ORIGINAL ARTICLE

CONTENTS

Impact of genetic diversity of PCSK9 polymorphisms on coronary artery disease in a sample of Iraqi individuals

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ABSTRACT

Aim: To assess the association of *PCSK9* SNPs (rs2483205, rs2479394) with CAD susceptibility in an Iraqi population.

Materials and Methods: A case-control study recruited 110 CAD patients, angiographically confirmed, and 110 age-/sex-matched healthy controls. Serum PCSK9 was quantified by ELISA, and lipid profiles were analyzed enzymatically. Genotyping for rs2483205 (C/T) and rs2479394 (G/A) was performed using ARMS-PCR. Associations were evaluated using logistic regression, haplotype analysis, and genotype-phenotype correlations, adjusting for age and sex.

Results: CAD patients exhibited significantly higher PCSK9 levels (54.12 ± 19.04 vs. 20.94 ± 17.72 ng/mL, $p < 0.0001$), LDL-C, TG, and lower HDL-C ($p \leq 0.005$) versus controls. Genotype analysis revealed a significant protective effect of the rs2479394 G/A genotype against CAD (OR = 0.21, 95% CI: 0.04–1.08, $p = 0.024$). The rs2483205 SNP showed no significant association. Haplotype analysis identified four major haplotypes; T-A demonstrated significant protection (OR = 0.45, 95% CI: 0.21–0.88, $p = 0.020$). No significant associations were found between rs2479394 genotypes and phenotypic parameters (blood pressure, lipids, PCSK9) in CAD patients.

Conclusions: The rs2479394 G/A genotype and T-A haplotype are associated with reduced CAD risk in Iraqis, suggesting a protective role for specific *PCSK9* variants. Elevated serum PCSK9 levels and dyslipidemia were confirmed in CAD patients; however, rs2479394 did not correlate with these phenotypic traits.

KEY WORDS: PCSK9, rs2483205, rs2479394, CAD, ARMS-PCR, association

Wiad Lek. 2026;79(1):69-77. doi: 10.36740/WLek/216770 DOI

INTRODUCTION

CAD is characterized by atherosclerotic plaque accumulation in coronary arteries. It is the leading global cause of mortality, responsible for 32% of worldwide deaths [1]. In Iraq, CAD prevalence has surged, affecting 5–10% of adults and manifesting 8–10 years earlier than in Western populations, with hyperlipidemia and smoking as predominant risk factors [2]. The pathophysiology involves endothelial dysfunction, lipid-driven plaque formation, and thrombosis, ultimately leading to myocardial ischemia or infarction [3]. By 2018, CAD caused 18.92% of deaths in Iraq, highlighting an urgent public health crisis [4]. Proprotein Convertase Subtilisin/Kexin Type 9 (PCSK9), a serine protease encoded on chromosome 1p32.3, regulates lipid metabolism by degrading hepatic low-density lipoprotein receptors (LDLRs) [5]. This process elevates circulating LDL cholesterol (LDL-C), accelerating atherosclerosis [6]. Beyond lipid modulation, PCSK9 amplifies inflammation via toll-like receptor activation and cytokine release, exacerbating plaque instability [7]. Its dual role positions PCSK9 as a therapeutic target, with inhibitors like evolocumab, reducing LDL-C by 60% and cardiovascular events [6]. Specific PCSK9 single-nucleotide polymorphisms (SNPs) influence CAD susceptibility; rs615563 and rs2479394 associate with subclinical atherosclerosis and hypercholesterolemia [8]. The rs2483205 correlates with elevated LDL-C and CAD risk in multi-ethnic studies [9]. Gain-of function variants increase LDLR degradation, thereby raising LDL-C levels and increasing atherosclerosis risk, while loss-of-function mutations confer protection [10]. Despite evidence from global cohorts, these SNPs remain unstudied in Iraqis, a population with unique genetic and environmental CAD risks [2].

AIM

This study aims to assess the association of *PCSK9* SNPs (rs2483205, rs2479394) with CAD susceptibility in an Iraqi population.

Table 1. Primer Sequence of AS-PCR Genotyping of *PCSK9*

SNP	Type of allele and primer	Sequence (5′ –3′)
rs2483205	Allele C(wild)Fwd	ATGTGGTCCTTGTGTTCGTC
	Allele T(mutant)Fwd	ATGTGGTCCTTGTGTTCGTT
	Reverse primer common	CACTCTGGTTCTCTGGCTCT
rs2479394	Allele G(wild)Rev	GGGAGTCCTCTATGTGACAATG
	Allele A(mutant)Rev	GGGAGTCCTCTATGTGACAATA
	Forward primer common	AGCTTTCTGCCTCCACAAC

Source: compiled by the authors of this study

MATERIALS AND METHODS

STUDY DESIGN AND PARTICIPANT RECRUITMENT

This case-control genetic association study was conducted between August 2024 and March 2025 at the University of Kufa, Iraq. We enrolled 110 patients with coronary artery disease (CAD) (79 males, 31 females) and 110 age-, sex-, and geographically matched healthy controls (81 males, 29 females). CAD diagnosis was confirmed by coronary angiography showing ≥70% obstruction in major coronary arteries or branches at Al-Najaf Center for Cardiac Surgery and Transcatheter Therapy. Patients were recruited from Al-Sadr Teaching Hospital cardiology center following strict inclusion criteria: age >30 years, angiographic confirmation of CAD, and absence of autoimmune disorders or hepatic dysfunction. Controls were asymptomatic individuals randomly selected from the general population with no personal history of CAD, diabetes mellitus, or hypertension, and no family history of CAD. Demographic matching between groups was systematically implemented to minimize confounding effects.

ETHICAL APPROVAL

The study protocol received full approval (MEC-1280) from the Ethical Committee of the Faculty of Medicine, University of Kufa. All participants provided written informed consent before their inclusion. Procedures were conducted in accordance with the principles outlined in the Declaration of Helsinki for human biomedical research.

SAMPLE COLLECTION AND BIOCHEMICAL ANALYSIS

After a standardized 12-hour overnight fast, 5 mL venous blood was collected via peripheral venipuncture. For phenotypic characterization, 3 mL was transferred to plain tubes, incubated at 37°C for 15 minutes for coagulation, centrifuged at 2000 ×g for 10-15 minutes, and serum was stored at -20°C. Biochemical analyses were performed at the Postgraduate Laboratory, Department of Biochemistry. Serum PCSK9 concentrations were quantified using a sandwich ELISA kit (BT LAB, Korea) and measured spectrophotometrically at 450 nm. Serum lipid concentrations were determined by enzymatic methods following the manufacturer's protocols (Randox, UK). They included triglycerides (TG), total cholesterol (TC), and HDL-C. LDL-C and VLDL-C were calculated using the Friedewald equations:

VLDL-C (mg/dL) = TG/5

LDL-C (mg/dL) = TC - (HDL-C + VLDL-C)

GENETIC ANALYSIS

Genomic DNA was extracted from EDTA-treated whole blood using Geneaid purification kits (Taiwan), yielding 20-30 kb fragments. DNA concentration and purity (A260/A280 ratio 1.8-2.0) were verified using a BioDrop spectrophotometer (UK). Two PCSK9 SNPs on chromosome 1, rs2483205 (C>T), and rs2479394 (G>A), were genotyped using Allele-Specific PCR (ARMS-PCR). Primers were designed with Primer3 software based on GenBank sequences, Table 1.

PCR amplification utilized AccuPower® PreMix (Bioneer, Korea) containing Taq DNA polymerase, dNTPs, and optimized buffer components. Reactions contained 6 µL genomic DNA, 2 µL each of allele-specific primers (Macrogen, Korea), and common primers in 20 µL reactions. Thermocycling conditions (Biometra T-professional, Germany) included: initial denaturation at 95°C for 5 minutes; 35 cycles of denaturation (95°C, 20 s), allele-specific annealing (rs2483205: 58.5°C, rs2479394: 59.5°C for 45 s), and extension (72°C, 1 min); with final extension at 72°C for 7 minutes. PCR products were resolved on 2% agarose gels in 1X TBE buffer, stained with Diamond dye (Pioneer, Korea). Electrophoresis was run at 75-80V for 120 minutes alongside 100 bp DNA ladders (Simgen, China). Genotypes were determined by UV visualization of allele-specific amplicons. For the rs2483205 SNP, the amplicons size was 309 bp, and for the rs2479394 SNP, it was 272 bp. homozygous wild-type samples showed bands only in wild-type primer lanes, homozygous mutants only in mutant lanes, and heterozygotes in both lanes, Figure 1 for rs2483205 SNP and Figure 2 for rs2479394 SNP.

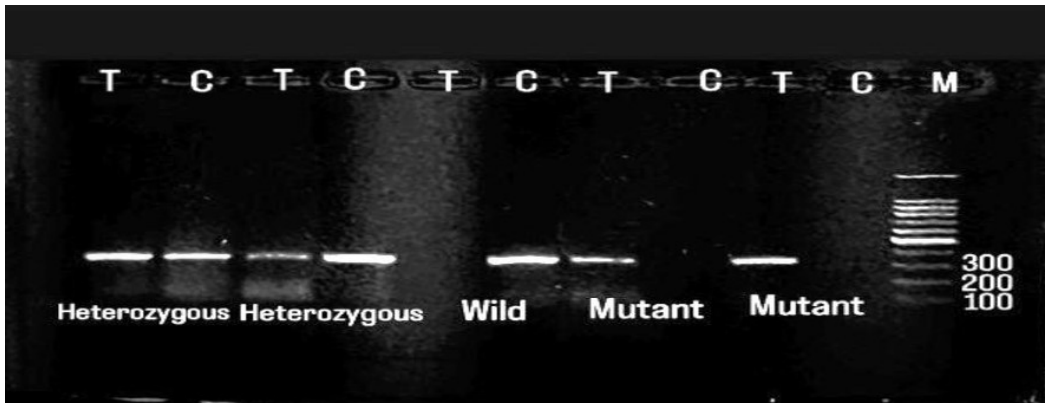

Figure 1: The PCR product of *PCSK9* analyzed for rs2483205 SNP by electrophoresis on an agarose gel. M: Ladder of DNA 100 bp. The PCR product is an amplicons of 309 bp
Source: compiled by the authors of this study

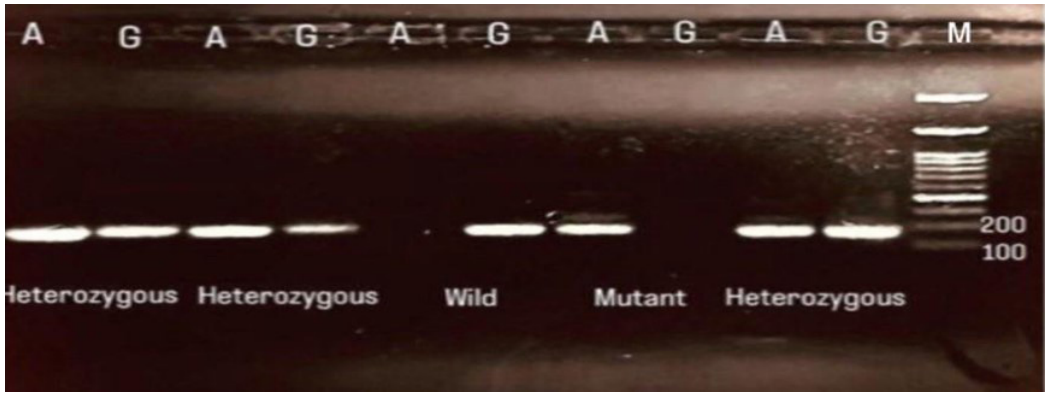

Figure 2: The PCR product of *PCSK9* analyzed for rs2479394 SNP by electrophoresis on an agarose gel. M: Ladder of DNA 100 bp. The PCR product is an amplicons of 272 bp
Source: compiled by the authors of this study

STATISTICAL ANALYSIS

Data are expressed as mean ± standard deviation. Continuous variables were compared using Student's *t*-test. Genotype and allele frequencies were compared using chi-square tests with Yates' correction where appropriate. Odds ratios (ORs) with 95 % confidence intervals (CIs) were estimated from logistic regression models adjusted for age and sex. To ensure consistency between point estimates and hypothesis tests, both *p*-values and CIs were obtained from the same likelihood-based model using profile-likelihood (rather than Wald) methods; this approach reduces the conservatism of CIs in sparse cells. Statistical significance for the prespecified primary analysis (rs615563 under its primary inheritance model in the overall cohort) was set at two-sided $p \leq 0.05$. All other inheritance models, additional SNPs, sex-stratified analyses, and haplotypes were treated as exploratory; for these, the false discovery rate (FDR) was controlled at $q = 0.05$ using the Benjamini–Hochberg procedure. All analyses were performed in SPSS v26 (IBM, USA).

RESULTS

CHARACTERISTICS OF PATIENTS AND CONTROL INDIVIDUALS

The anthropometric and biochemical characteristics of the study groups are summarized in Table 2. There were no significant differences in sex distribution or mean age, however, significant differences were observed in several clinical parameters. Compared to controls, CAD patients had a significantly higher mean BMI, systolic and diastolic blood pressure, FBG levels (195.57 ± 45.41 *vs*. 123.35 ± 11.23 mg/dL, P < 0.0001) and higher serum PCSK9 concentrations (54.12 ± 19.04 *vs*. 20.94 ± 17.72 ng/mL, P < 0.0001). Additionally, total cholesterol (184.70 ± 30.43 *vs*. 155.09 ± 26.76 mg/dL, P < 0.0001), TG (183.89 ± 45.42 vs. 104.25 ± 30.77 mg/dL, P < 0.0001), LDL-C (107.85 ± 24.50 *vs.* 92.63 ± 21.34 mg/dL, P = 0.0035), and VLDL-C (37.12 ± 17.45 *vs.* 21.87 ± 10.23 mg/dL, P < 0.0001) were significantly higher in the CAD patient group, whereas HDL-C was significantly lower in CAD patients (42.84 ± 9.57 *vs.* 46.30 ± 8.50 mg/dL; P = 0.005). These findings indicate significant differences in metabolic and lipid parameters between patients with CAD and healthy controls.

ASSOCIATION OF PCSK9 SNPS WITH CAD

A post hoc power calculation was carried out using the observed genotype frequencies. For rs615563 under the recessive model (G/G *vs*. A/A–A/G; frequency ≈ 0.57 in cases *vs.* 0.33 in controls), at α = 0.05 (two-sided), our study has approximately 85% power to detect an odds ratio of ≥ 2.5. For rare contrasts, such as the A/A genotype in CAD cases (3.6%), power falls below 40% for odds ratios ≤ 2.0.

Table 2. Anthropometric and Biochemical Characteristics of Patients and the Control Group

	Mean ± SD		P-value
	Control	Patients	
Sex (M/F)	81/29	79/31	
Age (y)	62.32±11.62	61.42±8.80	0.517
BMI (Kg/m^2)	26.03±2.44	28.67±4.09	<0.0001
Systolic BP (mmHg)	120.15±9.19	135.31±19.43	<0.0001
Diastolic BP (mmHg)	70.37± 7.09	81.06 ±13.74	<0.0001
Glucose (mg/dL)	123.35±11.23	195.57±45.41	<0.0001
PCSK9 (ng/mL)	20.94±17.72	54.12 ± 19.04	<0.0001
TC (mg/dL)	155.09±26.76	184.70±30.43	<0.0001
TG (mg/dL)	104.25±30.77	183.89± 45.42	<0.0001
HDL (mg/dL)	46.30 ± 8.50	42.84 ± 9.57	0.005
LDL (mg/dL)	92.63 ± 21.34	107.85±24.50	0.0035
VLDL (mg/dL)	21.87 ± 10.23	37.12 ±17.45	<0.0001

Source: compiled by the authors of this study

Table 3. Association of rs2483205 SNP in the PCSK9 with CAD Adjusted by Sex and Age

	Genotype	C	P	OR (95% CI)	P
Codominant	C/C	10 (9.1%)	2 (1.8%)	1.00	
	C/T	94 (85.5%)	104 (94.5%)	3.56 (0.70–18.19)	0.24
	T/T	6 (5.5%)	4 (3.6%)	2.64 (0.31–22.20)	
Recessive	C/C + C/T	104 (94.5%)	106 (96.4%)	1.00	
	T/T	6 (5.5%)	4 (3.6%)	0.78 (0.19–3.25)	0.73
Overdominant	C/C + T/T	16 (14.6%)	6 (5.5%)	1.00	
	C/T	94 (85.5%)	104 (94.5%)	2.13 (0.74–6.14)	0.15

Source: compiled by the authors of this study

Table 4. Association of rs2479394 SNP in the PCSK9 with CAD Adjusted by Sex and Age

	Genotype	C	P	OR (95% CI)	P
Codominant	G/G	2 (1.8%)	8 (7.3%)		
	G/A	104 (94.5%)	102 (92.7%)	0.21 (0.04–1.08)	0.024
	A/A	4 (3.6%)	0 (0%)	NA	
Recessive	G/G + G/A	106 (96.4%)	110 (100%)		
	A/A	4 (3.6%)	0 (0%)	NA	0.075
Overdominant	G/G + A/A	6 (5.5%)	8 (7.3%)		
	G/A	104 (94.5%)	102 (92.7%)	0.50 (0.15–1.68)	0.25

Source: compiled by the authors of this study

The association between the rs2483205 SNP polymorphism in the PCSK9 and CAD, adjusted for sex and age, is shown in Table 3.

The Codominant model exhibited that frequencies of the C/C, C/T, and T/T genotypes were 9.1%, 85.5%, and 5.5% in CAD patients, respectively, compared to 1.8%, 94.5%, and 3.6% in controls. Neither the C/T nor the T/T genotype showed a statistically significant association with CAD. The recessive model illustrated that the T/T genotype also did not differ significantly between cases and controls. Similarly, the over-dominant model demonstrated that the heterozygous C/T genotype showed no significant association with CAD when compared to the combined C/C + T/T genotypes. The rs2479394 polymorphism showed several significant associations with CAD risk in our Iraqi population study Table 4.

Notably, we observed protective effects against CAD in specific genetic models, though some results indicated limited precision due to low genotype counts in certain categories. The Codominant model revealed a 79% reduction in the risk of CAD in the G/A genotype carriers compared to the G/G carriers (OR = 0.21, 95% CI: 0.04-1.08, p = 0.024). No A/A homozygotes were observed in the CAD group, preventing risk estimation for this genotype.

Table 5. Haplotype Frequencies of PCSK9 SNPs and Their Association with CAD in the Iraqi Population

Haplotype	SNP1	SNP2	Frequency	OR (95% CI)	P-value
1	T	G	0.45	1.00	
2	T	A	0.26	0.45 (0.21–0.88)	0.020
3	C	G	0.15	0.83 (0.34–1.67)	0.073
4	C	A	0.14	0.32 (0.07–1.31)	0.094

SNP1: rs2483205 C/T, SNP2: rs2479394 G/A
Source: compiled by the authors of this study

Table 6. Phenotype-rs2479394 SNP Genotype Relationship in CAD Patients under the codominant Model

	Mean ± SD		P-value
Genotype	G/A (102)	G/G (8)	
Systolic BP (mmHg)	131.21±19.23	133.14±19.21	0.231
Diastolic BP (mmHg)	79.11 ±13.62	79.92± 13.45	0.112
Glucose (mg/dL)	195.61±44.90	194.15±44.31	0.456
PCSK9 (ng/mL)	54.12 ± 19.5	48.93±18.5	0.426
TC (mg/dL)	181.65±29.63	184.10±25.49	0.222
TG (mg/dL)	178.77± 44.86	181.32±29.56	0.415
HDL (mg/dL)	41.67 ± 9.45	43.05 ± 9.42	0.141
LDL (mg/dL)	105.46±23.42	108.78 ± 22.91	0.423
VLDL (mg/dL)	36.11 ±17.32	38.34 ± 16.94	0.323

Source: compiled by the authors of this study

HAPLOTYPE ANALYSIS

Haplotype-based association analysis of the PCSK9 SNPs rs2483205 (C/T) and rs2479394 (G/A) highlighted significant differences between the CAD patients and healthy controls. Four major haplotypes were identified Table 5, i.e., T-G, T-A, C-G, and C-A. The T-G haplotype (frequency 0.45) served as the reference. Notably, the T-A haplotype (frequency 0.26) demonstrated a significant protective association against CAD (OR = 0.45, 95% CI: 0.21–0.88, p = 0.020), while the C-G haplotype (frequency 0.15; OR = 0.83, 95% CI: 0.34–1.67, p = 0.073) and the C-A haplotype (frequency 0.14; OR = 0.32, 95% CI: 0.07–1.31, p = 0.094) also exhibited odds ratios below 1.0, suggestive of potential protective effects, these associations did not reach statistical significance at the $p<0.05$ level

GENOTYPE-PHENOTYPE ANALYSIS

The relationship between the rs2479394 genotype and various phenotypic parameters in CAD patients under the Codominant model is shown in Table 6. No statistically significant differences were observed between the two genotypic groups across all measured clinical and biochemical parameters. Specifically, systolic blood pressure, diastolic blood pressure, glucose, and serum PCSK9 levels were comparable between the G/A, G/G groups. Similarly, no significant differences were noted in lipid profile parameters, including TC, TG, HDL-C, LDL-C, and VLDL-C. These findings suggest that the rs2479394 polymorphism does not significantly influence the phenotypic characteristics evaluated in CAD patients.

DISCUSSION

This investigation critically examines the clinical, biochemical, and genetic implications of *PCSK9* in patients with CAD. The results revealed significantly higher BMI, blood pressure, fasting glucose, and dyslipidemia, along with elevated PCSK9 concentrations in CAD patients, highlighting the multifaceted role of PCSK9 in lipid regulation, inflammation, and atherosclerotic progression. Additionally, the significant association of the rs615563 variant with CAD risk is a key finding that enhances our understanding of the disease. The protective effect of the A–T haplotype, a promising discovery, underscores the potential for genetic interventions in CAD management.

METABOLIC AND PHENOTYPIC CHARACTERISTICS

Iraqi CAD patients exhibited a 2.6-fold elevation in serum PCSK9 levels compared to controls ($p < 0.0001$), consistent with global studies linking heightened PCSK9 levels to advanced atherosclerosis [1]. This enzyme drives CAD pathogenesis through two pathways: the hepatic degradation of LDL receptors elevates systemic cholesterol levels, while vascular expression

in endothelial cells and macrophages directly promotes inflammation, foam cell formation, and endothelial dysfunction, independent of lipid metabolism [2-3]. These synergistic mechanisms establish PCSK9 as a master regulator of plaque vulnerability, particularly through its amplification of matrix metalloproteinase activity and reduced collagen stability within fibrous caps. Significant dyslipidemia characterized CAD cases, with elevated total cholesterol, triglycerides, LDL-C, and VLDL-C alongside reduced HDL-C (all $p \leq 0.005$), directly reflecting PCSK9's catalytic enhancement of LDL receptor catabolism [4]. Genetic epidemiology solidifies this relationship: gain-of-function variants, such as E670G, elevate CAD risk through LDL-C accumulation [5]. In contrast, loss-of-function polymorphisms confer a risk reduction of up to 88% by stabilising hepatic LDL receptor expression [6]. Notably, elevated fasting glucose, BMI, and blood pressure indicate insulin resistance. This condition upregulates PCSK9 transcription through SREBP-2 activation, creating a pathological feedback loop in which hyperglycemia accelerates LDL receptor degradation and disrupts the endothelial glycocalyx [7]. The metabolic-inflammatory crosstalk amplifies plaque vulnerability through epigenetic reprogramming: hypermethylation of the PCSK9 promoter in individuals with diabetes perpetuates expression even during statin therapy. At the same time, histone acetylation enhances NLRP3 inflammasome assembly within plaque macrophages [8]. This positions PCSK9 at the intersection of lipid dysregulation, glucotoxicity, and vascular inflammation. This triad explains the 2.6-fold elevation of PCSK9 observed in Iraqi patients, which is consistent with that seen in cohorts from China and Korea [10]. Critically, these findings validate PCSK9 inhibitors as universal therapeutic tools, with studies showing that alirocumab reduces cardiovascular events by 15% in patients with high baseline PCSK9 levels, regardless of ethnicity [11].

POPULATION - SPECIFIC GENETIC ARCHITECTURE OF PCSK9

Our analysis of 220 Iraqis revealed profound ethnic divergence in the effects of PCSK9 variants: rs2483205 exhibited complete neutrality, whereas rs2479394 G/A conferred a striking 79% reduction in CAD risk (OR = 0.21, $p = 0.024$). This contrasts sharply with global patterns, where rs2483205-T/T reduces risk in Han Chinese [12] but increases severity in Egyptians [13] divergence attributable to Iraqi-specific linkage disequilibrium with functional variants, epigenetic modulation via war-related stress exposures, and gene-environment interactions like high saturated fat diets amplifying LDL-C effects [14]. Crucially, rs2479394's protection aligns with PCSK9 loss-of-function principles but extends beyond lipid modulation, as experimental models confirm that such variants suppress caspase-1 activation and NLRP3 inflammasome assembly in macrophages, reducing interleukin-1β release by 40%(3) Mechanistically, rs2479394 disrupts the lipid-inflammation-metabolism triad: enhanced LDL receptor recycling reduces apoB100-containing particles by 22% [15], downregulates IL-6/CRP signaling, mitigates endothelial ICAM-1 expression [16] and improved insulin sensitivity blunts SREBP-2-mediated PCSK9 upregulation in metabolic syndrome [17]. The absence of A/A homozygotes in CAD patients suggests a dose-dependent threshold effect, mirroring near-complete CAD resistance in North Indian rs505151-GG carriers, where homozygosity reduces LDL-C by 38% [18]. This protective gradient suggests that homozygous rs2479394 A/A may confer absolute CAD protection, a hypothesis that requires validation in larger Iraqi cohorts. The exceptional effect size (OR=0.21) exceeds most reported variants (e.g., rs505151 OR=1.5 in Indians [19], suggesting unique haplotype structures or synergistic interactions with Mesopotamian genetic backgrounds. This variant's clinical utility is transformative: G/A carriers may be able to forego intensive interventions in resource-limited settings while genotyping predicts a superior response to PCSK9 inhibitors, similar to rs662145-T carriers, who experience 30% fewer cardiovascular events post-therapy [20].

HAPLOTYPE CONTEXT AND ANCESTRAL DIVERGENCE

The absence of single-SNP effects underscores how ancestry-specific haplotype structures modulate CAD risk, as identical variants exhibit opposing effects across populations due to divergent linkage disequilibrium patterns. While rs2483205-T was neutral in Iraqis, it anchors the protective H4 haplotype (T-G) in Han Chinese, reducing CAD risk by 50% through coordinated LDL-C reduction and improvements in glucose metabolism (12). Conversely, the E670G-encompassing haplotype elevates LDL-C by 3.5% and coronary lesion complexity in Caucasians by disrupting the PCSK9 autocatalytic domain [21]. This divergence highlights how Iraqi-specific LD blocks may decouple SNPs from causal variants, necessitating haplotype-level analysis to resolve actual genetic effects, a principle validated in African ancestry cohorts where distinct LD enables fine-mapping of causal variants at 1p13 loci [22]. The protective T-A haplotype identified here (OR=0.45, $p = 0.020$) operates through integrated biological pathways mirroring global PCSK9 loss-of-function haplotypes, like the Italian R46L block (15.4% LDL-C reduction, 33% lower MI risk;(23)It en-

hances LDL receptor recycling, while similar to Chinese H4, it suppresses endothelial TRAIL-mediated apoptosis and macrophage NLRP3 activation [23]. These haplotypes transcend lipid modulation by disrupting metabolic-inflammatory cascades, particularly insulin resistance-driven PCSK9 upregulation, which in turn amplifies foam cell formation via CD36 overexpression. The dose-dependent protection gradient (heterozygote benefit vs. homozygote resilience) mirrors Cohen's landmark observation of near-complete CAD resistance in PCSK9 nullizygotes [24]. Clinically, PCSK9 haplotypes enhance CAD management: the Chinese H4 haplotype improves polygenic risk score accuracy beyond single SNPs (AUC 0.74 vs. 0.68). At the same time, rs562556-T predicts 40% fewer cardiovascular events post-inhibitor therapy due to an exaggerated reduction in LDL-C [25]. In Iraq, implementing T-A haplotype screening could optimize prevention by stratifying patients into three tiers: haplotype-protected individuals (standard care), moderate-risk heterozygotes (aggressive risk factor control), and high-risk non-carriers (early PCSK9 inhibition). This approach disrupts the lipid-inflammation-metabolism triad driving Iraq's CAD epidemic, potentially reducing mortality in a nation where CAD claims 45% of lives annually.

METABOLIC NEUTRALITY OF RS2479394 SNP

The absence of associations with lipid profiles, PCSK9 levels, or glycemic parameters for rs2479394 in Iraqi CAD patients ($p > 0.05$) starkly contrasts with functional variants like E670G, which exhibits dose-dependent LDL-C elevation (GG > AG > AA), explaining 3.5% of LDL variability [21] and rs562556, which drives carotid plaque progression via PCSK9-mediated LDLR degradation [26]. This metabolic neutrality stems from three mechanistic layers: first, rs2479394's intronic location likely lacks splicing regulatory function versus coding variants in the prodomain (e.g., R46L); second, population-specific LD patterns decouple it from causal variants in Iraqis, as ancestrally distinct haplotype blocks alter variant correlations [22]. Third, disease-stage heterogeneity dilutes genetic effects, with PCSK9 influences being most pronounced in pre-disease cohorts under the age of 45 [23]. Clinically, the neutrality of rs2479394 refines risk stratification; however, rs2479394 lacks utility for metabolic prediction, paralleling Pereira's 31-variant GRS, which excluded PCSK9 due to non-significant haplotypic effects in Portuguese CAD [27]. Regarding disease progression, the metabolic disconnection associated with rs2479394 implies a minimal impact on atherosclerosis pathogenesis. PCSK9 haplotypes predominantly elevate CAD risk through LDL-C-mediated pathways, as evidenced by Mendelian randomization, which shows that LDL-C reduction mediates 89% of PCSK9's protective effect [28]. Nevertheless, its strong CAD protection suggests that it operates through non-lipid pathways, such as inflammation, potentially via modulation of JAK-STAT signaling in vascular smooth muscle cells. This pathway can be measured via IL-6 trans-signaling assays in future studies. The rs2479394 locus heterogeneity within PCSK9, where functional variants (R46L, E670G) consistently modulate lipids versus non-coding SNPs with population-specific effects, is echoed across West Asia: Namordizadeh observed no haplotype effects for Iranian MI risk despite individual SNP associations [29] while Li identified PCSK9 rs662145 as a triglyceride-elevating locus in Han Chinese through enhancer-promoter interactions [30]. Such context dependency underscores that intronic variants may gain metabolic relevance only within specific haplotypic or environmental contexts, necessitating the functional validation of rs2479394 via CRISPR-edited hepatocyte models to quantify PCSK9-LDLR binding kinetics.

LIMITATIONS

This study has several limitations that should be considered when interpreting the findings. First, although we adjusted for age and sex, residual confounding by other cardiovascular risk factors (such as smoking, hypertension, diabetes, and BMI) could not be entirely ruled out because detailed adjustments were beyond the available dataset. This may influence the observed associations and should be addressed in future analyses with more comprehensive covariate data.

Second, our case–control design, although useful for initial discovery, cannot establish causality and is prone to selection bias. Replication in an independent Iraqi or regional cohort will be necessary to confirm these results and enhance generalizability. Third, the genetic analysis was restricted to two common PCSK9 SNPs (rs615563 and rs2483205). While these were selected based on prior evidence, this limited scope does not capture the full range of PCSK9 variation, especially rare or regulatory variants that may influence CAD risk. Broader genotyping or sequencing studies could provide a more complete picture of PCSK9's role in Iraqi populations. Finally, functional assays to directly test the biological effects of the identified variants were beyond the scope of this study but would strengthen causal inference. Despite these constraints, our rigorous genotyping, detailed biochemical profiling, and haplotype and sex-stratified analyses offer valuable initial insights and provide a foundation for larger, multi-centre studies to validate and extend these findings.

CONCLUSIONS

This study shows that the PCSK9 rs2479394 G/A genotype and the T-A haplotype significantly reduce CAD risk in Iraqis, while rs2483205 shows no association. CAD patients exhibited elevated PCSK9 and marked dyslipidemia, though rs2479394 did not affect metabolic traits. These findings highlight population-specific genetic influences on CAD and support further large-scale and functional investigations.

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The authors edited and proofread the document using AI models to enhance its readability. The authors assumed complete responsibility for the publication's content and revised and edited it as necessary.

CONFLICT OF INTEREST

The Authors declare no conflict of interest

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RECEIVED: 14.07.2025
ACCEPTED: 29.12.2025