD [67,68]. Our findings may be consistent with previous studies reporting an association between *TCF7L2* rs12255372 and T2D [69–71]. Insulin resistance, which characterizes T2D, is common in atherosclerotic CVD, including the CAD [72]. To our knowledge, this is the first study to demonstrate a direct association between *TCF7L2* rs12255372 and CAD in a Saudi population.

Limitations of this study include the relatively small sample size and the cross-sectional study design. Future large-scale case-control studies and functional analysis of proteins are warranted to validate these findings.

Conclusion

In conclusion, we examined the association between serum vitamin D levels and CAD development, as well the potential linkage of the *CYP2R1* rs1562902 C > T, TCF7L2 rs12255372 G > T, and *CCR5* Δ32 (rs333) genetic variants with CAD development. Our findings indicated that low serum vitamin D levels are associated with an increased risk of CAD and that the *CYP2R1* rs1562902 C > T variant is linked to vitamin D concentration. Additionally, the *CCR5* Δ32 mutation and *TCF7L2* rs12255372 G > T SNP were identified as potential genetic loci associated with CAD susceptibility. These findings warrant further validation in large-scale studies. Once verified, they could be valuable for genetic testing to identify individuals at higher risk for early detection and prevention of CAD.

Availability of Data and Materials

All data generated or analyzed during this study are included in this published article.

Author Contributions

Conceptualization: JJ, RM, IE, RA, JB, NAA, MMJ, MAAla, MAAlt, SOA, TB, EH, FMA. Samples collection: JJ, SOA and FMA. Methodology: JJ, RM, and IE. Validation and analysis: RA, JB, NAA, MMJ, MAAla, MAAlt, SOA, TB, EH, FMA. Writing of the manuscript draft: JJ, RM, IE, RA, JB, NAA, MMJ, MAAla, MAAlt, SOA, TB, EH, FMA. All authors contributed significantly to editorial changes of important content. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

This study was conducted in accordance with the guidelines outlined in the Declaration of Helsinki and was ethically approved by the University of Tabuk Research Ethics Committee (Registration number UT-91-23-2020). All participants provided informed written consent in this study.

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Conflict of Interest

The authors declare no conflict of interest.

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