

# Incidence of Factor V Leiden and Prothrombin Gene Polymorphisms as Risk Factors for Coronary Artery Disease in a Libyan Population: A Case–Control Study

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## Research Article

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# Abstract

## Background

Cardiovascular diseases (CVDs) remain the leading cause of mortality worldwide. Although inherited thrombophilia polymorphisms have been implicated in thrombotic disorders, their contribution to coronary artery disease (CAD) remains controversial, particularly in understudied populations such as Libya.

## Methods

A case–control study was conducted among 82 recruited participants. Following quality control, 61 individuals were included in the final analysis, comprising 49 CAD patients and 12 healthy controls. Genomic DNA was extracted from peripheral blood and genotyped using the CVD StripAssay® (ViennaLab Diagnostics). Biochemical parameters including lipid profile and D-dimer levels were assessed. Statistical analyses were performed using SPSS version 27 (significance threshold:  $p < 0.05$ ).

## Results

CAD patients exhibited significantly higher D-dimer levels ( $p = 0.001$ ), whereas total cholesterol ( $p = 0.017$ ) and HDL cholesterol ( $p = 0.008$ ) were significantly higher among controls. The heterozygous Factor V Leiden (GA) genotype was strongly associated with CAD susceptibility (OR = 12.44, 95% CI estimated;  $p = 0.005$ ). The Factor V H1299R (HR2) AG genotype showed a significant association with disease presence ( $p = 0.035$ ). Although the Prothrombin G20210A variant was more frequently detected among patients, no statistically significant association was observed ( $p = 0.109$ ).

## Conclusions

Factor V Leiden and Factor V H1299R polymorphisms were identified as potential genetic determinants of CAD susceptibility in the studied Libyan population, while no significant contribution of Prothrombin G20210A was detected. These findings provide novel evidence on inherited thrombophilia-related risk factors in Libya and support the integration of genetic and biochemical markers into future CAD risk assessment strategies.

## Background

Cardiovascular diseases (CVDs) represent one of the most pressing global health challenges and remain the leading cause of mortality worldwide. In 2022, approximately 19.8 million deaths were attributed to CVDs, accounting for approximately one-third of all global deaths [1]. A substantial proportion of these deaths in low- and middle-income countries are attributable to myocardial infarction and stroke [2]. CVDs

encompass a wide spectrum of disorders including coronary artery disease (CAD), cerebrovascular disease, peripheral artery disease, congenital heart defects, arrhythmias, and heart failure.

Atherosclerosis constitutes the principal pathological substrate underlying most of these disorders, characterized by lipid accumulation, inflammatory responses, and structural alterations in the arterial intima [3]. The pathophysiology of atherosclerotic CVD involves complex interactions between blood cells, platelets, plasma proteins, coagulation factors, and the vascular endothelium. Disruption of the delicate balance between the coagulation and anticoagulation systems can lead to intravascular thrombus formation and, in severe cases, myocardial infarction or stroke [4].

Both modifiable and non-modifiable risk factors influence CVD development. Non-modifiable risk factors include age, sex, and genetic predisposition, whereas modifiable factors encompass smoking, physical inactivity, unhealthy diet, hypertension, type 2 diabetes mellitus, dyslipidemia, and obesity [5]. Among genetic determinants, single-nucleotide polymorphisms (SNPs) in coagulation factor genes are of particular clinical relevance, as they can modulate gene expression and influence vascular homeostasis. Mutations in factor V and factor II (prothrombin) genes are among the most clinically significant inherited thrombophilia risk factors [6].

The Factor V Leiden mutation (G1691A) causes resistance to activated protein C (APC), resulting in prolonged thrombin generation and a hypercoagulable state [7]. A second non-synonymous variant in exon 13 of the factor V gene—the A4070G (His1299Arg, HR2) polymorphism—has also been linked to hereditary thrombosis, with some studies reporting a two- to threefold increased risk of venous thrombosis in carriers [8]. Similarly, the G20210A mutation in the prothrombin gene is associated with elevated plasma prothrombin levels and an increased risk of venous thromboembolism [9].

Despite growing interest in the genetic basis of CVD, data specific to the Libyan population remain limited. To address this gap, the present study aimed to investigate the prevalence of Factor V Leiden (G1691A), Prothrombin (G20210A), and Factor V H1299R (HR2) polymorphisms among Libyan CAD patients and healthy controls, and to evaluate their association with CAD susceptibility.

## Methods

### Study Design and Setting

A case–control study was conducted among adult Libyan nationals recruited from clinical centers in Tripoli, Libya. Cases comprised patients with clinically confirmed cardiovascular disease, predominantly CAD, diagnosed according to established cardiology guidelines. Controls were apparently healthy individuals with no documented history of cardiovascular disease. This design enabled comparison of genetic and biochemical risk factors between groups.

### Participants

Eligible participants were Libyan nationals aged  $\geq 18$  years with complete clinical, biochemical, and genetic data. Cases were required to have a confirmed diagnosis of CVD (CAD, myocardial infarction, or stroke); controls must have had no history of CVD. Exclusion criteria included non-Libyan nationality, incomplete clinical or laboratory data, poor DNA quality or inconclusive PCR results, withdrawal, or severe comorbidities likely to confound cardiovascular risk assessment.

A total of 82 participants were initially recruited. Following quality control, 23 participants were excluded due to incomplete data, ineligibility, or withdrawal, and 21 samples were excluded due to inconclusive PCR results. The final analytical cohort comprised 61 participants: 49 CAD cases and 12 healthy controls.

## Data Collection

Clinical and demographic data—including age, sex, cardiovascular status, and medical history (e.g., diabetes mellitus, hypertension)—were collected through structured interviews and medical record review. Fasting venous blood samples were collected under sterile conditions and distributed into EDTA tubes (DNA extraction), citrate tubes (coagulation analysis), and plain tubes (biochemical analysis). Samples were processed and stored under appropriate conditions until analysis.

## Biochemical Analysis

Biochemical parameters including total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides (TG), and D-dimer were measured from fasting blood samples using standard laboratory methods.

## DNA Extraction

Genomic DNA was extracted from whole blood using the Magic Pure® Blood Genomic DNA Kit (TransGen Biotech, China) based on magnetic bead technology. Briefly, samples were lysed with binding buffer and Proteinase K, DNA was bound to magnetic beads, washed sequentially to remove contaminants, and eluted. Extracted DNA was stored at  $-20^{\circ}\text{C}$  until further analysis.

## Genotyping by CVD StripAssay®

Genotyping of thrombophilia-related polymorphisms was performed using the CVD StripAssay® Kit (ViennaLab Diagnostics GmbH, Austria) according to the manufacturer's instructions. The assay is based on PCR followed by reverse hybridisation of amplified DNA fragments onto allele-specific oligonucleotide probes immobilised on test strips. PCR amplification was performed in two separate reaction mixes at  $94^{\circ}\text{C}$  initial denaturation for 2 minutes, followed by 35 cycles of denaturation ( $94^{\circ}\text{C}/15$  s), annealing ( $58^{\circ}\text{C}/30$  s), and extension ( $72^{\circ}\text{C}/30$  s), with a final extension at  $72^{\circ}\text{C}$  for 3 minutes. Hybridisation was performed at  $45^{\circ}\text{C}$ , and detection was achieved by enzymatic colour development.

Polymorphisms analysed included: Factor V Leiden (G1691A), Prothrombin (G20210A), Factor V H1299R (R2), Factor XIII V34L,  $\beta$ -fibrinogen – 455 G/A, PAI-1 (4G/5G), GPIIIa L33P (HPA-1), MTHFR (C677T, A1298C), ACE (I/D), Apo B (R3500Q), and Apo E (E2/E3/E4). The primary polymorphisms of interest were Factor V G1691A, Prothrombin G20210A, and Factor V H1299R.

## Validation of Molecular Results

To confirm genotyping accuracy, ten randomly selected samples representing different genotypic patterns were re-tested using the CVD StripAssay® T kit (same manufacturer). Results were fully concordant (100% agreement), confirming reproducibility and validity of the molecular findings.

## Statistical Analysis

Data were analysed using SPSS version 27. Categorical variables were presented as frequencies and percentages; continuous variables as means  $\pm$  standard deviations (SD). Between-group comparisons for continuous variables were performed using independent-samples t-tests; comparisons across more than two groups used one-way ANOVA with post hoc tests. Associations between categorical variables were assessed using the chi-square ( $\chi^2$ ) test or Fisher's exact test when expected cell counts were small. Odds ratios (ORs) were calculated to estimate association strength. Hardy–Weinberg equilibrium (HWE) was assessed in the control group using a chi-square goodness-of-fit test. All tests were two-tailed;  $p < 0.05$  was considered statistically significant.

## Ethical Approval

The study was conducted in accordance with the Declaration of Helsinki. Informed consent was obtained from all participants. Ethical approval was obtained from the relevant institutional review committee.

## Results

### *Demographic and Clinical Characteristics*

The final study population comprised 61 participants: 49 CAD cases (80.3%) and 12 healthy controls (19.7%). Female participants constituted the majority (62.3%;  $n = 38$ ), while males accounted for 37.7% ( $n = 23$ ). The largest age stratum was 50–69 years (37.7%), followed by  $\geq 70$  years (34.4%) and  $< 50$  years (27.9%).

**Table 1. Demographic distribution of the study population**

| Characteristic | Category    | n  | %    |
|----------------|-------------|----|------|
| Study Group    | CAD Cases   | 49 | 80.3 |
|                | Controls    | 12 | 19.7 |
| Sex            | Male        | 23 | 37.7 |
|                | Female      | 38 | 62.3 |
| Age Group      | <50 years   | 17 | 27.9 |
|                | 50–69 years | 23 | 37.7 |
|                | ≥70 years   | 21 | 34.4 |

### ***Genotype Distributions***

Regarding the FV G1691A locus, the GG genotype was observed in 55.7% and the GA genotype in 44.3% of participants; no AA homozygotes were detected. For FV H1299R, the AA genotype predominated (77.0%) versus AG (23.0%), with a significantly non-random distribution ( $\chi^2 = 17.852$ ,  $p < 0.001$ ). For Prothrombin G20210A, the GG genotype was most frequent (80.3%), followed by GA (16.4%) and AA (3.3%), also showing significant departure from expected frequencies ( $\chi^2 = 62.197$ ,  $p < 0.001$ ).

### ***Hardy–Weinberg Equilibrium***

HWE was assessed in the control group. For FV G1691A (observed: GG = 11, GA = 1, AA = 0; expected: GG = 11.02, GA = 0.96, AA = 0.02), no significant deviation was detected ( $\chi^2 = 0.023$ ,  $p = 0.880$ ), supporting validity of the genotype data. For Prothrombin G20210A, a statistically significant deviation was observed ( $\chi^2 = 12.000$ ,  $p = 0.001$ ), driven by the presence of a single AA homozygote in the complete absence of GA heterozygotes among only 12 controls. Given the very small sample size and expected cell counts below 1, this deviation most likely reflects sampling variance rather than true population stratification or genotyping error.

### ***Association Between Genetic Polymorphisms and CAD***

The heterozygous GA genotype of Factor V Leiden was markedly more frequent among cases (53.1%) than controls (8.3%), yielding a strong and statistically significant association (OR = 12.44;  $\chi^2 = 7.817$ ,  $p = 0.005$ ; Table 3). The FV H1299R AG genotype was present exclusively among cases (28.6%) and absent in all controls, reaching statistical significance by Fisher's exact test ( $p = 0.035$ ; Table 4). In contrast, the Prothrombin G20210A GA genotype was observed only among cases (20.4%), but the overall association with CAD was not statistically significant (Fisher's exact test,  $p = 0.109$ ; Table 5).

**Table 3. Association between Factor V Leiden (G1691A) polymorphism and CAD**

| Genotype | Cases n (%) | Controls n (%) | OR    | $\chi^2$ | p-value |
|----------|-------------|----------------|-------|----------|---------|
| GA       | 26 (53.1)   | 1 (8.3)        | 12.44 | 7.817    | 0.005   |
| GG (Ref) | 23 (46.9)   | 11 (91.7)      | 1.00  |          |         |
| Total    | 49 (100.0)  | 12 (100.0)     |       |          |         |

**Table 4. Association between Factor V H1299R (HR2) polymorphism and CAD (Fisher's exact test)**

| Genotype | Cases n (%) | Controls n (%) | Fisher's Exact | p-value |
|----------|-------------|----------------|----------------|---------|
| AA       | 35 (71.4)   | 12 (100.0)     | 4.450          | 0.035   |
| AG       | 14 (28.6)   | 0 (0.0)        |                |         |
| Total    | 49 (100.0)  | 12 (100.0)     |                |         |

**Table 5. Association between Prothrombin (G20210A) polymorphism and CAD (Fisher's exact test)**

| Genotype | Cases n (%) | Controls n (%) | Fisher's Exact | p-value |
|----------|-------------|----------------|----------------|---------|
| AA       | 1 (2.0)     | 1 (8.3)        | 4.033          | 0.109   |
| GA       | 10 (20.4)   | 0 (0.0)        |                |         |
| GG       | 38 (77.6)   | 11 (91.7)      |                |         |
| Total    | 49 (100.0)  | 12 (100.0)     |                |         |

### Secondary Analyses

No statistically significant associations were observed between any of the three polymorphisms and sex or age group among CAD patients (all  $p > 0.05$ ), indicating that genotype distributions were independent of these demographic variables. Among FV G1691A genotypes, LDL levels were significantly lower in GA carriers compared to GG carriers (74.58 vs. 95.49 mg/dL;  $p = 0.024$ ), while no significant differences were observed for other biochemical parameters. No significant biochemical differences were detected across FV H1299R or Prothrombin G20210A genotypes. Lipid profiles (total cholesterol, LDL, triglycerides) differed significantly across anticoagulant medication groups ( $p < 0.001$ ,  $< 0.001$ , and  $0.005$ , respectively), likely reflecting lipid-lowering therapy effects.

## Discussion

This case–control study investigated the association of three inherited thrombophilic polymorphisms—Factor V Leiden (G1691A), Factor V H1299R (HR2), and Prothrombin (G20210A)—with CAD susceptibility in a Libyan population, providing novel data from an underrepresented geographic and ethnic group.

The principal finding of this study is a strong and statistically significant association between the heterozygous Factor V Leiden (GA) genotype and CAD (OR = 12.44,  $p = 0.005$ ). This finding is broadly consistent with regional evidence from Libya, where Factor V Leiden heterozygosity was identified in 15% of patients with deep vein thrombosis [10], and with data from neighboring populations. Almawi et al. [11] reported relatively high frequencies of Factor V Leiden among Lebanese (13.8% heterozygosity) and Tunisian populations, underscoring the elevated prevalence in Mediterranean-adjacent regions. Similarly, a recent Iranian case–control study among 944 patients with premature CAD demonstrated a significant association between Factor V Leiden and disease risk [12]. In contrast, a Turkish study involving 287 participants found no significant association between Factor V Leiden and angiographically confirmed CAD [13], and an Indian study among patients with acute coronary syndromes detected no carriers at all [14]. Such heterogeneity is consistent with the established ethnic and geographical variation in Factor V Leiden prevalence, which is highest in Caucasian populations and lower in Asian and African groups [7].

The Factor V H1299R (HR2) AG genotype was observed exclusively among cases and was absent in all controls, yielding a statistically significant association ( $p = 0.035$ ). The HR2 haplotype, comprising more than 12 linked polymorphisms including His1299Arg, may exert a mild prothrombotic effect through reduced sensitivity to APC and potentially elevated Factor V levels [15]. Although its individual clinical effect is weaker than that of Factor V Leiden, the HR2 haplotype may potentiate thrombotic risk when co-inherited with FVL [15]. The complete absence of the AG genotype among controls, while biologically plausible, should be interpreted cautiously given the small control sample size.

Prothrombin G20210A was not significantly associated with CAD in this study ( $p = 0.109$ ), despite a higher frequency of the GA genotype among cases. This finding aligns with regional data from Libya and neighboring countries reporting low prevalence and weak or inconsistent associations with arterial thrombosis [10, 11]. Globally, the mutation is more firmly established as a risk factor for venous rather than arterial thrombosis; a phenotype-specific meta-analysis pooling 12,034 subjects found only a modest and subgroup-dependent association with ischaemic heart disease, with a stronger signal in individuals under 55 years [16]. Insufficient statistical power related to the small sample and low mutation frequency in the current study may also have contributed to the null finding.

Elevated D-dimer levels in CAD cases confirm enhanced coagulation activation and fibrinolytic activity in this population, consistent with the established role of D-dimer as a biomarker of thrombotic activity. Paradoxically, total cholesterol and HDL were higher among controls than cases; this unexpected finding is most plausibly explained by the widespread use of lipid-lowering therapy, dietary modification following diagnosis, and the small control sample size. The significant association of higher LDL levels with the FV G1691A GG genotype is an exploratory finding of uncertain biological significance and requires confirmation in larger studies.

Several limitations should be considered. First, the relatively small sample—particularly the limited control group ( $n = 12$ )—reduces statistical power and may have affected the precision of OR estimates and HWE analysis. The marked case–control imbalance further increases the risk of type I and II errors. Second, the cross-sectional case–control design precludes inference of causality. Third, the absence of multivariate adjustment means that conventional cardiovascular risk factors (smoking, obesity, hypertension, diabetes, dyslipidemia) cannot be controlled for, and their potential confounding influence cannot be excluded. Fourth, population stratification was not formally assessed. Fifth, exclusion of samples with inconclusive PCR results introduces potential selection bias. Finally, the findings pertain to a single Libyan center and may not generalize to other populations.

Despite these limitations, this study provides preliminary but important molecular epidemiological evidence from Libya—a population notably underrepresented in the thrombophilia literature. The significant association of Factor V Leiden with CAD, together with the suggestive association of Factor V H1299R, supports a potential role of inherited thrombophilia in arterial disease pathogenesis in this cohort and warrants further investigation.

## Conclusion

The present study identifies Factor V Leiden (G1691A) as a significant genetic risk factor for CAD in the studied Libyan population ( $OR = 12.44$ ,  $p = 0.005$ ), and provides suggestive evidence that the Factor V H1299R (HR2) polymorphism may also contribute to disease susceptibility. Prothrombin G20210A was not significantly associated with CAD in this cohort. These findings add to the limited molecular genetic data available from Libya and highlight the need for large-scale, multicenter studies incorporating multivariate adjustment and broader genetic profiling to validate these associations and improve CAD risk stratification strategies in this population.

## Declarations

### Ethics Approval and Consent to Participate

The study was conducted in accordance with the principles of the Declaration of Helsinki. Written informed consent was obtained from all participants. Ethical approval was obtained from the institutional review committee of The Libyan Academy.

## Competing Interests

The authors declare that they have no competing interests.

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# Author Contribution

Ahlam M. Ahbeel (AMA): Conceptualization, methodology, investigation, participant recruitment, sample collection, laboratory experiments, molecular analyses, data curation, formal analysis, visualization, writing—original draft. Ahmed M. Eshwika (AME): Supervision, conceptualization, methodology, validation, interpretation of data, critical review and editing of the manuscript. Lutfi M. Bakar (LMB): Investigation, clinical coordination, data collection, resources, manuscript review and editing. Mohamed S. Abughren (MSA): Methodology, validation, laboratory supervision, interpretation of molecular findings, manuscript review and editing. Abdulrzag F. Ahmed (AFA): Conceptualization, supervision, project administration, funding acquisition (personal financial support), methodology, formal analysis, interpretation of results, writing—review and editing, final approval of the manuscript, and corresponding author. All authors read and approved the final manuscript and agree to be accountable for all aspects of the work.

## Availability of Data and Materials

The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

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