Article

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Diversity of the Duffy Blood Group Gene (DARC/ACKR1) and Its Association With the Risk of Developing Coronary Artery Disease

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Background: The Duffy Antigen Receptor for Chemokines (DARC/ACKR1) regulates inflammatory chemokine levels. The functional ACKR1 rs12075 G>A (p.Gly42Asp) polymorphism influences this activity and varies across populations, but studies on its association with human diseases such as coronary artery disease (CAD) are limited. The purpose of this study was to investigate the potential genetic link between this specific variation in the Duffy blood group gene ACKR1 and the risk of developing CAD within a Saudi Arabian population cohort.

Methods: A case-control study of 100 confirmed CAD patients and 100 matched healthy controls was conducted. Genomic DNA was extracted from peripheral blood and genotyped for the ACKR1 rs12075 variant using the Amplification Refractory Mutation System PCR (ARMS-PCR). Allelic and genotypic frequencies were compared using Chi-square tests, and associations with CAD risk were estimated via logistic regression, calculating odds ratios (ORs) with 95% confidence intervals (CI).

Results: The genotype distribution differed significantly between cases and controls ($p = 0.0004$). The AA genotype frequency was markedly higher in CAD patients (15%) versus controls (2%). In a recessive inheritance model, the AA genotype conferred a significantly increased risk of CAD (OR = 8.64, 95% CI: 1.92–38.90, $p = 0.004$). The GA genotype was also associated with elevated risk (OR = 2.06, 95% CI: 1.04–4.11, $p = 0.037$). Furthermore, the AA and GA genotypes showed significant associations with key CAD comorbidities, including hypertension, hyperlipidemia, diabetes mellitus, and a history of myocardial infarction ($p < 0.05$).

Conclusion: The ACKR1 rs12075 A allele, particularly in the homozygous state, is strongly associated with an increased risk of CAD in a Saudi Arabian cohort and is linked to a more severe clinical phenotype. These findings suggest that this genetic variant may serve as a potential biomarker for CAD susceptibility and severity in this population.

Keywords: DARC; ACKR1 polymorphism; chemokines; coronary artery disease; inflammation

Introduction

Coronary artery disease (CAD) is the leading single cause of mortality and disability globally, accounting for nearly 7 million deaths and 129 million disability-adjusted life years annually [1]. In 2022, 315 million cases of CAD were prevalent globally [2]. Complex interactions between genetic and environmental risk factors underlie the disease, and both modifiable and non-modifiable determinants play a crucial role in its development. Epidemiological studies, such as the seminal Framingham Heart Study, have provided a foundational understanding of CAD risk factors [3] and identified critical contributors, including cigarette smoking, hypertension, elevated cholesterol, and diabetes mellitus. Contemporary genome-wide association studies have identified over 200 genetic loci linked to CAD [4], but these variants account for only a fraction of disease heritability, emphasizing that disease susceptibility is polygenic. The genetic architecture of CAD indicates that inherited predisposition significantly influences individual risk profiles [5], and family history is a key risk factor, especially in younger patients. In addition, lifestyle factors such as smoking exacerbate disease risk, with genetic predisposition and smoking exposure interacting synergistically [6]. Consequently, individuals with high genetic risk scores experience disproportionately elevated CAD risk from smoking exposure. Conventional risk factors, including dyslipidemia, metabolic syndrome, are significant contributors to the overall disease burden [7]. Single-nucleotide polymorphisms (SNPs) are fundamental genetic variants that serve as critical biomarkers for identifying individuals at increased risk of CAD and for predicting clinical outcomes [8]. When SNPs are integrated into weighted genetic risk scores (wGRS), it substantially improves risk stratification,

establishing that patients with a high-burden wGRS experience approximately two-fold greater risk of major adverse cardiovascular events than those with low wGRS [9]. The functional effects of these variants extend beyond risk prediction to include sex-specific effects and interactions with environmental factors. For example, SNP-based genotype–phenotype correlations reveal that certain variants modulate abdominal fat distribution and lipid profiles, partially mediating CAD risk [10].

Duffy Antigen Receptor for Chemokines (DARC)

The DARC, also known as atypical chemokine receptor 1 (ACKR1), is expressed as distinct transcript isoforms and plays a crucial role in regulating circulating levels of proinflammatory chemokines [11]. Its product, the Duffy antigen, is a transmembrane protein expressed on erythrocytes and endothelial cells. This antigen functions as a "decoy receptor" by binding and internalizing several chemokines, thereby modulating immune responses and inflammation. The varying combinations of isoforms and alleles produce the diverse range of Duffy antigens observed across populations and likely influence disease risk and susceptibility [12]. Chemokines are also key mediators of chronic inflammatory damage in cardiovascular diseases such as atherosclerosis, with their concentrations, combinations, and oligomerization contributing to the initiation and progression of vascular lesions [13]. This study investigates the influence of the ACKR1 rs12075 G>A variant on CAD risk and susceptibility in a cohort of patients with CAD from a Saudi Arabian ethnic population.

Methods

Study Population

The study was conducted at the Prince Fahd Bin Sultan Research Chair for Biomedical Research at the University of Tabuk. The subjects were recruited from the hematology OPD at King Fahad Special Hospital, Tabuk, and all subjects completed a consent form before sample collection. The study was conducted in accordance with the guidelines outlined in the Declaration of Helsinki and was approved by the University of Tabuk Research Ethics Committee (Registration Number UT-91-23-2020). A total of 100 patients with confirmed CAD and 100 matched healthy controls were enrolled in this study. All the participants signed the consent form.

Inclusion Criteria of CAD Patients

Patients with new-onset acute chest pain undergoing coronary angiography were selected for this study. Based on their coronary angiographic findings, subjects classified as significant CAD (stenosis ≥50%) were included. The following tests were conducted,

- X-rays, exercise stress,
- myocardial perfusion imaging,
- ambulatory electrocardiography,
- Holter monitoring,
- chest echocardiogram,
- computerized tomography coronary angiography, and
- multigated acquisition scans (MUGA).

Exclusion Criteria for Patients

Patients with a history of non-coronary cardiac disorders, percutaneous transluminal coronary angioplasty (PTCA), or previously performed coronary bypass surgery were excluded from this study since their coronary conditions had been previously treated.

Inclusion Criteria for Controls

Healthy controls were selected from among the individuals attending the hospital for a routine checkup. The healthy controls selected are required to be devoid of a previous history of heart attack or angina. Some blood biochemistry analyses were performed on the healthy controls.

Sample Collection

Three milliliters of venous blood were aseptically extracted from individuals who had given their consent. Thereafter, 2 mL of the blood sample was collected EDTA tube for DNA extraction, 1 mL of blood sample was kept in an EDTA vial for analyses of serum markers.

Genomic DNA Extraction

In this study, venipuncture was used to obtain a 3 ml sample of peripheral blood in EDTA tubes from CAD patients and healthy donor subjects. Using the DNeasy Blood Kit (Qiagen, Germany), genomic DNA was extracted in accordance with the vendor's instructions. The extracted DNA was dissolved in 200 µL of TE buffer. DNA quality was examined using 1% gel electrophoresis and purity was checked by NanoDrop™ (Thermo Scientific, USA).

Genotyping of ACKR1 rs12075 G>A

ACKR1 rs12075 G>A genotyping was done by optimizing ARMS-PCR. Primers to assess ACKR1 rs12075 G>A genotyping were designed using Primer1 Software (https://primer1.soton.ac.uk/primer1.html), as depicted in Table 1. A reaction volume of 15 µL was used for the ARMS-PCR. It contained 60 ng of template DNA, 7 µL of GoTaq® Green Master Mix (cat no. M7122) from Promega, USA, Fo-0.12 µL, Ro-0.12 µL, FI-0.12 µL, and RI-0.12 µL of each primer.

Thermocycling Conditions

An ideal annealing temperature was determined with the fewest possible steps by using gradient PCR method. It is frequently possible to accomplish this optimization in a

Table 1. ARMS-PCR primers for ACKR1 rs12075 G>A genotyping.

Gene	Sequence	PCR product size
ACKR1FO	5'-GAGACCTTGTTCTCCCACCCGACCTT-3'	370 bp
ACKR1RO	5'-AGCTGCCAGCGGAAGAGAGGTCTGAAAA-3'	
ACKR1-GFI	5'-GAATGATTCCTTCCCAGATGGAGACTAGGG-3'	180 bp
ACKR1-ARI	5'-GGGGCAGCTGCTTCCAGGTTGGAAT-3'	244 bp

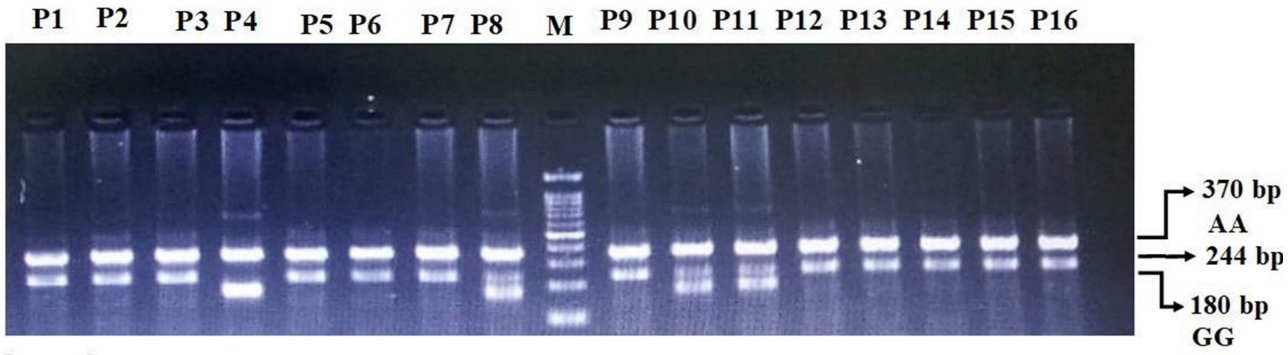

Fig. 1. PCR amplification of ACKR1 rs12075 A>G polymorphism by ARMS-PCR in CAD patients. M-100 bp DNA ladder, Heterozygous G/A: P8, P10 and P11; Homozygous AA: P1, P2, P3, P5,P6, P7, P9, P12, P13, P14, P15 and P16; Homozygous GG: P4

single trial. The best temperature was determined to be 60 °C for ACKR1 rs12075 G>A using a gradient PCR thermocycler and testing in the 55 °C to 64 °C temperature range. The yields of all three PCR products were greatly boosted when the number of cycles was raised from 35 to 45. The relative strengths of the relevant bands on agarose gel electrophoresis demonstrated that these modifications together produced a more robust amplification of the mutant allele and a less competitive response from the control.

The cycling conditions were as follows: a 7-minute hot start at 95 °C; 30 amplification cycles at 95 °C for 30 seconds; 35 seconds at a variable annealing temperature (Table 1); 40 seconds at 72 °C; 8 minutes at 72 °C for one elongation step; and 4 °C for storage. The ACKR1 outer region is amplified by the outer primers FO/RO, yielding a band of 370 bp that serves as a DNA control check for PCR. A band of 244 bp is produced by primers FO/RI amplifying the A allele and a band of 180 bp is produced by primers FI/RO amplifying the G allele (Fig. 1).

Statistical Analysis

The frequency of Duffy alleles was examined using the odds ratio approach. Additionally, two-tailed Fisher-Exact or Chi-Squared tests were employed. The association of ACKR1 rs12075 G>A gene variations were estimated by odds ratios with 95% confidence intervals. Graph Pad version 8.0 (GraphPad Software, San Diego, CA, USA) and SPSS version 25 (IBM Corp, Armonk, New York, USA) software's were used for statistical analyses and a *p*-value of less than 0.05 was considered statistically significant.

Results

Comparative Analysis of Patients With CAD and Healthy Controls: Demographic and Clinical Profiles

A total of 200 participants were enrolled in the study: 100 with confirmed CAD and an equal number of matched healthy controls, selected according to specific inclusion and exclusion criteria. The demographic and clinical characteristics of both groups are summarized in Table 2. The CAD group was predominantly male (80%), with a mean age of 55.1 years (range 26–87). The control group was also largely male (83%) but considerably younger, with a mean age of 37.5 years (range 18–55). Several cardiovascular risk factors were prevalent in patients with CAD: 44% were smokers, 41% had hypertension, 27% had hyperlipidemia, 16% were classified as obese, and 34% had diabetes mellitus. In terms of clinical presentation, the majority (88%) had stable angina, whereas 12% had unstable angina. A history of myocardial infarction was reported in 30% of patients with CAD, including 18% with ST-elevation myocardial infarction (STEMI) and 12% with non-ST-elevation myocardial infarction (NSTEMI). In addition, 24% of patients with CAD reported a positive family history of cardiovascular disease. These findings present a clear profile of the CAD cohort: older and carrying a higher burden of modifiable and non-modifiable risk factors compared with the younger, healthier control group. The data demonstrate associations among advancing age, cardiometabolic risk factors, and the presence of CAD.

Table 2. General characteristics of study population.

Characteristics		CAD cases (%)	Healthy controls (%)
Study population		100 (100%)	100 (100%)
Gender	Males	80 (80%)	83 (83%)
	Females	20 (20%)	17 (17%)
Age (Years)	Mean (Range)	55.1 (26–87)	37.5 (18–55)
Age group	≤55 years	57 (57%)	97 (97%)
	>55 years	43 (43%)	3 (3%)
Smoking status	Non-smoker	56 (56%)	70 (70%)
	Smoker	44 (44%)	30 (30%)
Hypertension	No	59 (59%)	89 (80%)
	Yes	41 (41%)	11 (11%)
Hyperlipidemia	No	73 (73%)	95 (95%)
	Yes	27 (27%)	5 (5%)
Obesity	No	84 (84%)	
	Yes	16 (16%)	
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%)	

Table 3. Distribution of Allele and genotype frequencies of ACKR1 (rs12075 125G>A) p.Gly42Asp in CAD and healthy controls.

ACKR1	GG	GA	AA	G	A	χ^2	*p*-value
rs12075 G>A	Gly42 Fy(a+)	Gly42Asp Fy(a+b)	Asp42 Fy(b+)	Asp42 FY*01	Gly42 FY*02		
CAD cases	58 (58%)	27 (27%)	15 (15%)	(0.72)	(0.28)	15.85	0.0004
Controls	80 (80%)	18 (18%)	2 (2%)	(0.89)	(0.11)		

Distribution of Allele and Genotype Frequencies of ACKR1 (rs12075 125 G>A) p.Gly42Asp in Patients With CAD and Healthy Controls

As reported in Table 3, the GG, GA, and AA genotype frequencies were 58%, 27%, and 15%, respectively, in patients with CAD, whereas in healthy controls, they were 80%, 18%, and 2%, respectively. The distribution of ACKR1 (rs12075 125G>A) p.Gly42Asp genotypes in patients with CAD and controls was significantly different (p = 0.0004). Moreover, the frequency of *FY*01* A allele (fA) was higher in patients with CAD than in controls (0.28 vs. 0.11). In contrast, the frequency of the G allele (fG) was lower in patients with CAD than in controls (0.89 vs. 0.72), as presented in Table 3.

Multivariate Analysis to Estimate the Association of ACKR1 (rs12075 125G>A) p.Gly42Asp Genotypes With the Risk of Disease Development

Statistical analyses based on logistic regression, including odds ratio (OR) and risk ratio (RR) with 95% confidence intervals (CIs), were performed using multivariate analysis for each group to estimate the association between ACKR1 (rs12075 125G>A) p.Gly42Asp genotypes and the risk of developing CAD (Table 4). Codominant inheritance model: The findings demonstrated that in the codominant model, the heterozygous ACKR1-GA genotype was strongly associated with CAD susceptibility, with OR = 2.06 (95% CI = 1.04–4.11), RR = 1.44 (0.99–2.13), and p = 0.037. Similarly, the ACKR1-AA genotype was strongly linked to CAD susceptibility [OR = 10.3 (95% CI = 2.28–47.00), RR = 4.92 (1.33–18.25), and p = 0.002]. Recessive inheritance model: A significant correlation was observed between the ACKR1 (GA + GG) vs. ACKR1-AA genotypes and CAD susceptibility, with OR = 8.64 (95% CI: 1.92–38.90), RR = 4.55 (1.23–16.85), and p = 0.004 in the recessive model. Additive inheritance model [Allele]: The results also showed that in the allelic comparison, the A allele was not associated with CAD susceptibility, with OR = 1.00, 95% CI = (0.68–1.48), RR = 1.00 (0.82–1.22), and p = 1.000. Over-dominant inheritance model: No significant

Table 4. Logistic regression to estimate the association of ACKR1 (rs12075 125 A>G) gene polymorphism with the risk of CAD susceptibility.

Genotypes	Controls (100)	Cases (100)	Odd ratio (OR) (95% CI)	Risk ratio (RR) (95% CI)	*p*-value
Codominant inheritance model					
ACKR1-GG	80	58	(1 (ref.))	(1 (ref.))	
ACKR1-GA	18	27	2.06 (1.04 to 4.11)	1.44 (0.99 to 2.13)	0.037
ACKR1-AA	2	15	10.30 (2.28 to 47.00)	4.92 (1.33 to 18.25)	0.002
Dominant inheritance model					
ACKR1-GG	80	58	(1 (ref.))	(1 (ref.))	
ACKR1 (GA + AA)	20	42	2.89 (1.54 to 5.44)	1.79 (1.22 to 2.65)	0.0009
Recessive inheritance model					
ACKR1 (GA + GG)	98	85	(1 (ref.))	(1 (ref.))	
ACKR1-AA	2	15	8.64 (1.92 to 38.90)	4.55 (1.23 to 16.85)	0.004
Allele					
ACKR1-G	100	100	(1 (ref.))	(1 (ref.))	
ACKR1-A	100	100	1.00 (0.68 to 1.48)	1.00 (0.82 to 1.21)	1.000
Ove dominant model					
ACKR1 (GG + AA)	82	73	(1 (ref.))	(1 (ref.))	
ACKR1 (GA)	18	27	1.68 (0.86 to 3.31)	1.32 (0.90 to 1.95)	0.129

p-values > 0.05 were considered significant.

association was reported between the ACKR1 (GG + AA) vs. ACKR1-GA genotypes and CAD susceptibility, with OR = 1.68 (95% CI: 0.86–3.31), RR = 1.32 (0.90–1.95), and $p = 0.129$ in the recessive model.

Association of ACKR1 (rs12075 125 G>A) Genotypes With Clinical Characteristics of Patients With CAD

Abdominal obesity, high blood pressure, high triglycerides, low high-density lipoprotein cholesterol, and high blood sugar (insulin resistance) are the cardiometabolic risk factors that increase the risk of type 2 diabetes, heart disease, and stroke. These conditions and behaviors are often caused by poor diet, sedentary lifestyle, smoking, stress, and, in some cases, genetics or ethnicity, all of which cause inflammation and vascular damage.

Association of ACKR1-rs12075 G>A Genotypes With Sex

The distribution of ACKR1-rs12075 G>A genotypes between males and females in patients with CAD was not statistically significant ($p = 0.289$). However, heterozygosity (Fy(a+b) GA genotype) was more prevalent in female patients with CAD than in males (30.00% vs. 26.25%). Similarly, the frequency of the polymorphic genotype AA (Fy(b+)) was more prevalent among females than the males (25.00% vs. 12.25%) (Table 5).

Association of ACKR1-rs12075 Genotypes With Age and CAD Patients

As shown in Table 5 and Fig. 2, there was a significant association between the ACKR1-rs12075 G>A genotype and age in patients with CAD ($p = 0.042$). However, the heterozygous Fy(a+b) GA genotype was more prevalent in older patients with CAD than in younger patients (30.2% vs. 24.6%). The polymorphic AA (Fy(b+) genotype was more frequent in younger patients with CAD than in older patients (4.7% vs. 22.8%) (Fig. 2).

Association of ACKR1-rs12075 Genotypes With Hyperlipidemia and CAD

High blood lipid levels, such as cholesterol and triglycerides, are known as hyperlipidemia. This widespread disorder, which is sometimes asymptomatic, is a significant risk factor for heart attack, stroke, and arterial disease owing to plaque accumulation. Statistical analysis showed a significant association between ACKR1-rs12075 genotypes and hyperlipidemia in patients with CAD ($p = 0.015$). Furthermore, the heterozygous Fy(a+b) GA genotype was more prevalent among patients with hyperlipidemia than among those with lower lipid levels (48.14% vs. 19.17%). However, the frequency of the polymorphic genotype AA (Fy(b+)) was more prevalent in non-hyperlipidemia group (16.43% vs. 11.11%).

Association of ACKR1-rs12075 Genotypes With Smoking

No association was found between ACKR1-rs12075 genotypes and smoking or CAD risk.

Association of ACKR1 (rs12075 125 G>A) Genotypes With CAD and Hypertension

A strong association was reported for ACKR1-rs12075 genotypes with hypertensive cases and CAD susceptibility ($p = 0.006$) (Table 5). Also, the heterozygous

Table 5. Distribution of ACKR1-rs12075 G>A genotypes in association with the clinical characteristics of coronary artery disease patients.

Clinical features	Variables	N =	GG	GA	AA	χ^2	*p*-value
			(58)	(27)	(15)		
Gender	Male	80	49 (61.25%)	21 (26.25%)	10 (12.25%)	2.15	0.289
	Female	20	9 (45.00%)	6 (30.00%)	5 (25.00%)		
Age group	≤55	57	30 (52.63%)	14 (24.56%)	13 (22.80%)	6.34	0.042
	>55	43	28 (65.11%)	13 (30.23%)	2 (4.66%)		
Hyperlipidemia	No	73	47 (64.38%)	14 (19.17%)	12 (16.43%)	8.4	0.015
	Yes	27	11 (40.74%)	13 (48.14%)	3 (11.11%)		
Smoking status	No	56	33 (58.92%)	13 (23.21%)	10 (17.85%)	0.673	0.714
	yes	44	25 (56.81%)	14 (31.81%)	5 (11.36%)		
Hypertension	No	59	40 (67.79%)	9 (15.25%)	10 (16.94%)	10.1	0.006
	Yes	41	18 (43.90%)	18 (43.90%)	5 (12.19%)		
Obesity	No	84	50 (59.52%)	22 (26.19%)	12 (14.28%)	0.52	0.771
	Yes	16	8 (50.00%)	5 (31.25%)	3 (18.75%)		
Diabetes mellitus	No	66	44 (66.66%)	12 (18.18%)	10 (15.51%)	8.11	0.017
	Yes	34	14 (41.17%)	15 (44.11%)	5 (14.70%)		
Angina	Stable	88	52 (59.09%)	23 (26.13%)	13 (14.77%)	0.38	0.827
	Unstable	12	6 (50.00%)	4 (33.33%)	2 (16.66%)		
Myocardial infarction (MI)	No	70	44 (62.85%)	23 (32.85%)	3 (4.28%)	21.77	0.0001
	Stemi	30	14 (46.66%)	4 (13.33%)	12 (40.00%)		
Familial history	No	76	43 (56.57%)	21 (27.63%)	12 (15.78%)	0.29	0.865
	Yes	24	15 (62.50%)	6 (25.00%)	3 (12.50%)		

ST-elevation MI (STEMI) or a non-ST elevation MI (NSTEMI).

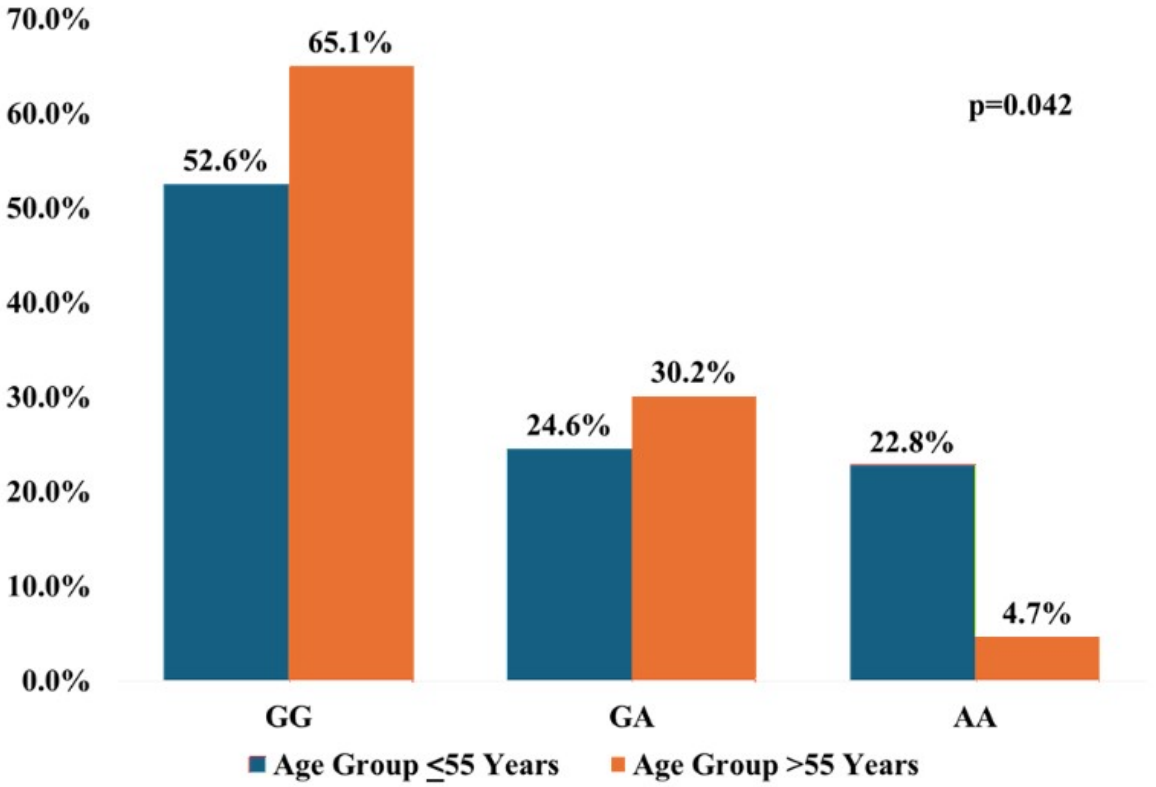

Fig. 2. Association between the ACKR1-rs12075 G>A genotypes and age in patients with CAD patients.

Fy(a+b) GA genotype was more prevalent among patients with hypertension than among those with normal blood pressure (43.90% vs. 15.25%). However, the frequency of the polymorphic genotype AA (Fy(b+)) was less prevalent in the hypertensive group than in the non-hypertensive group (12.19 vs 16.94%).

Association of ACKR1 (rs12075 125 G>A) Genotypes With Obesity

A non-significant association was reported between the ACKR1-rs12075 125 G>A genotypes and obesity and CAD susceptibility (p = 0.771). The GA heterozygosity (Fy(a+b)) was more prevalent in patients with CAD who had obesity than in those who did not have obesity (31.25% vs. 26.19) (Table 5).

Association of ACKR1 (rs12075 125 G>A) Genotypes With Myocardial Infarction

A strong association was reported between the ACKR1-rs12075 125 G>A genotypes and myocardial infarction and CAD susceptibility (p = 0.0001). The GA heterozygosity (Fy(a+b)) was more frequent among patients with CAD and STEMI than among those with CAD and NSTEMI (32.85% vs. 13.33%). Moreover, the polymorphic genotype (AA) (Fy(b+)) was more frequent in patients with NSTEMI than in those with STEMI (40.00% vs. 4.28%) (Table 5).

Association of ACKR1 (rs12075 125 G>A) Genotypes With Diabetes

A strong association was reported between ACKR1-rs12075 125 G>A genotypes and diabetes and CAD susceptibility (p = 0.017). The GA heterozygosity (Fy(a+b)) was more prevalent in patients with CAD and type 2 diabetes mellitus (T2D) than in those with CAD without T2D (44.11% vs. 18.18%) (Table 5).

Discussion

CAD, a complex multifactorial disorder, is the leading cause of global mortality. Although conventional risk factors such as smoking, hypertension, and dyslipidemia are well established, a considerable portion of individual risk results from genetic predisposition. Although genome-wide association studies have identified over 200 loci linked to CAD, a large fraction of heritability remains unexplained, highlighting the need to investigate specific candidate genes within defined populations and their interactions with environmental factors [14]. This case–control study examined the association between the ACKR1 rs12075 G>A (p.Gly42Asp) polymorphism, which encodes the Duffy antigen/chemokine receptor, and susceptibility to CAD in a Saudi Arabian cohort. The observations indicated a significant divergence in genotype distribution and showed that specific ACKR1 genotypes may considerably increase the risk of CAD, particularly when co-occurring with conventional cardiometabolic risk factors.

The ACKR1 gene, located on chromosome 1q23.2, encodes DARC, which is also classified as an ACKR1. This receptor is uniquely expressed on red blood cells and endothelial cells, particularly on postcapillary venules [15]. Its primary physiological role is as a “decoy” or “silent” receptor, binding proinflammatory chemokines of both the CC (e.g., CCL2, CCL5) and CXC (e.g., CXCL8) families with high affinity but does not signal via G-proteins to induce leukocyte migration. Instead, DARC internalizes and clears these chemokines from the circulation, acting as a biological sink that regulates their plasma concentrations and inflammatory gradients [16]. The rs12075 SNP is a missense variant (c.125 G>A) that results in a glycine-to-aspartic acid substitution at position 42 (Gly42Asp) of the protein. This change determines the serological Duffy blood group antigens: the A allele encodes the Fy(b) antigen (Asp42), whereas the G allele encodes the Fy(a) antigen (Gly42). The Fy(a-b-) null phenotype, common in populations of African descent and conferring resistance to *Plasmodium vivax* malaria, arises from a separate promoter mutation (T-33C) that disrupts erythroid expression but preserves endothelial expression [17].

The fundamental role of inflammation in atherosclerosis, the pathological basis of CAD, is well established [18]. Chemokines, crucial regulators of leukocyte trafficking, are central mediators of this chronic inflammatory process within the vascular wall [19]. This polymorphism has profound functional effects as DARC is a critical regulator of chemokine bioavailability and inflammatory homeostasis. Evidence suggests that this polymorphism influences receptor expression levels, chemokine-binding kinetics, or intracellular trafficking, thus altering the efficiency of chemokine clearance [20]. Given the central role of chemokines such as CCL2 (MCP-1) and CXCL8 (IL-8) in monocyte recruitment and neutrophil activation within atherosclerotic plaques, any variation affecting DARC’s scavenging capacity may directly influence vascular inflammation and CAD risk.

The protective GG genotype was significantly more frequent in controls (80%) than in cases (58%), whereas the high-risk AA genotype was rare in controls (2%) but more common in cases (15%). This distribution led to a notable deviation from the Hardy–Weinberg equilibrium in the case group (p = 0.0004), supporting the association between the A allele and disease. Multivariate logistic regression across various genetic models clarified the risk profile. The recessive model (GA+GG vs. AA) showed a strong OR of 8.64 (p = 0.004), highlighting homozygosity for A as a significant risk factor. The dominant model (GG vs. GA+AA) also indicated a notable association (OR = 2.89, p = 0.0009), meaning that even one A allele increases the risk of CAD. Conversely, the additive model was not significant, suggesting that the association is not purely dose-dependent but may involve threshold effects or interactions, especially for homozygotes. The absence of

significance in the over-dominant model suggests that the risk is driven by the presence of the A allele itself rather than heterozygote effects. The most meaningful findings involve the links between ACKR1 genotypes and specific CAD phenotypes and comorbidities. A strong association with hypertension ($p = 0.006$) is mechanistically plausible, as chemokines such as CCL2 are involved in vascular remodeling, monocyte infiltration, and oxidative stress, all pathways implicated in hypertension [21]. Reduced clearance of such chemokines in FY*A carriers could worsen these processes. Similarly, associations with hyperlipidemia ($p = 0.034$), and diabetes ($p = 0.017$) suggest a link between Duffy antigen function and metabolic inflammation. Adipose tissue produces numerous chemokines, and aberrant chemokine signaling is a key contributor to insulin resistance and dyslipidemia [22]. Therefore, the ACKR1 polymorphism might influence CAD risk directly via vascular inflammation and indirectly by affecting systemic inflammation from metabolic disorders. The strong association with a history of myocardial infarction ($p = 0.0001$) is especially crucial. The genotype distribution differed notably between patients with CAD with and without MI; the AA genotype was very rare (4.28%) in those without MI but was present in 40% of MI cases. Hence, the ACKR1 AA genotype may not only promote atherosclerosis but also increase plaque vulnerability and rupture, leading to acute coronary events. Chemokines play a key role in recruiting inflammatory cells into unstable plaques and encouraging intraplaque neovascularization and hemorrhage [18]. The FY*A antigen binds certain chemokines, including CCL2 (MCP-1), less efficiently than FY*B. Thus, individuals with the FY*A variant (GA or AA) may have less effective chemokine clearance, resulting in higher persistent levels of inflammatory mediators in the blood and vascular tissues [22]. Impaired clearance in AA homozygotes could create a local environment that accelerates destabilization processes.

Conclusion

This study provides robust evidence that the ACKR1 rs12075 G>A (p.Gly42Asp) polymorphism is significantly associated with an increased risk of CAD in a Saudi Arabian cohort. The FY*A allele, particularly in the homozygous state, appears to be a strong genetic risk factor, potentially acting via mechanisms that impair regulation of proatherogenic chemokines. Furthermore, this variant shows compelling associations with key clinical features of CAD, including hypertension, metabolic comorbidities, and a history of myocardial infarction, suggesting its role in disease severity and progression. These findings contribute to the growing understanding of the genetic underpinnings of CAD and underscore the importance of population-specific genetic studies.

Limitations of the Study

While this research provides novel and valuable insights into the association between the ACKR1 rs12075 variant and CAD risk, some limitations should be acknowledged. First, the relatively small sample size of 200 participants, while adequate for an initial investigation, may limit the statistical power. Replication in a larger, independent cohort is required to confirm and generalize these findings. Second, this was a single-center study conducted within a specific Saudi Arabian population. The genetic architecture and disease risk factors can vary across different ethnic and geographical groups. Therefore, the observed associations may not be directly applicable to other populations, highlighting the need for multi-center, multi-ethnic studies. Finally, this study was restricted to genetic association analysis and did not include the measurement of functional biochemical indicators, such as circulating chemokine levels. Although the rs12075 polymorphism is known to be functional, directly correlating genotype data with phenotypic chemokine activity and inflammatory status in the study participants would strengthen the mechanistic link between the genetic variant and the observed increase in CAD risk.

Availability of Data and Materials

We have included the data associated with the study in the manuscript. In case of specific queries, the corresponding authors can be contacted.

Author Contributions

Conceptulization: MAA, RM; Methodology: RM; Data analysis: RM, JJ; Data curation and interpretation: JJ, MMJ, MFU, AY; Manuscript writing,reviewing and editing: MAA, RM, MMJ, JJ, MFU, AY; Tables and Figures preparation: RM, JJ. 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 has been approved by the University of Tabuk Research Ethics Committee (Registration Number UT-91-23-2020). The study was conducted in accordance with the Declaration of Helsinki. All the participants signed the consent form.

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Conflict of Interest

The authors declare no conflict of interest.

References

[1] Ralapanawa U, Sivakanesan R. Epidemiology and the Magnitude of Coronary Artery Disease and Acute Coronary Syndrome: A Narrative Review. Journal of Epidemiology and Global Health. 2021; 11: 169–177. https://doi.org/10.2991/jegh.k.201217.001.

[2] Stark B, Johnson C, Roth G. Global Prevalence of Coronary Artery Disease: An Update From The Global Burden of Disease Study. Journal of the American College of Cardiology. 2024; 83 (13_Supplement): 2320. https://doi.org/10.1016/S0735-1097(24)04310-9.

[3] KANNEL WB, DAWBER TR, KAGAN A, REVOTSKIE N, STOKES J, 3rd. Factors of risk in the development of coronary heart disease–six year follow-up experience. The Framingham Study. Annals of Internal Medicine. 1961; 55: 33–50. https://doi.org/10.7326/0003-4819-55-1-33.

[4] Turner AW, Hu SS, Mosquera JV, Ma WF, Hodonsky CJ, Wong D, *et al*. Single-nucleus chromatin accessibility profiling highlights regulatory mechanisms of coronary artery disease risk. Nature Genetics. 2022; 54: 804–816. https://doi.org/10.1038/s41588-022-01069-0.

[5] Di Lenarda F, Balestrucci A, Terzi R, Lopes P, Ciliberti G, Marchetti D, *et al*. Coronary Artery Disease, Family History, and Screening Perspectives: An Up-to-Date Review. Journal of Clinical Medicine. 2024; 13: 5833. https://doi.org/10.3390/jcm13195833.

[6] Huang Y, Hui Q, Gwinn M, Hu YJ, Quyyumi AA, Vaccarino V, *et al*. Interaction between genetics and smoking in determining risk of coronary artery diseases. Genetic Epidemiology. 2022; 46: 199–212. https://doi.org/10.1002/gepi.22446.

[7] Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, *et al*. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. Circulation. 2005; 112: 2735–2752. https://doi.org/10.1161/CIRCULATIONAHA.105.169404.

[8] Sitinjak BDP, Murdaya N, Rachman TA, Zakiyah N, Barliana MI. The Potential of Single Nucleotide Polymorphisms (SNPs) as Biomarkers and Their Association with the Increased Risk of Coronary Heart Disease: A Systematic Review. Vascular Health and Risk Management. 2023; 19: 289–301. https://doi.org/10.2147/VHRM.S405039.

[9] Zhao C, Zhu P, Shen Q, Jin L. Prospective association of a genetic risk score with major adverse cardiovascular events in patients with coronary artery disease. Medicine. 2017; 96: e9473. https://doi.org/10.1097/MD.0000000000009473.

[10] Wang XB, Han YD, Zhang S, Cui NH, Liu ZJ, Huang ZL, *et al*. Associations of polymorphisms in TXNIP and gene-environment interactions with the risk of coronary artery disease in a Chinese Han population. Journal of Cellular and Molecular Medicine. 2016; 20: 2362–2373. https://doi.org/10.1111/jcmm.12929.

[11] Torphy RJ, Yee EJ, Schulick RD, Zhu Y. Atypical chemokine receptors: emerging therapeutic targets in cancer. Trends in Pharmacological Sciences. 2022; 43: 1085–1097. https://doi.org/10.1016/j.tips.2022.09.009.

[12] Crawford KS, Volkman BF. Prospects for targeting ACKR1 in cancer and other diseases. Frontiers in Immunology. 2023; 14: 1111960. https://doi.org/10.3389/fimmu.2023.1111960.

[13] Apostolakis S, Chalikias GK, Tziakas DN, Konstantinides S. Erythrocyte Duffy antigen receptor for chemokines (DARC): diagnostic and therapeutic implications in atherosclerotic cardiovascular disease. Acta Pharmacologica Sinica. 2011; 32: 417–424. https://doi.org/10.1038/aps.2011.13.

[14] Khera AV, Kathiresan S. Genetics of coronary artery disease: discovery, biology and clinical translation. Nature Reviews. Genetics. 2017; 18: 331–344. https://doi.org/10.1038/nrg.2016.160.

[15] Pruenster M, Mudde L, Bombosi P, Dimitrova S, Zsak M, Middleton J, *et al*. The Duffy antigen receptor for chemokines transports chemokines and supports their promigratory activity. Nature Immunology. 2009; 10: 101–108. https://doi.org/10.1038/ni.1675.

[16] Rot A. Contribution of Duffy antigen to chemokine function. Cytokine & Growth Factor Reviews. 2005; 16: 687–694. https://doi.org/10.1016/j.cytogfr.2005.05.011.

[17] Tournamille C, Colin Y, Cartron JP, Le Van Kim C. Disruption of a GATA motif in the Duffy gene promoter abolishes erythroid gene expression in Duffy-negative individuals. Nature Genetics. 1995; 10: 224–228. https://doi.org/10.1038/ng0695-224.

[18] Ajoolabady A, Pratico D, Lin L, Mantzoros CS, Bahijri S, Tuomilehto J, *et al*. Inflammation in atherosclerosis: pathophysiology and mechanisms. Cell Death & Disease. 2024; 15: 817. https://doi.org/10.1038/s41419-024-07166-8.

[19] Zhang H, Dhalla NS. The Role of Pro-Inflammatory Cytokines in the Pathogenesis of Cardiovascular Disease. International Journal of Molecular Sciences. 2024; 25: 1082. https://doi.org/10.3390/ijms25021082.

[20] Schnabel RB, Baumert J, Barbalic M, Dupuis J, Ellinor PT, Durda P, *et al*. Duffy antigen receptor for chemokines (Darc) polymorphism regulates circulating concentrations of monocyte chemoattractant protein-1 and other inflammatory mediators. Blood. 2010; 115: 5289–5299. https://doi.org/10.1182/blood-2009-05-221382.

[21] Rudemiller NP, Crowley SD. The role of chemokines in hypertension and consequent target organ damage. Pharmacological Research. 2017; 119: 404–411. https://doi.org/10.1016/j.phrs.2017.02.026.

[22] Makki K, Froguel P, Wolowczuk I. Adipose tissue in obesity-related inflammation and insulin resistance: cells, cytokines, and chemokines. ISRN Inflammation. 2013; 2013: 139239. https://doi.org/10.1155/2013/139239.