Article

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Molecular Characterization and Impact of the Genetic Variants *GSTP1*, LncRNA *H19*, *TCF7L2* and *HNF1A* on the Risk of Coronary Artery Disease With and Without T2DM Comorbidity: A Genomic Biomarker Study

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Background: Cardiovascular diseases, including coronary artery disease (CAD), represent one of the leading causes of death worldwide. Individuals with type 2 diabetes mellitus (T2DM) are susceptible to more severe forms of CAD. Given the strong genetic basis underlying the pathogenesis of T2DM and CAD, this study aimed to investigate the potential significance of the following genetic variants in the pathogenesis of CAD with and without T2DM comorbidity: hepatocyte nuclear factor-1 alpha (*HNF1A*) [p.I27L] rs1169288, glutathione S-transferase Pi 1 (*GSTP1*) rs1695 (A>G; Ile→Val), transcription factor 7-like 2 (*TCF7L2*) rs7903146 (C>T), and long non-coding RNA H19 (LncRNA *H19*) rs217727 (C>T).

Methods: Three hundred subjects were enrolled in this study, containing 200 cases of CAD (100 non-diabetic and 100 diabetic) and 100 healthy individuals as controls. The genotyping studies were conducted using amplification-refractory mutation system polymerase chain reaction (ARMS-PCR).

Results: Demographic and clinical characteristics associated with the two CAD groups demonstrated significant differences. Additionally, a significant difference in genotype frequencies of the *GSTP1* (rs1695 A>G) gene polymorphism was detected between CAD patients and healthy controls, with the GG genotype being more common in CAD patients (18% in non-diabetic and 20% in diabetic) than in the healthy controls (3%) ($p < 0.0001$ and $p = 0.00003$). Those with the GG genotype had a notably higher risk of developing CAD, regardless of T2DM comorbidity. The LncRNA *H19* (rs217727 C>T) gene polymorphism displayed significant differences in genotype frequencies between healthy controls and non-diabetic CAD patients, but not in diabetic CAD patients. The TT genotype was much more common in non-diabetic CAD patients (20%) compared to healthy controls (6%, $p = 0.0006$), with nondiabetic patients also having a higher frequency of the T allele (0.42 vs. 0.23 in controls). Similarly, the *TCF7L2* (rs7903146 C>T) gene polymorphism displayed significant differences in genotype frequencies between healthy controls and non-diabetic CAD patients, instead of diabetic CAD patients. The CT heterozygous genotype was more common in non-diabetic CAD patients (63%) than in healthy controls (35%, $p = 0.0001$). The *HNF1A* (rs1169288 G>T) gene polymorphism showed significant differences in genotype frequencies between healthy controls and diabetic CAD patients, but not between non-diabetic CAD patients. The TT genotype was notably overrepresented in diabetic CAD patients (8%) than in healthy controls (1%, $p = 0.04$), and diabetic patients also had a higher frequency of the T allele (0.38 vs. 0.33 in controls).

Conclusion: The data from the current study have identified certain genetic variants of interest in the given population as risk markers of CAD with and without T2DM comorbidity.

Keywords: coronary artery disease; type 2 diabetes mellitus; *GSTP1*; LncRNA *H19*; *TCF7L2*; *HNF1A*; genomic biomarkers; comorbidity

Introduction

There is considerable variation in coronary artery disease (CAD)'s manifestations, severity, and the underlying pathological changes in individuals with type 2 diabetes mellitus (T2DM) when compared to those who do not have a history of diabetes [1]. This variation can be attributed to several factors, including differences in metabolic processes, the presence of comorbid conditions, and the impact of insulin resistance associated with T2DM. As a result, individuals with T2DM are susceptible to more severe forms of CAD, exhibit atypical symptoms, and show distinct patterns of arterial plaque development and progression [2]. T2DM significantly affects both survival and quality of life, particularly for patients diagnosed at a younger age. The disorder is defined by the presence of insulin resistance and elevated blood sugar levels, which are typically, but not consistently, associated with abnormal lipid metabolism. Insulin resistance often manifests early in the development of both T2DM and cardiovascular disease [3].

Cardiovascular disease (CVD) encompasses a range of conditions related to the heart and blood vessels, including CAD, cerebrovascular (CBV) disease, and peripheral vascular disease. Studies have reported that among these various forms of CVD, CAD emerged as the most commonly reported type, accounting for 21.2% of the cases [4]. In the observed data, higher prevalence rates of CVD were detected in male patients compared to their female counterparts. Additionally, this disease was also more prevalent in older individuals than in younger patients, demonstrating the impact of age on cardiovascular health. Furthermore, patients diagnosed with T2DM face a significantly increased risk of developing CAD (2- to 4-fold) compared to those without this condition [5]. This association reflects the critical link between diabetes management and cardiovascular health. Accordingly, a substantial proportion of individuals with T2DM face fatal complications related to cardiovascular diseases, including CAD and myocardial infarction [6]. Currently, approximately 8.8% of the global population is affected by diabetes, and the International Diabetes Federation projects a significant increase marked by an estimated rise in diabetes cases to 642 million by the year 2040 (IDF Factsheet) [7]. In individuals diagnosed with T2DM, mortality rates vary significantly based on their cardiovascular history. Specifically, the death rates among those with T2DM who have not previously experienced a myocardial infarction (MI) stand at 15.4% [4]. This figure advances dramatically for patients with a history of MI, where the death rate escalates to 42.0%. In comparison, individuals without T2DM exhibit considerably lower mortality rates due to cardiovascular issues. Similarly, the Framingham Heart Study revealed that as the prevalence of T2DM rises, the associated risk of cardiovascular disease attributable to T2DM is also increased [8]. The efficacy of specific medications used in the management of CAD can vary significantly among individuals of different ethnic backgrounds, which is largely attributed to variations in genetic architecture between these populations.

Genetic factors can influence how individuals metabolize drugs and respond to treatment, leading to disparities in therapeutic outcomes. Recent research indicates a positive genetic correlation between CAD and T2DM, with both diseases sharing a significant overlapping set of genetic components [9–11]. Single-nucleotide polymorphisms (SNPs) known to be associated with T2DM also increase the risk for CAD. In terms of heritability, T2DM has an estimated genetic heritability of approximately 72%, while CAD has a heritability of about 58%, demonstrating the strong genetic basis for both conditions [12]. In this study, we utilized several advanced methodologies, including genetic correlation estimations, bivariate genetic analyses focusing on individual SNPs, and gene-level association analyses for the following gene variants: hepatocyte nuclear factor-1 alpha (*HNF1A*) [p.I27L] (rs1169288), glutathione S-transferase Pi 1 (*GSTP1*) rs1695 (A>G; Ile→Val), transcription factor 7-like 2 (*TCF7L2*) rs7903146 (C>T), and long non-coding RNA H19 (LncRNA *H19*) rs217727 C>T. *HNF1A* is a transcription factor essential for the development and function of pancreatic β cells, as it regulates the expression of numerous genes, such as insulin and *HNF1A* [13]. As a member of the *GST* gene family, *GSTP1* functionally encodes a range of GSTP1 variant proteins, including the rs1695 (c.313A>G) variant, harboring an SNP associated with an altered catalytic activity and playing a role in metabolic phenotypic manifestations [14]. *TCF7L2* encodes a transcription factor that contains a high mobility group (HMG) box and is crucial for the Wnt signaling pathway. The protein is involved in regulating blood glucose levels, and genetic variations of this gene are linked to a higher risk of developing T2DM [15]. LncRNA *H19* is the first discovered long non-coding RNA, and interestingly, it is transcribed only from maternally inherited alleles and contributes to the regulation of gene expression through chromatin modifications [16]. Through this study, several potential causal loci with certain implications for the risk for and susceptibility to CAD with or without T2DM comorbidity were identified.

Materials and Methods

Study Population

The prospective cross-sectional study was conducted at the Department of Biochemistry of the G.B. Pant Institute of Postgraduate Medical Education & Research (GIPMER) in New Delhi, India. In the study, 200 age- and sex-matched, angiography-confirmed patients diagnosed with CAD from both the Outpatient Department (OPD) and Inpatient Department (IPD) of the Department of Cardiology, G.B. Pant Institute of Postgraduate Medical Education & Research (GIPMER) in New Delhi, India. All patients provided their informed consent in this study.

Inclusion and Exclusion Criteria

Inclusion Criteria for 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 $\geq$50%) were included.

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, 1 mL of the blood sample was kept in an ethylene diamine tetra acetic acid (EDTA) vial for DNA extraction, 1 mL for glycated hemoglobin (HbA1c) analysis, while the remaining 1 mL sample was kept in an EDTA vial for analyses of serum markers. Patients were classified as having diabetic-CAD (Group I) if their HbA1c level was $\geq$6.5% or they had previously been diagnosed with diabetes; patients were classified as non-diabetic-CAD (Group II) if their HbA1c level was less than 6.5%. Non-diabetic CAD patients with neither a history of diabetes nor anti-diabetic therapy were categorized into Group II. A completely automated analyzer (Cobas c 513 analyzer, Roche Diagnostics, Indianapolis, IN, USA) was used to measure the serum levels of HbA1c, and for the detection of other markers, enzyme-linked immunosorbent assays such as Human Total Cholesterol enzyme-linked immunosorbent assay (ELISA) Kit (Cat No. orb782215, Biorbyt, Durham, NC, USA), Triglyceride Microplate Assay Kit (Cat. No. orb390792, Biorbyt, Durham, NC, USA), high-density lipoprotein (HDL) and low-/very-low-density lipoprotein (LDL/VLDL) Cholesterol Assay Kit (Cat. No. STA-391, Cell Biolabs, Inc., San Diego, CA, USA), Human Urea ELISA Kit (Cat. No. MBS2601488, MyBioSource, Inc., San Diego, CA, USA) and Human Creatinine (Cr) ELISA Kit (Cat. No. orb1669340, Biorbyt, Durham, NC, USA) were used.

Sample Size

The sample size of 200 CAD patients was determined by taking into account a 90% confidence level and 8% absolute precision, and by measuring the expected percentage of CAD as 50% (assumed proportion). The formula used in sample size determination is shown in the following:

$$n = \left(Z^2 \times p(1-p)\right)/e^2$$

where Z = value from standard normal distribution corresponding to desired confidence level (Z = 1.96 for 95% confidence interval (CI)); p = expected true proportion of desired precision (half desired CI width), and e = margin of error (0.05).

Cardiovascular Risk Factor Assessment

This research comprised patients over the age of 18 who had a resting electrocardiography (ECG) or a coronary angiography showing more than 50% stenosis and were diagnosed with CAD. The participants were seated and allowed to relax while their blood pressure was monitored. Two readings of their blood pressure were measured at least five minutes apart and then averaged. Having a history of hypertension, a systolic blood pressure of 140 mmHg, or a diastolic blood pressure of 90 mmHg was considered to have hypertension. Dyslipidemia was defined as having triglycerides (>150 mg/dL), high-density lipoprotein cholesterol (HDL-C; <40 mg/dL), low-density lipoprotein cholesterol (LDL-C; >100 mg/dL), or total cholesterol (TC; >200 mg/dL).

Genotyping Analysis of HNF1A, GSTP1, LncRNA H19, and TCF7L2

Using a spin column-based DNA extraction kit (Cat. No. 51104, Qiagen, Germantown, MD, USA), genomic DNA was isolated from every blood sample collected in EDTA vials. Amplification-refractory mutation system polymerase chain reaction (ARMS-PCR) primers previously used to genotype the gene variations in *HNF1A* rs1169288 G>T (Ile27Leu) [17], *GSTP1* Ile105val [18], LncRNA *H19* rs217727 C>T [19], and *TCF7L2* rs12255372 G>T [20], were selected for use in this study (Table 1).

Preparation of PCR Mix

Four primers—forward outer primer (Fo, 0.10 µL), reverse outer primer (Ro, 0.10 µL), forward inner primer (FI, 0.10 µL), and reverse inner primer (RI, 0.10 µL)—along with 25 pmol of each primer (Sigma, India) and 6 µL of green PCR Master Mix (2×) (Cat M712C, Promega, Madison, WI, USA) made up the 12 µL reaction volume used for the PCR. Supplementation with nuclease-free ddH_2O produced a final volume of 12 µL. Finally, 50 ng of template DNA was introduced into the mixture.

PCR Thermocycling Conditions

The T100 Thermal Cycler (BioRad, Hercules, CA, USA). The thermocycling process involved the following

Table 1. Primers for genotyping *HNF1A* rs1169288 G>T (Ile27Leu), *GSTP1* Ile105val (A313G), LncRNA *H19* rs217727 C>T, and *TCF7L2* rs12255372 G>T.

ARMS primers for *HNF1A* rs1169288 A>C (I27L)				
HNF1A Fo		5′-GTGCCCACAGGGCTTGGCTAG-3′	387 bp	62 °C
HNF1A Ro		5′-CCATCGTCGTCCGTCTCGTCCTCG-3′		
HNF1A FI	G	5′-GGGCTGAGCAAAGAGGCACCG-3′	176 bp	
HNF1A RI	T	5′-CCCGGCTCACCCAGTGCCTGAAT-3′	257 bp	
ARMS primers of *GSTP1* Ile105val (A313G)				
GSTP1 Fo		5′-AGGTTACGTAGTTTGCCCAAGGTC-3′	563 bp	64 °C
GSTP1 Ro		5′-CGTTACTTGGCTGGTTGATGTCC-3′		
GSTP1 FI	G	5′-GAGGAC-CTCCGCTGCAAATTCG-3′	260 bp	
GSTP1 RI	A	5′-CATAGTTGGTGTAGATGAGGGAGCT-3′	360 bp	
ARMS primers of *LncRNA H19* rs217727 C>T				
LncRNA H19 Fo		5′-ATGACTCAGGAATCGGCTCTGGAAGGTG-3′	397 bp	63 °C
LncRNA H19 Ro		5′-GGGGAAACAGAGTCGTGGAGGCTTTGA-3′		
LncRNA H19 FI	T	5′-TCATCTTCATGGCCACCCCCTGCTGT-3′	200 bp	
LncRNA H19 RI	C	5′-ATATGGTGGCTGGTGGTCAACCGTACG-3′	248 bp	
ARMS-PCR primers for *TCF7L2* rs12255372 G>T				
TCF7L2 Fo		5′-GGGCAATAGATACATTTTAAGA-3′	760 bp	59 °C
TCF7L2 Ro		5′-GAGATAGATGATAGGCTGTT-3′		
TCF7L2 FI	G	5′-GGAATATCCAGGCAAGAATG-3′	494 bp	
TCF7L2 RI	T	5′-CCTGAGTAATTATCAGAATATGGTA-3′	310 bp	

Note: ARMS-PCR, amplification-refractory mutation system polymerase chain reaction; *HNF1A*, hepatocyte nuclear factor-1 alpha; *GSTP1*, glutathione S-transferase Pi 1; LncRNA *H19*, long non-coding RNA H19; *TCF7L2*, transcription factor 7-like 2; Fo, forward outer primer; Ro, reverse outer primer; FI, forward inner primer; RI, reverse inner primer.

steps: initial denaturation at 95 °C for 11 minutes, followed by 30 cycles of denaturation at 95 °C for 35 seconds; annealing at 62 °C for 35 seconds (*HNF1A* rs1169288 A>C 62 °C), 64 °C for 35 seconds (*GSTP1* Ile105val 64 °C), 59 °C for 35 seconds (*TCF7L2* rs12255372 G>T 59 °C), 65 °C for 35 s LncRNA *H19* rs217727 C>T; extension at 72 °C for 34 seconds, followed by final extension at 72 °C for 8 minutes before storage at 4 °C.

Agarose Gel Electrophoresis and PCR Product Visualization

Following separation on 2% agarose gel electrophoresis (Gel electrophoresis unit; BioRad, Hercules, CA, USA), the PCR-amplified products were stained with SYBR Safe dye and examined on a gel documentation system (BioRad, Hercules, CA, USA).

HNF1A rs1169288 G>T (Ile27Leu) genotyping: The external primers Fo and Ro amplify the external region of the *HNF1A* gene, producing a band of 387 bp that serves as a control for DNA soundness. Primers Fo and RI amplify the A allele, producing a band of 257 bp, and primers FI and Ro generate a band of 176 bp from the C allele.

GSTP1 (Ile105val) genotyping: PCR products following the genotyping of *GSTP1* Ile105val (A313G) were separated on a 2% agarose gel and stained with ethidium bromide. The primer for this polymorphism amplifies a control product of 563 bp, while the PCR products of 360 bp and 260 bp identify the AA (Ile) and GG (Val) variants, respectively.

LncRNA *H19* rs217727 C>T genotyping: Outer primers Fo and Ro amplify the outer region of the gene site and generate a band of 397 bp, which acts as a control. Primers FI and Ro amplify the T allele and generate a band of 248 bp, whereas primers Fo and RI generate a band of 200 bp for the C allele.

TCF7L2 rs12255372G/T genotyping: A band of 760 bp produced by the amplification primers Fo and Ro act as the control band to indicate DNA quality and quantity. A band of 310 bp (T allele) is produced by the amplification of primers Fo and RI, and a band of 494 bp was produced by the amplification of primers Ro and FI (T allele).

Statistical Analysis

The Statistical Program for Social Science (SPSS) version 25 (IBM Corp., Armonk, NY, USA) was used to analyze the data. The Kolmogorov–Smirnov test was applied to test the normality distribution of the data. Continuous variables and categorical variables are represented as mean ± standard deviation (SD) and count with percentages, respectively. Analysis of variance (ANOVA) with Tukey's post-hoc analysis and Chi–square tests were applied for analyzing continuous and categorical variables, respectively. The Chi–square test or Fisher's exact test was

used for genotype analysis, and the Hardy–Weinberg equilibrium was used to analyze the allele frequency among CAD patients. A logistic regression analysis was performed to examine the influence of *GSTP1* rs1695 A>G, LncRNA *H19* rs217727 C>T, *TCF7L2* rs7903146 C>T, and *HNF1A* rs1169288 G>T gene polymorphisms on the development of CAD with or without diabetes. p-values < 0.05 were considered statistically significant.

Results

Demographic Characteristics of Study Subjects

The demographic characteristics of the subjects are depicted in Table 2. In brief, a total of 300 study subjects, including 200 CAD patients and 100 healthy controls, were analyzed. The patient group was constituted by 50% of non-diabetic CAD patients and 50% of diabetic CAD patients. Most of the patients in both the patient groups were males (Group I = 81% and Group II = 89%). The mean age of non-diabetic CAD patients was 53.2 $\pm$ 10.3 years, while the mean age for diabetic CAD patients was 54.2 $\pm$ 10.2 years. Most of the CAD patients were in the age range of 41–60 years (Group I = 63% and Group II = 62%). The Group I patients showed a higher level of glycated hemoglobin (HbA1c >6.4%) with a mean duration of diabetes of about 4.9 $\pm$ 2.2 years, and most of the patients had diabetes for a duration of more than 5 years (71%). Hypertension was more prevalent in diabetic CAD patients (59%) than in nondiabetic CAD patients (20%). The Group I CAD patients showed higher total cholesterol levels (>200 mg/dL) than the Group II CAD patients (16% vs 8%). Regarding triglycerides, the Group I CAD patients showed higher levels (>150 mg/dL) than the Group II CAD patients (49% vs 25%). More patients of Group I than those of Group II had normal high-density lipoprotein (HDL) levels (40% vs 25%). Both the patient groups showed almost similar values of high low-density lipoprotein (LDL) level (21% and 24%, respectively). High very-low-density lipoprotein (VLDL) levels were observed among the Group I patients compared to the Group II patients (48% vs 28%). High blood urea levels were observed among both the patient groups (94% and 88%); however, higher creatinine levels were noted in the diabetic CAD patients (27% vs 15%).

Genotyping and Agarose Gel Electrophoresis for Allele Identification

Genotyping of the selected four genes was performed using the ARMS-PCR, a technique that allows for the specific amplification of DNA sequences containing mutations of interest. After completing the PCR amplification, the resulting products were subjected to agarose gel electrophoresis at a concentration of 2.5%. This method facilitated the separation of the DNA fragments based on their size. The bands that appeared on the gel were carefully analyzed to differentiate between the wild-type alleles and the mutant alleles present in the samples. The gel images are presented in Fig. 1, which highlights the distinct band patterns associated with each allele type.

Comparative Analysis of the GSTP1, LncRNA H19, TCF7L2 and HNF1A Gene Polymorphisms

Analysis of genotype frequencies (Table 3) for the *GSTP1* (rs1695 A>G) gene polymorphism reflected a significant difference between CAD patients (diabetic or non-diabetic) and healthy controls. Specifically, the GG genotype was considerably more prevalent in both the CAD patients compared to healthy controls (Group I = 20% and Group II = 18% vs healthy controls = 3%, $p < 0.0001$ and $p = 0.00003$, respectively). The allelic frequency supports this association, with the non-diabetic and diabetic CAD patients exhibiting a higher frequency of the G allele (0.47 and 0.46) compared to the controls (0.24). In the analysis of genotype frequencies for the LncRNA *H19* (rs217727 C>T) gene polymorphism, there was a significant difference between non-diabetic CAD patients and healthy controls. Specifically, the TT genotype was considerably more prevalent in non-diabetic CAD patients compared to healthy controls (20% vs 6%, respectively, $p = 0.0006$). The allelic frequency supports this association, with the non-diabetic CAD patients exhibiting a higher frequency of the T allele (0.42) compared to the controls (0.23). In the analysis of genotype frequencies for the *TCF7L2* (rs7903146 C>T) gene polymorphism, there was a significant difference between non-diabetic CAD patients and healthy controls. Specifically, the CT heterozygous genotype was considerably more prevalent in the non-diabetic CAD patients compared to healthy controls (63% vs 35%, respectively, $p = 0.0001$). In the analysis of genotype frequencies for the *HNF1A* (rs1169288 G>T) gene polymorphism, there was a significant difference between diabetic CAD patients and healthy controls. Specifically, the TT genotype was considerably more prevalent in the diabetic CAD patients compared to the healthy controls (8% vs 1%, respectively, $p = 0.04$). The allelic frequency supports this association, with the diabetic CAD patients exhibiting a higher frequency of the T allele (0.38) compared to the controls (0.33).

Comparative Risk Analysis of Non-Diabetic CAD Development Associated With GSTP1, LNRHP19, TCF7L2 and HNF1A Gene Polymorphisms

Comparative risk analysis results presented in Table 4 highlight a significant correlation between CAD development and the GG genotype of the *GSTP1* rs1695 A>G polymorphism. Individuals with the GG genotype demonstrated a markedly higher risk of developing CAD, with an odds ratio (OR) of 12.9 (95% CI: 3.5–48.1, $p < 0.0001$). The same comparative risk analysis also revealed a significant correlation between the development of CAD and the TT genotype of the LncRNA *H19* rs217727 C>T. Individuals with the TT genotype demonstrated a significantly higher

Table 2. Demographic characteristics of study subjects.

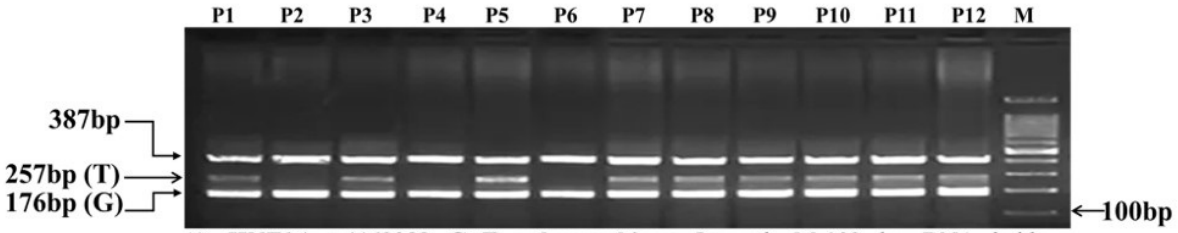
Parameters		Group I: Diabetic CAD (%)	Group II: Non-diabetic CAD (%)	Healthy controls	F/χ^2	*p*-value
		n = 100	*n* = 100	*n* = 100		
Age	Mean ± SD (years)	54.2 ± 10.2	53.2 ± 10.3	43.9 ± 11.9	27.4	<0.001
	20–40	9 (9%)	13 (13%)	32 (32%)		
	41–60	63 (63%)	62 (62%)	62 (62%)	31.26	<0.0001
	>61	28 (28%)	25 (25%)	6 (6%)		
Gender	Male	81 (81%)	89 (89%)	66 (66%)	16.25	0.0003
	Female	19 (19%)	11 (11%)	34 (34%)		
Diet	Non-vegetarian	81 (81%)	72 (72%)		2.25	0.1336
	Vegetarian	19 (19%)	28 (28%)			
Smoking	No	35 (35%)	39 (39%)		0.34	0.559
	Yes	65 (65%)	61 (61%)			
Alcoholic consumption	No	74 (74%)	75 (75%)		0.03	0.863
	Yes	26 (26%)	25 (25%)			
Tobacco chewer	No	51 (51%)	61 (61%)		2.03	0.154
	Yes	49 (49%)	39 (39%)			
Hypertensive	No	41 (41%)	80 (80%)		31.82	<0.0001
	Yes	59 (59%)	20 (20%)			
Total cholesterol	Normal (<200 mg/dL)	84 (84%)	92 (92%)		3.03	0.0817
	High (>200 mg/dL)	16 (16%)	8 (8%)			
Triglyceride	Normal (<150 mg/dL)	51 (51%)	75 (75%)		12.36	0.0004
	High (>150 mg/dL)	49 (49%)	25 (25%)			
HDL	Normal (>40 mg/dL)	40 (40%)	25 (25%)		5.13	0.0235
	High (<40 mg/dL)	60 (60%)	75 (75%)			
LDL	Normal (<100 mg/dL)	76 (76%)	79 (79%)		0.26	0.610
	High (>100 mg/dL)	24 (24%)	21 (21%)			
VLDL	Normal (<30 mg/dL)	52 (52%)	72 (72%)		8.49	0.0036
	High (>30 mg/dL)	48 (48%)	28 (28%)			
FBS	Normal (<100 mg/dL)	9 (9%)	25 (25%)		9.07	0.0026
	High (>100 mg/dL)	91 (91%)	75 (75%)			
HbA1c	Normal (<6.4%)	0 (0%)	100 (100%)		200.0	<0.0001
	High (>6.4%)	100 (100%)	0 (0%)			
Urea	Normal (<20 mg/dL)	12 (12%)	6 (6%)		2.2	0.138
	High (>20 mg/dL)	88 (88%)	94 (94%)			
Creatinine	Normal (<1.2 mg/dL)	73 (73%)	85 (85%)		4.34	0.037
	High (>1.2 mg/dL)	27 (27%)	15 (15%)			

Note: CAD, coronary artery disease; FBS, fasting blood sugar; HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very-low-density lipoprotein; SD, standard deviation; HbA1c, glycated hemoglobin.

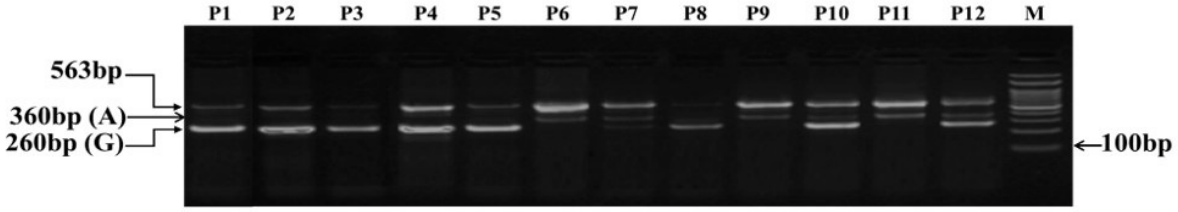
risk of developing CAD (OR = 5.6; 95% CI: 2.04–15.1, p = 0.0003). In the case of *TCF7L2* rs7903146 C>T polymorphism, comparative risk analysis revealed a significant association between CAD development and the heterozygous CT genotype, which predisposes the carriers to a markedly increased risk of CAD (OR = 3.4; 95% CI: 1.86–6.12; $p < 0.0001$). Comparative risk analysis highlights a borderline significant correlation of CAD development with the heterozygous GT genotype of the *HNF1A* rs1169288 G>T polymorphism. Individuals with the GT genotype demonstrated a slightly increased risk for CAD (OR = 1.96; 95% CI: 1.07–3.57; p = 0.027).

Comparative Risk Analysis of Diabetic CAD Development Associated With GSTP1, LncRNA H19, TCF7L2 and HNF1A Polymorphisms

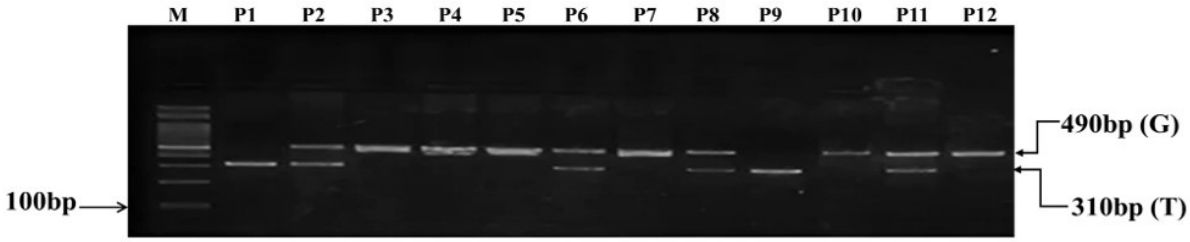
The comparative risk analysis results presented in Table 5 highlight a significant correlation between diabetic CAD development and the GG genotype of the *GSTP1* rs1695 A>G polymorphism. Individuals with the *GG* genotype demonstrated a markedly increased risk of developing CAD (OR = 12.4; 95% CI: 3.4–45.3; $p < 0.0001$). In addition, individuals with the heterozygous GA genotype also demonstrated a slightly higher risk of developing diabetic CAD (OR = 2.2; 95% CI: 1.2–4.1, p = 0.001). On the other hand, the heterozygous CT genotype of the LncRNA *H19* rs217727 C>T polymorphisms, as revealed by the comparative risk analysis, presented a marginally signif-



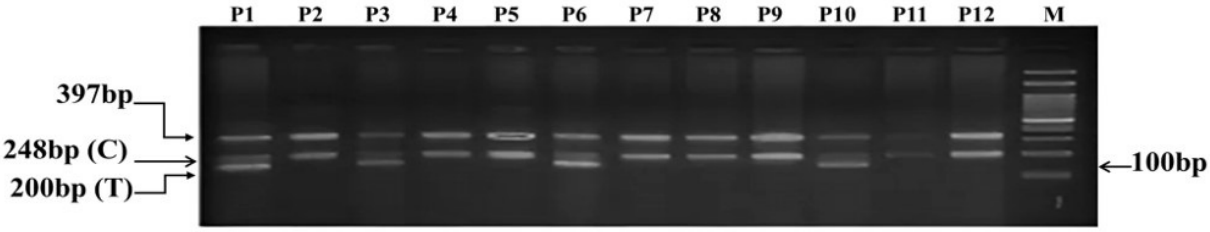
A) *HNF1A* rs1169288 G>T polymorphism: Legend: M-100 bp DNA ladder; homozygous GG -P2, P4 and P6 ; homozygous TT-0; heterozygous GT-P1, P3, P5, P7, P8, P9, P10, P11, P12.



B) *GSTP1* rs1695 A>G polymorphism : Legend: M-100 bp DNA ladder; homozygous GG –P1, P2, P3, P4, P5; homozygous AA-P6, P9,P11; heterozygous GA-P7, P10, P12.



C) *TCF7L2* rs12255372 G>T polymorphism: Legend: M-100 bp DNA ladder; homozygous GG-P3,P4,P5,P7,P10,P12; homozygous TT-P1,P9; heterozygous GT-P2, P6, P8, P11.



D) *LncRNA H19* rs217727 C>T polymorphism: Legend: M-100 bp DNA ladder; homozygous CC-P2, P4, P5, P7, P8, P9, P11, P12; homozygous TT-0; heterozygous GT-P1, P3, P6, P10

Fig. 1. Agarose gel electrophoresis image of (A) *HNF1A* rs1169288 G>T, (B) *GSTP1* rs1695 A>G, (C) *TCF7L2* rs12255372 G>T and (D) *LncRNA H19* rs217727 C>T polymorphism.

icant correlation with the development of diabetic CAD. Individuals with the CT genotype demonstrated a slightly higher risk of developing diabetic CAD (OR = 1.96; 95% CI: 1.09–3.51; $p = 0.02$). However, no significant correlation was observed with the homozygous TT genotype ($p = 0.86$). The comparative risk analysis for the development of diabetic CAD also reflects a significant correlation with the homozygous TT genotype of the *TCF7L2* rs7903146 C>T, which renders carriers more susceptible to diabetic CAD (OR = 3.1; 95% CI: 1.04–9.4; $p = 0.03$). Comparative risk analysis also revealed that the homozygous TT genotype of the *HNF1A* rs1169288 G>T polymorphism presented a significant correlation with the development of diabetic CAD, with individuals harboring the TT genotype demonstrating an increased risk for diabetic CAD (OR = 9.9; 95% CI: 1.18–83.54; $p = 0.035$).

Table 3. Genotype frequencies among CAD patients and healthy controls.

GSTP1 (rs1695 A>G)							
	AA	GA	GG	A allele	G allele	χ^2	*p*-value
Controls	54 (54%)	43 (43%)	3 (3%)	0.76	0.24		
Non-diabetic CAD	25 (25%)	57 (57%)	18 (18%)	0.53	0.47	23.3	**<0.0001**
Diabetic CAD	29 (29%)	51 (51%)	20 (20%)	0.54	0.46	20.8	**0.00003**
LncRNA *H19* (rs217727 C>T)							
	CC	CT	TT	C allele	T allele	χ^2	*p*-value
Controls	60 (60%)	34 (34%)	6 (6%)	0.77	0.23		
Non-diabetic CAD	36 (36%)	44 (44%)	20 (20%)	0.58	0.42	14.8	**0.0006**
Diabetic CAD	45 (45%)	50 (50%)	5 (5%)	0.70	0.30	5.28	0.07
TCF7L2 (rs7903146 C>T)							
	CC	CT	TT	C allele	T allele	χ^2	*p*-value
Controls	60 (60%)	35 (35%)	5 (5%)	0.78	0.22		
Non-diabetic CAD	32 (32%)	63 (63%)	5 (5%)	0.63	0.37	17.04	**0.0001**
Diabetic CAD	50 (50%)	37 (37%)	13 (13%)	0.68	0.32	4.7	0.09
HNF1A (rs1169288 G>T)							
	GG	GT	TT	G allele	T allele	χ^2	*p*-value
Controls	41 (41%)	58 (58%)	1 (1%)	0.67	0.33		
Non-diabetic CAD	26 (26%)	72 (72%)	2 (2%)	0.62	0.38	5.2	0.074
Diabetic CAD	33 (33%)	59 (59%)	8 (8%)	0.62	0.38	6.3	**0.04**

Note: Significant *p* values are denoted in boldface.

Table 4. The risk of developing non-diabetic CAD associated with *GSTP1*, LncRNA *H19*, *TCF7L2* and *HNF1A* polymorphisms.

	Controls	Non-diabetic CAD (%)	β	SE	Wald	OR (95% CI)	χ^2	*p*-value
GSTP1 (rs1695 A>G)								
AA (ref.)	54 (54%)	25 (25%)				1.0		
GA	43 (43%)	57 (57%)	1.05	0.32	11.14	2.9 (1.5–5.3)	11.4	**0.0007**
GG	3 (3%)	18 (18%)	2.56	0.67	14.67	12.9 (3.5–48.1)	19.8	**<0.0001**
LncRNA *H19* (rs217727 C>T)								
CC (ref.)	60 (60%)	36 (36%)				1.0		
CT	34 (34%)	44 (44%)	0.77	0.31	6.12	2.2 (1.2–3.96)	6.2	**0.01**
TT	6 (6%)	20 (20%)	1.72	0.51	11.26	5.6 (2.04–15.1)	12.8	**0.0003**
TCF7L2 (rs7903146 C>T)								
CC (ref.)	60 (60%)	32 (32%)				1.0		
CT	35 (35%)	63 (63%)	1.22	0.30	16.02	3.4 (1.86–6.12)	16.5	**<0.0001**
TT	5 (5%)	5 (5%)	0.63	0.67	0.88	1.9 (0.51–6.96)	0.9	0.34
HNF1A (rs1169288 G>T)								
GG (ref.)	41 (41%)	26 (26%)				1.0		
GT	58 (58%)	72 (72%)	0.67	0.31	4.80	1.96 (1.07–3.57)	4.9	0.027
TT	1 (1%)	2 (2%)	1.15	1.25	0.84	3.2 (0.27–36.6)	0.9	0.34

Note: Significant *p*-values are denoted in boldface. ref., reference group; β, regression coefficient; SE, standard error; OR, odds ratio; CI, confidence interval.

Genotype Distribution and Clinical Characteristics of Diabetic CAD Patients

Upon analyzing the correlation between the clinical characteristics of individuals with diabetic CAD and the genotype distributions (Table 6), a significant correlation was observed with respect to the LncRNA *H19* genotypes and the LDL level among diabetic CAD patients ($p = 0.01$). Specifically, the LncRNA *H19* TT genotype was found in 16.7% of the diabetic CAD patients with high-level LDL, compared to just 1.3% of the patient group with normal LDL blood levels. Diabetic CAD patients with LncRNA *H19* TT genotype showed higher blood levels of LDL (>100 mg/dL). In addition, a significant correlation was also observed with respect to the *HNF1A* genotypes and the blood urea level among diabetic CAD patients ($p = 0.026$). The *HNF1A* TT genotype was found in 9.1% of the diabetic

Table 5. The risk of developing diabetic CAD associated with *GSTP1*, LncRNA *H19*, *TCF7L2* and *HNF1A* genotypes.

	Controls	Diabetic CAD (%)	β	SE	Wald	OR (95% CI)	χ^2	*p*-value
GSTP1 (rs1695 A>G)								
AA (ref.)	54 (54%)	29 (29%)				1.0		
GA	43 (43%)	51 (51%)	0.79	0.31	6.55	2.2 (1.2–4.1)	6.6	**0.009**
GG	3 (3%)	20 (20%)	2.52	0.66	14.54	12.4 (3.4–45.3)	19.6	**<0.0001**
LncRNA *H19* (rs217727 C>T)								
CC (ref.)	60 (60%)	45 (45%)				1.0		
CT	34 (34%)	50 (50%)	0.67	0.29	5.14	1.96 (1.09–3.5)	5.2	**0.02**
TT	6 (6%)	5 (5%)	0.11	0.64	0.03	1.1 (0.31–3.9)	0.03	0.86
TCF7L2 (rs7903146 C>T)								
CC (ref.)	60 (60%)	50 (50%)				1.0		
CT	35 (35%)	37 (37%)	0.24	0.30	0.61	1.3 (0.69–2.3)	0.6	0.43
TT	5 (5%)	13 (13%)	1.14	0.56	4.13	3.1 (1.04–9.4)	4.4	0.03
HNF1A (rs1169288 G>T)								
GG (ref.)	41 (41%)	33 (33%)				1.0		
GT	58 (58%)	59 (59%)	0.23	0.29	0.62	1.3 (0.70–2.3)	0.6	0.43
TT	1 (1%)	8 (8%)	2.29	1.09	4.47	9.9 (1.18–83.54)	4.65	**0.035**

Note: Significant *p*-values are denoted in boldface.

CAD patients with high-level urea; in contrast, the homozygous TT genotype was not detected in any of the patients with normal urea level. Diabetic CAD patients with *HNF1A* GT (62.5%) and TT (9.1%) genotypes showed higher blood levels of urea (>20 mg/dL). However, no such correlation was observed with other clinical characteristics of diabetic CAD patients such as hypertension, diabetes duration, total cholesterol, triglyceride, HDL, VLDL, fasting blood sugar (FBS), and creatinine.

Genotype Distribution and Clinical Characteristics of Non-Diabetic CAD Patients

Upon analyzing the correlation between the clinical characteristics of individuals with non-diabetic CAD and the genotype distributions (Table 7), a significant correlation was observed with respect to the *TCF7L2* genotypes and blood urea level among the non-diabetic CAD patients (p = 0.02). The *TCF7L2* CT genotype was observed in 66.3% of non-diabetic CAD patients with high blood urea level, compared to just 25% of the patient group with normal blood urea level. The *TCF7L2* TT genotype was observed in 5.4% of non-diabetic CAD patients with high blood urea level; on the contrary, the *TCF7L2* TT genotype was not detected in any of the patients with normal urea level. A significant correlation was also observed with respect to the *HNF1A* genotypes and the blood urea level among non-diabetic CAD patients (p = 0.04). The *HNF1A* GT genotype was observed in 75% of non-diabetic CAD patients with high blood urea level, which was markedly higher than 37.5% of the patients with normal blood urea level. In addition, 2.2% of the non-diabetic CAD patients with high blood urea level harbor the *HNF1A* TT genotype, but this genotype was not detected in the patients with normal urea level. A significant correlation was also observed with respect to the *HNF1A* genotypes and VLDL level among non-diabetic CAD patients (p = 0.01). A higher *HNF1A* GT genotype frequency was observed in the non-diabetic CAD patients with high VLDL level (82.1%), as compared to those with normal VLDL level (68.1%). In addition, 7.1% of non-diabetic CAD patients with high VLDL levels harbor the *HNF1A* TT genotype, which was not detected in patients with normal urea levels carrying the *HNF1A* TT genotype.

Discussion

The occurrence of CVD often precedes the onset of T2DM, which significantly increases the risk of complications related to CVDs. This correlation indicates that both conditions share an overlapping set of genetic and environmental factors, which raises the disease susceptibility, consistent with the postulation by Stern that these conditions arise from a shared foundational context or "common soil" [21]. The phenotypic manifestations of T2DM and CAD share several common risk factors, including insulin resistance, dyslipidemia, hyperglycemia, and obesity [22]. According to the Genome-Wide Association Study (GWAS), T2DM-associated loci showing positive correlations between body mass index (BMI) and disease risk suggest that the effects are primarily driven by adiposity. [23]. Similarly, in liver samples from CAD patients, the fine-mapping of adiposity-associated MAF BZIP Transcription Factor B (MAFB) loci suggested that the CAD association is mediated by changes in MAFB expression [24].

Table 6. Genotype distribution and clinical characteristics of diabetic CAD patients.

Clinical features	Variables	*GSTP1* (rs1695 A>G)			χ^2	*p*-value	LncRNA *H19* (rs217727 C>T)			χ^2	*p*-value	T*CF7L2* (rs7903146 C>T)			χ^2	*p*-value	H*NF1A* (rs1169288 G>T)			χ^2	*p*-value
	Genotypes	AA	GA	GG			CC	CT	TT			CC	CT	TT			GG	GT	TT		
Hypertension	No	9 (22.0%)	21 (51.2%)	11 (26.8%)	2.8	0.25	20 (48.8%)	18 (43.9%)	3 (7.3%)	1.48	0.47	17 (41.5%)	17 (41.5%)	7 (17.1%)	2.27	0.32	11 (26.8%)	26 (63.4%)	4 (9.8%)	1.29	0.52
	Yes	20 (33.9%)	30 (50.8%)	9 (15.3%)			25 (42.4%)	32 (54.2%)	2 (3.4%)			33 (55.9%)	20 (33.9%)	6 (10.2%)			22 (37.3%)	33 (55.9%)	4 (6.8%)		
Diabetes duration	≤5 years	24 (33.8%)	36 (50.7%)	11 (15.5%)	4.4	0.11	28 (39.4%)	40 (56.3%)	3 (4.2%)	3.95	0.14	37 (52.1%)	26 (36.6%)	8 (11.3%)	0.79	0.67	23 (32.4%)	42 (59.2%)	6 (8.5%)	0.09	0.96
	>5 years	5 (17.2%)	15 (51.7%)	9 (31.0%)			17 (58.6%)	10 (34.5%)	2 (6.9%)			13 (44.8%)	11 (37.9%)	5 (17.2%)			10 (34.5%)	17 (58.6%)	2 (6.9%)		
Total cholesterol	<200 mg/dL	22 (25.6%)	46 (53.5%)	18 (20.9%)	3.5	0.18	35 (40.7%)	47 (54.7%)	4 (4.7%)	5.3	**0.06**	41 (47.7%)	32 (37.2%)	13 (15.1%)	2.9	0.25	29 (33.7%)	51 (59.3%)	6 (7.0%)	0.91	0.63
	≥200 mg/dL	7 (50.0%)	5 (35.7%)	2 (14.3%)			10 (71.4%)	3 (21.4%)	1 (7.1%)			9 (64.3%)	5 (35.7%)	0 (0.0%)			4 (28.6%)	8 (57.1%)	2 (14.3%)		
Triglyceride	<150 mg/dL	14 (27.5%)	26 (51.0%)	11 (21.6%)	0.21	0.89	20 (39.2%)	29 (56.9%)	2 (3.9%)	1.99	0.37	22 (43.1%)	21 (41.2%)	8 (15.7%)	2.04	0.36	17 (33.3%)	31 (60.8%)	3 (5.9%)	0.64	0.73
	>150 mg/dL	15 (30.6%)	25 (51.0%)	9 (18.4%)			25 (51.0%)	21 (42.9%)	3 (6.1%)			28 (57.1%)	16 (32.7%)	5 (10.2%)			16 (32.7%)	28 (57.1%)	5 (10.2%)		
HDL	<60 mg/dL	25 (28.7%)	45 (51.7%)	17 (19.5%)	0.16	0.93	38 (43.7%)	44 (50.6%)	5 (5.7%)	1.1	0.59	44 (50.6%)	31 (35.6%)	12 (13.8%)	0.71	0.70	27 (31.0%)	52 (59.8%)	8 (9.2%)	2.1	0.36
	>60 mg/dL	4 (30.8%)	6 (46.2%)	3 (23.1%)			7 (53.8%)	6 (46.2%)	0 (0.0%)			6 (46.2%)	6 (46.2%)	1 (7.7%)			6 (46.2%)	7 (53.8%)	0 (0.0%)		
LDL	<100 mg/dL	21 (27.6%)	40 (52.6%)	15 (19.7%)	0.38	0.83	36 (47.4%)	39 (51.3%)	1 (1.3%)	9.1	**0.01**	35 (46.1%)	32 (42.1%)	9 (11.8%)	3.5	0.17	26 (34.2%)	45 (59.2%)	5 (6.6%)	0.94	0.62
	>100 mg/dL	8 (33.3%)	11 (45.8%)	5 (20.8%)			9 (37.5%)	11 (45.8%)	4 (16.7%)			15 (62.5%)	5 (20.8%)	4 (16.7%)			7 (29.2%)	14 (58.3%)	3 (12.5%)		
VLDL	<30 mg/dL	14 (26.9%)	25 (48.1%)	13 (25.0%)	1.7	0.43	21 (40.4%)	28 (53.8%)	3 (5.8%)	0.96	0.62	21 (40.4%)	21 (40.4%)	10 (19.2%)	5.6	**0.06**	16 (30.8%)	33 (63.5%)	3 (5.8%)	1.2	0.55
	>30 mg/dL	15 (31.3%)	26 (54.2%)	7 (14.6%)			24 (50.0%)	22 (45.8%)	2 (4.2%)			29 (60.4%)	16 (33.3%)	3 (6.3%)			17 (35.4%)	26 (54.2%)	5 (10.4%)		
FBS	<100 mg/dL	1 (11.1%)	7 (77.8%)	1 (11.1%)	2.9	0.24	3 (33.3%)	6 (66.7%)	0 (0.0%)	1.34	0.51	6 (66.7%)	3 (33.3%)	0 (0.0%)	1.9	0.39	5 (55.6%)	4 (44.4%)	0 (0.0%)	2.7	0.36
	>100 mg/dL	28 (30.8%)	44 (48.4%)	19 (20.9%)			42 (46.2%)	44 (48.4%)	5 (5.5%)			44 (48.4%)	34 (37.4%)	13 (14.3%)			28 (30.8%)	55 (60.4%)	8 (8.8%)		
Urea	<20 mg/dL	6 (50.0%)	4 (33.3%)	2 (16.7%)	2.98	0.23	5 (41.7%)	6 (50.0%)	1 (8.3%)	0.34	0.85	7 (58.3%)	4 (33.3%)	1 (8.3%)	0.47	0.79	8 (66.7%)	4 (33.3%)	0 (0.0%)	**7.3**	**0.026**
	>20 mg/dL	23 (26.1%)	47 (53.4%)	18 (20.5%)			40 (45.5%)	44 (50.0%)	4 (4.5%)			43 (48.9%)	33 (37.5%)	12 (13.6%)			25 (28.4%)	55 (62.5%)	8 (9.1%)		
Creatinine	<1.2 mg/dL	24 (32.9%)	35 (47.9%)	14 (19.2%)	1.98	0.37	30 (41.1%)	38 (52.1%)	5 (6.8%)	2.9	0.22	35 (47.9%)	29 (39.7%)	9 (12.3%)	0.87	0.65	24 (32.9%)	42 (57.5%)	7 (9.6%)	0.95	0.62
	>1.2 mg/dL	5 (18.5%)	16 (59.3%)	6 (22.2%)			15 (55.6%)	12 (44.4%)	0 (0.0%)			15 (55.6%)	8 (29.6%)	4 (14.8%)			9 (33.3%)	17 (63.0%)	1 (3.7%)		

Note: Significant *p*-values are denoted in boldface.

Table 7. Genotype distribution and clinical characteristics of non-diabetic CAD patients.

Clinical features	Variables	*GSTP1* (rs1695 A>G)			χ^2	*p*-value	LncRNA *H19* (rs217727 C>T)			χ^2	*p*-value	T*CF7L2* (rs7903146 C>T)			χ^2	*p*-value	H*NF1A* (rs1169288 G>T)			χ^2	*p*-value
	Genotypes	AA	GA	GG			CC	CT	TT			CC	CT	TT			GG	GT	TT		
Hypertension	No	21 (26.3%)	46 (57.5%)	13 (16.3%)	0.95	0.62	29 (36.3%)	32 (40.0%)	19 (23.8%)	4.3	0.12	25 (31.3%)	51 (63.7%)	4 (5.0%)	0.11	0.95	21 (26.3%)	57 (71.3%)	2 (2.5%)	0.54	0.76
	Yes	4 (20.0%)	11 (55.0%)	5 (25.0%)			7 (35.0%)	12 (60.0%)	1 (5.0%)			7 (35.0%)	12 (60.0%)	1 (5.0%)			5 (25.0%)	15 (75.0%)	0 (0.0%)		
Total cholesterol	<200 mg/dL	23 (25.0%)	53 (57.6%)	16 (17.4%)	0.31	0.86	34 (37.0%)	41 (44.6%)	17 (18.5%)	1.71	0.43	29 (31.5%)	58 (63.0%)	5 (5.4%)	0.52	0.72	25 (27.2%)	65 (70.7%)	2 (2.2%)	1.1	0.59
	≥200 mg/dL	2 (25.0%)	4 (50.0%)	2 (25.0%)			2 (25.0%)	3 (37.5%)	3 (37.5%)			3 (37.5%)	5 (62.5%)	0 (0.0%)			1 (12.5%)	7 (87.5%)	0 (0.0%)		
Triglyceride	<150 mg/dL	18 (24.0%)	43 (57.3%)	14 (18.7%)	0.20	0.91	26 (34.7%)	33 (44.0%)	16 (21.3%)	0.42	0.81	26 (34.7%)	45 (60.0%)	4 (5.3%)	1.2	0.56	23 (30.7%)	51 (68.0%)	1 (1.3%)	3.8	0.15
	>150 mg/dL	7 (28.0%)	14 (56.0%)	4 (16.0%)			10 (40.0%)	11 (44.0%)	4 (16.0%)			6 (24.0%)	18 (72.0%)	1 (4.0%)			3 (12.0%)	21 (84.0%)	1 (4.0%)		
LDL	<100 mg/dL	18 (22.8%)	46 (58.2%)	15 (19.0%)	1.04	0.59	31 (39.2%)	34 (43.0%)	14 (17.7%)	2.15	0.34	23 (29.1%)	51 (64.6%)	5 (6.3%)	2.45	0.29	21 (26.6%)	56 (70.9%)	2 (2.5%)	0.65	0.99
	>100 mg/dL	7 (33.3%)	11 (52.4%)	3 (14.3%)			5 (23.8%)	10 (47.6%)	6 (28.6%)			9 (42.9%)	12 (57.1%)	0 (0.0%)			5 (23.8%)	16 (76.2%)	0 (0.0%)		
VLDL	<30 mg/dL	18 (25.0%)	41 (56.9%)	13 (18.1%)	0.001	1.00	26 (36.1%)	30 (41.7%)	16 (22.2%)	0.95	0.62	26 (36.1%)	42 (58.3%)	4 (5.6%)	2.40	0.30	23 (31.9%)	49 (68.1%)	0 (0.0%)	9.1	**0.01**
	>30 mg/dL	7 (25.0%)	16 (57.1%)	5 (17.9%)			10 (35.7%)	14 (50.0%)	4 (14.3%)			6 (21.4%)	21 (75.0%)	1 (3.6%)			3 (10.7%)	23 (82.1%)	2 (7.1%)		
FBS	<100 mg/dL	5 (20.0%)	13 (52.0%)	7 (28.0%)	2.33	0.31	11 (44.0%)	13 (52.0%)	1 (4.0%)	5.34	0.06	6 (24.0%)	17 (68.0%)	2 (8.0%)	1.39	0.49	5 (20.0%)	20 (80.0%)	0 (0.0%)	1.43	0.49
	>100 mg/dL	20 (26.7%)	44 (58.7%)	11 (14.7%)			25 (33.3%)	31 (41.3%)	19 (25.3%)			26 (34.7%)	46 (61.3%)	3 (4.0%)			21 (28.0%)	52 (69.3%)	2 (2.7%)		
Urea	<20 mg/dL	1 (12.5%)	5 (62.5%)	2 (25.0%)	0.83	0.66	3 (37.5%)	4 (50.0%)	1 (12.5%)	0.32	0.85	6 (75.0%)	2 (25.0%)	0 (0.0%)	7.5	**0.02**	5 (62.5%)	3 (37.5%)	0 (0.0%)	6.1	**0.04**
	>20 mg/dL	24 (26.1%)	52 (56.5%)	16 (17.4%)			33 (35.9%)	40 (43.5%)	19 (20.7%)			26 (28.3%)	61 (66.3%)	5 (5.4%)			21 (22.8%)	69 (75.0%)	2 (2.2%)		
Creatinine	<1.2 mg/dL	20 (23.5%)	48 (56.5%)	17 (20.0%)	1.78	0.41	31 (36.5%)	37 (43.5%)	17 (20.0%)	0.06	0.96	29 (34.1%)	52 (61.2%)	4 (4.7%)	1.19	0.55	23 (27.1%)	60 (70.6%)	2 (2.4%)	0.75	0.69
	>1.2 mg/dL	5 (33.3%)	9 (60.0%)	1 (6.7%)			5 (33.3%)	7 (46.7%)	3 (20.0%)			3 (20.0%)	11 (73.3%)	1 (6.7%)			3 (20.0%)	12 (80.0%)	0 (0.0%)		

Note: Significant *p*-values are denoted in boldface.

Demographic Characteristics

In our study, the demographic characteristics such as mean age, dietary preferences, and lifestyle factors (e.g., alcohol intake) were comparable between the non-diabetic and diabetic CAD patients. Some of these are independent risk factors for both CAD and diabetes and might have cumulative effects on the disease outcome in diabetic CAD patients. The majority of the CAD patients in this study, regardless of their diabetes status, indicated a non-vegetarian diet as their primary choice of diet. Dietary risks have been recognized as the second most impactful CVD factor [25]. Previous studies have reported significantly lower risks of ischemic heart disease and the associated mortality in vegetarians than in non-vegetarians [25,26]. Traditionally, the global trend shows that men have a higher risk of coronary heart disease than women [27]. However, women with T2DM experience a higher relative risk of cardiovascular disease and mortality compared to men. Additionally, young women with type 2 diabetes are currently less likely than their male counterparts to receive the recommended treatments and interventions aimed at reducing CVD risk [28]. In our study cohorts, the percentage of male patients was significantly higher than female patients, irrespective of diabetic or non-diabetic CAD, implicating the gender bias of CAD risk associated with male sex. It is to be noted that strategies for the prevention and management of CAD in the existing medical guidelines lack sex-specific or gender-sensitive nuances for optimizing the treatment effects. Separately, in this study, smoking habit was found to be prevalent among subjects in both the CAD patient groups. It has long been recognized that smoking can elicit oxidative processes, as well as affect platelet function, fibrinolysis, inflammation, and vasomotor function, causing detrimental effects on endothelial function, which effectively doubles the 10-year risk of fatal events in smokers compared to non-smokers [29]. Moreover, a recent GWAS consisting of 1.2 million participants reported that smoking initiation was positively related to CAD risk in patients with diabetes by analyzing the genetic signatures for smoking [30].

Clinical Characteristics

In our study, the majority of diabetic CAD patients were in a hypertensive state. In the hypertensive context, endothelial dysfunction may play a role in triggering and advancing vascular inflammation, vascular remodeling, and atherosclerosis, and it is also independently linked to higher cardiovascular risk. Endothelial dysfunction may also augment peripheral resistance through various mechanisms, resulting in greater constriction and vascular remodeling of resistance arteries, which are linked to the development and complications of hypertension [31]. It is well established that hypertension and T2DM are common comorbidities, with the incidence of hypertension being twice that in patients with diabetes compared with non-diabetic people. Additionally, the major cause of morbidity and mortality in diabetes is CVD, which is exacerbated by hypertension [32]. Lipid profile showed the triglycerides and VLDL levels were higher in the diabetic CAD patient group in comparison to the non-diabetic CAD patient group. Studies have indicated that a higher triglyceride level tends to increase the risk of coronary heart disease and that the impact of triglycerides on CVD showed prominence among T2DM individuals with a mean age $<$65 year [33,34], which might be a significant factor in the current study as approximately 75% of them were between 20 and 60 years of age. The progression of coronary atherosclerosis is strongly influenced by the interplay between diabetes-related factors and other factors, such as abnormal lipid metabolism [34]. Hypertriglyceridemia aggravates diabetes through the impairment of the pancreatic β-cell function, which decreases the glucose-induced insulin secretion and raises the glycemic level in T2DM patients, thereby significantly increasing the risk of cardiovascular disease [35]. The Reduction of Cardiovascular Events with Icosapent Ethyl-Intervention Trial (REDUCE-IT) revealed that patients with elevated triglyceride levels experienced a significantly reduced risk of major ischemic events, including cardiovascular death, when treated with icosapent ethyl compared to a placebo [36]. A recent Mendelian randomization analysis demonstrating a genetic association between T2DM and an increased coronary atherosclerosis risk also observed a suggestive causal link between T2DM and VLDL levels in coronary atherosclerosis pathogenesis [37]. The study further reported that T2DM was associated with a 13.35% increased risk for coronary atherosclerosis; in patients with T2DM, VLDL levels rose by 2.49%; and for each standard deviation increase in VLDL, the risk of heart disease increased by 29.6%. The urea levels were found to be significantly higher in both the CAD patient groups. Clinical studies have shown that blood urea nitrogen (BUN) levels are elevated in patients with cardiovascular disease, establishing it as a valuable prognostic biomarker for CVD compared to other indicators of kidney function [38]. An earlier prospective study involving 9420 patients indicated that higher BUN levels were associated with increased mortality in cases of acute coronary syndrome [39].

The most common type of genetic variation in the human genome is SNPs, which involve variations in the single-nucleotide base pair among individuals. SNPs located in gene promoter regions or coding regions can engender critical functional effects, and certain SNPs can serve as biomarkers for the pathogenesis of disease [40] by virtue of their ability to alter gene function and expression, thereby influencing disease susceptibility and treatment responses.

GSTP1 rs1695 A>G Polymorphism

GSTP1 is a member of the GST superfamily, which plays a significant role in neutralizing oxidative stress, a hallmark of cardiovascular disease [41]. One of its well-

analyzed polymorphisms is *GSTP1* rs1695 (313 A>G, Ile105Val), where guanine (G) replaces adenine (A) at the 313rd position of exon 5 within the *GSTP1* coding region. This change results in the substitution of valine (Val) for isoleucine (Ile) at the 105th amino acid position, and such genetic changes may alter the function of the GSTP1 metabolic enzyme. The rs1695 G allele contributes to poor thermal stability and catalytic activity in the enzyme. Enzyme activity in individuals carrying the GG genotype is lower than in those with the AG genotype, which is generally marked by reduced or even absent GSTP1 enzyme activity [42]. In our study, the GG genotype of the *GSTP1* (rs1695 A>G) gene polymorphism was considerably more prevalent in both the CAD patient groups compared to healthy controls, contributing to a higher frequency of the G allele and markedly higher risk of developing CAD in non-diabetic and diabetic CAD patients. Moreover, the heterozygous GA genotype was associated with a slightly higher risk of developing CAD in both the CAD patient groups in comparison to healthy individuals. This heterozygous genotype, and its constituent alleles, appear to predispose the carriers to CAD development, making it a common genetic biomarker irrespective of the associated risk of T2DM comorbidity.

LncRNA H19 rs217727 C>T Polymorphismp

Long non-coding RNAs (LncRNAs) are non-protein-coding RNAs that exceed 200 nucleotides in length and play a role in regulating gene expression both at the transcriptional and post-transcriptional levels [43]. The *H19* gene, which encodes LncRNA *H19*, is significantly expressed in human atherosclerotic plaques and perivascular adipose tissue of patients with CAD [44,45]. Studies have indicated that elevated plasma levels of LncRNA *H19* are linked to a higher risk for CAD, suggesting that LncRNA *H19* may play a role in the onset and development of atherosclerosis [45,46]. Angiogenesis plays a crucial role in the growth and stability of plaques within atherosclerotic lesions. Studies have shown that the inhibition of LncRNA *H19* significantly retards the growth of endothelial cells and reduces the formation of capillary-like structures [47]. LncRNA *H19* is also thought to promote lipid metabolic disorders arising from the foam cells, whose formation is considered a crucial step in the development of atherosclerosis, by inhibiting lipid metabolism and enhancing lipid accumulation [48,49]. This process contributes to the progression of atherosclerosis. Our results demonstrated that there was a significant difference in the LncRNA-*H19* rs217727 C>T gene polymorphism between non-diabetic CAD patients and healthy controls, with the TT genotype being considerably more prevalent among subjects in the non-diabetic CAD patient group compared to the healthy controls. The non-diabetic CAD patients harboring the TT genotype demonstrated a markedly higher risk of developing CAD, whereas the heterozygous CT genotype was found to be marginally associated with the increased risk for the disease among the diabetic CAD patients. This polymorphic variation might affect the expression levels of H19, which have been correlated with obesity indices and homeostasis model assessment of insulin resistance values [50].

To the best of our knowledge, this is the first study on the risk of CAD associated with the LncRNA-*H19* rs217727 C>T polymorphism, leaving behind implications for further functional characterization of LncRNA *H19* associated with atherosclerotic lesions.

TCF7L2 rs7903146 C>T Polymorphism

The *TCF7L2* gene encodes a transcription factor that plays a critical role in the Wnt signaling pathway, which is essential for the development of pancreatic islets. This gene also stimulates the expression of several important genes, including those for the insulinotropic hormone glucagon-like peptide 1 (GLP-1), the insulin gene, and other genes that encode proteins involved in the processing and exocytosis of insulin granules [51]. *TCF7L2* expression leads to the disruption of the pancreatic islet function, thereby lowering insulin secretion as it modifies the effect of incretins on insulin secretion by reducing sensitivity in beta cells [52]. Our findings did not show a significant correlation of *TCF7L2* rs7903146 C>T polymorphism with the risk and development of diabetic CAD. Interestingly, a significant difference was detected in genotype frequencies between non-diabetic CAD patients and healthy controls, with the heterozygous CT genotype considerably more prevalent in the non-diabetic CAD patients and associated with markedly increased risk for CAD. Although studies have shown the influence of this gene polymorphism on the development and progression of T2DM, we hypothesize that this genetic variation might be an independent risk factor associated with CAD, with the risk heightened through insulin resistance, a common pathological condition observed in both the T2DM and CVD cases [53,54]. An earlier study by Sousa and coworkers [55] demonstrated that while diabetic patients exhibited a higher prevalence of coronary lesions, no link was found between *TCF7L2* genotype and coronary lesions, whereas non-diabetic individuals carrying the T allele had a significantly greater frequency of coronary lesions compared to non-carriers. Additionally, non-diabetic individuals harboring the CT or TT genotypes were more susceptible to developing multi-vessel CAD, and experienced more composite cardiovascular events, particularly an increased incidence of death, compared to those carrying the CC genotype.

HNF1A rs1169288 G>T Polymorphism

HNF1A is a transcription factor involved in the development and functionality of pancreatic ß-islet cells. Rare mutations in the *HNF1A* gene lead to a monogenic type of diabetes known as maturity-onset diabetes of the young (MODY3), whereas polymorphic variations in the *HNF1A*

gene have shown a modest association with T2DM and glycemic characteristics across various populations [56]. Research has indicated a link between the *HNF1A* p.I27L (rs1169288) variant and the risk of T2DM and dysglycemia in individuals with normal weight; however, this association does not appear in those who are overweight. Additionally, among carriers of the 27L variant, age, rather than body mass index, was significantly correlated with the risk of dysglycemia [57]. However, in an earlier study, such an association with T2DM was observed in the overweight elderly subjects, indicating a potential correlation of BMI with diabetes [58]. In the current study, genotype frequencies for the *HNF1A* rs1169288 G>T polymorphism demonstrated a significant difference between healthy controls and diabetic CAD patients, but not non-diabetic CAD patients. The TT genotype was considerably more prevalent in diabetic CAD patients, who exhibited a higher frequency of the T allele compared to controls. On the other hand, the heterozygous GT genotype demonstrated an association with a slightly increased risk for CAD in the non-diabetic patients, while the homozygous TT genotype showed a correlation with significantly higher risk for CAD in the diabetic patients. It has been previously reported that this genetic variation was significantly associated with serum lipid levels in both controls and CAD patients [59]. Interestingly, the study further demonstrated significant linkage disequilibrium among the four SNPs ($r^2 > 0.5$, $D' > 0.8$) and the haplotype combining rs1169288A, rs2259820C, rs2464196G and rs2650000A was found to be associated with an increased risk of CAD.

Limitations

While this study provides valuable insights into the genetic and lifestyle factors associated with CAD, there are certain limitations that must be considered. The cross-sectional design of the study restricts the ability to establish causal relationships between genotype frequencies and CAD risk, as it does not account for longitudinal changes or temporal associations. The focus on a specific population may restrict the generalizability of the results to the diverse ethnic and demographic groups; therefore, this underscores the need for further research that includes larger cohorts consisting ethnically and demographically diverse sample to validate these findings.

Conclusion

In conclusion, the current study offers valuable insights into the associations of gene polymorphisms in certain key candidate genes with the risk and susceptibility to CAD with and without T2DM comorbidity. Furthermore, this distinctiveness of certain genetic variants within a specific population makes them potential genetic markers for diseases, which can serve as a tool for personalized medicine in both therapeutic approaches and disease prognosis. The findings from this study might have significant implications for the early diagnosis and targeted therapy of CAD, particularly in differentiating between diabetic and non-diabetic patients. The significant prevalence of specific genotypes, such as the GG genotype of the *GSTP1* gene among CAD patients, emphasizes the potential of genetic screening as a means to identify individuals at heightened risk of developing CAD, regardless of diabetic status. Moreover, the distinct genotype patterns in *LNRHP19* and *TCF7L2* genes observed in the non-diabetic CAD patients suggest a pathway for tailored preventive strategies in this patient subgroup. Besides, the association of *HNF1A* genotype with diabetic CAD patients augments the importance of considering metabolic conditions during CAD risk assessment.

Availability of Data and Materials

All data can be accessed on request from the corresponding author.

Author Contributions

Conceptualization, PKD, RM and MU; Data curation, PKD, MU, JB, RB and MAA; Formal analysis, JJ and EH; Investigation, PKD and VM; Methodology, RM, JJ, MMJ and IE; Software, JJ, EH, MMJ and RB; Supervision, RM and VM; Validation, RM, IE, MAA and SKM. All authors were involved in the drafting or critical revision of the manuscript. Further, all authors have 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 International and National regulations and in accordance with the Declaration of Helsinki. The study was approved by the Institutional Ethical Committee of Maulana Azad Medical College and associated hospitals, Delhi, India (F1/IEC/MAMC/85/03/21/no.422; Dt-30.08.2021). All patients provided their informed consent in this study.

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Conflict of Interest

The authors declare no conflict of interest.

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