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# The Impact Of Rs2066715 And Rs2230808 Polymorphisms In ABCA1 Gene With The Risk Of Diabetes And Coronary Artery Disease In North Indians

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**Abstract**
**Background:** Polymorphisms in the ABCA1 gene are linked to lipid metabolism, insulin resistance, and cardiovascular disease. This study assessed the correlation between ABCA1 rs2066715 and rs2230808 polymorphisms and Type 2 Diabetes Mellitus (T2DM), Coronary Artery Disease (CAD), and T2DM with CAD in a North Indian cohort.
**Material and Method:** A total of 600 participants were separated into four groups: healthy controls, T2DM, CAD, and T2DM with CAD patients (150 each). Clinical and biochemical data were analyzed. The rs2066715 and rs2230808 polymorphisms were genotyped using the PCR-RFLP method. The statistical analysis was carried out with SPSS version 31.
**Result:** T2DM, CAD, and T2DM with CAD patients had significantly higher FBG, TC, TG, LDL-C, HbA1c, blood pressure, and fasting insulin levels, but HDL-C levels were significantly lower ($p < 0.05$). There was no significant connection between the rs2066715 polymorphism and illness susceptibility. However, the rs2230808 polymorphism was significantly associated with T2DM, CAD, and T2DM with CAD. The A allele and TA+AA genotypes were linked to higher disease risk and a poor lipid profile. Haplotype study found that the CT haplotype is protective, whereas the CA and TA haplotypes enhance disease risk.
**Conclusion:** The ABCA1 rs2230808 polymorphism may serve as an important genetic marker for T2DM and CAD susceptibility in the North Indian population.

**Keywords:** ABCA1 gene, Coronary artery disease, Lipid metabolism, Haplotype analysis, North Indian Population
**Abbreviations:** ABCA1 = ATP-binding cassette transporter A1, T2DM = Type 2 Diabetes Mellitus, CAD = Coronary Artery Disease, CVD = Cardiovascular Disease, SNP = Single Nucleotide Polymorphism, PCR-RFLP = Polymerase Chain Reaction–Restriction Fragment Length Polymorphism, DNA = Deoxyribonucleic Acid, FBG = Fasting Blood Glucose

## 1. Introduction:

Diabetes mellitus is a chronic metabolic disorder in which there is hyperglycemia because of either less insulin or resistance to insulin [1]. The global burden of diabetes is quickly increasing, with over 536 million persons affected in 2021, and the figure is predicted to rise significantly by 2045 [2]. India is one of the countries with a maximum number of diabetic patients and a high percentage of the patients are not diagnosed [3]. In “Type 2 diabetes mellitus (T2DM)”, hyperglycemia and insulin resistance are associated with development of numerous comorbidities including cardiovascular disease (CVD), nephropathy, neuropathy, and retinopathy [4].
The “ATP-binding cassette transporter A1 (ABCA1)” gene plays a role in cholesterol homeostasis among the genetic variables involved in lipid metabolism and cardiovascular issues [5]. The ABCA1 gene resides at the 9q31.1 locus and is highly expressed in different organs such as liver, heart, pancreas, gut, macrophages, and endothelial cells [6]. It regulates reverse cholesterol transport by promoting the clearance of intracellular cholesterol and phospholipids to apolipoprotein A-I, leading to the production of nascent high-density lipoprotein (HDL) cholesterol [7]. ABCA1 gene polymorphisms have been associated with impaired lipid transport, low HDL-C and high risk for metabolic and cardiovascular diseases [8].
ABCA1 helps regulate pancreatic β-cell cholesterol levels and insulin release. Glucose metabolism may be impaired by cholesterol accumulation in β-cells that may result in T2DM [9]. Diabetics are at an increased risk for coronary artery disease (CAD) which is the leading cause of death in people with T2DM [10]. Different Single Nucleotide Polymorphisms

(SNPs) of the ABCA1 gene were reported to be associated with lipid metabolism and cardiovascular risk in different ethnic groups [11]. Of these, the rs2066715 and rs2230808 polymorphisms have been attracting considerable interest given their potential association with dyslipidaemia, insulin resistance and CAD risk [12].

### 1.1. Role of ABCA1 gene in lipid metabolism and disease susceptibility

The "ATP-binding cassette transporter A1 (ABCA1)" gene is a key regulator of cholesterol metabolism and reverse cholesterol transfer [13]. It promotes the transport of intracellular cholesterol and phospholipids to apolipoprotein A-I, which results in the synthesis of high-density lipoprotein cholesterol (HDL-C) [14]. Alterations to the ABCA1 gene may disrupt lipid transport, leading to dyslipidaemia and an increased risk of cardiovascular disease. ABCA1 regulates lipid levels, pancreatic β-cell activity, and insulin production [15]. Genetic variants in the ABCA1 gene, specifically rs2066715 and rs2230808, have been linked to aberrant lipid profiles, insulin resistance, Type 2 (T2DM), and (CAD) in many populations [16]. These polymorphisms may contribute to disease vulnerability by disrupting cholesterol balance and glucose metabolism [17].

## Material and Method

### 1.2. Study design

This cross-sectional, case-control molecular investigation was carried out at Kurukshetra University's Department of Biochemistry in Haryana, India. In a North Indian population, the study sought to determine if the ABCA1 gene polymorphisms rs2066715 and rs2230808 were associated with an increased risk of (T2DM), CAD), and T2DM with CAD**.**

### 1.3. Study Population

A total of 600 unrelated Indian subjects from different regions of Haryana and nearby North Indian states were recruited for the study. The participants were divided into four groups consisting of 150 healthy controls, 150 T2DM patients, 150 CAD patients, and 150 patients with both T2DM and CAD. Subjects aged above 35 years of either gender were included in the study.

- **Inclusion criteria**

- Individuals aged more than 35 years
- Clinically diagnosed T2DM patients
- Clinically diagnosed CAD patient
- Patients having both T2DM and CAD
- Subjects willing to participants and provide informed consent

- **Exclusion criteria**

- Type 1 Diabetes mellitus (T1DM)
- Chronic kidney disease (CKD)
- Maturity onset diabetes of the young
- Latent autoimmune diabetes in adults
- Diabetic neuropathy

### 1.4. Ethical approval

The Institutional Human Ethics Committee at Kurukshetra University provided ethical approval for the collection of blood samples. Prior to sample collection, all individuals provided written informed consent. In accordance with ICMR rules, trained medical personnel under the supervision of a qualified medical practitioner took blood samples.

### 1.5. Sample collection:

Approximately 5 mL of venous blood was collected from each participant in the EDTA vacutainer. Blood samples were then stored at 20 C until analyzed for biochemical and molecular parameters. Demographic data including medical history, social habits and past medical history with regard to drugs were recorded using a standardized questionnaire.
Sample size formula
n = reported sample size

$$n = \frac{z^2 \times p \times q}{d^2}$$

z = standard normal variate at 95% confidence interval (1.96)
p = estimated prevalence of disease
q = 1-p
d = allowable error or precision

#### 1.5.1.Biochemical analysis:

Fasting blood glucose (FBG), total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), and glycated hemoglobin (HbA1c) levels were assessed with established biochemical techniques. "Low-density lipoprotein cholesterol (LDL-C)" was determined via the Friedewald equation. Plasma insulin concentrations were

quantified using enzyme-linked immunosorbent assay (ELISA). “Body mass index (BMI), systolic blood pressure (SBP), and diastolic blood pressure (DBP)” were documented for all individuals.

**1.5.2.DNA Extraction and genotyping:**

The whole blood samples were used to isolate genomic DNA using a commercial genomic DNA extraction kit. UV spectrophotometry was used to evaluate the content and purity of the DNA. The ABCA1 gene polymorphisms rs2066715, rs2230808 were genotyped by the “Polymerase Chain Reaction–Restriction Fragment Length Polymorphism (PCR-RFLP)” method. Amplified PCR products were digested with the proper restriction enzymes and then characterized by agarose gel electrophoresis. Peripheral whole blood (1 mL) was collected in EDTA coated vacutainers and genomic DNA was extracted following manufacturer's instructions using mdi Genomic DNA Miniprep Kit (Advanced Microdevices Pvt. Ltd., India). DNA extraction was performed and its purity and concentration were determined by ultraviolet spectrophotometer. DNA samples with a purity ratio (OD260/OD280) of 1.7–2.0 were considered good for further molecular analysis. Extracted DNA was stored at −20°C until its genotyping was performed. The “Polymerase Chain Reaction–Restriction Fragment Length Polymorphism (PCR-RFLP)” method was used for genotyping of ABCA1 gene polymorphisms (rs2066715 (V825I) and rs2230808 (R1587K)). Genomic DNA, forward and reverse primers, commercially available “PCR master mix (GoTaq® Green Master Mix, Promega, India)”, nuclease-free water and Taq DNA polymerase were used in a total reaction volume of 20 µL.

**1.6. Statistical analysis**:

A p-value below 0.05 was considered statistically significant. The data was analysed using IBM SPSS software version 31. The chi-square test was used to analyse genotype and allele frequencies. To assess the connection with illness risk, the “odds ratio (OR) with a 95% confidence interval (CI)” was computed. To compare biochemical markers among the research groups, the Student's t-test was employed. For the purpose of analysing genotype and allele frequencies, the Chi-square test was utilised. To evaluate the association with disease risk, the odds ratio (OR) with a 95% CI was calculated. To compare biochemical markers among the research groups, the Student's t-test was used. A p-value below 0.05 was deemed statistically significant.

**Table 1. The forward and reverse primers sequence, annealing temperature for different SNPs and their restriction enzymes**

| SNP | Primer Sequence | Product Length (bp) | Annealing Temperature | Restriction Enzyme |
|---|---|---|---|---|
| V825I (rs2066715) | Forward Primer: 5′-CCCATGCACTGCAGAGATTC-3′ Reverse Primer: 5′-GCAAATTCAAATTTCTCCAGG-3′ | 386 bp Digested fragments: 237 bp + 149 bp | 58°C | BsaI |
| R1587K (rs2230808) | Forward Primer: 5′-TGGAGATAGGGCAGGATGG-3′ Reverse Primer: 5′-CTTCCTTCCTCCTCCTCCCT-3′ | 145 bp Digested fragments: 114 bp + 31 bp | 55°C | BssSI |

## 2. Result

### 2.1. Clinical characteristics of study population

The clinical and biochemical parameters of healthy controls, T2DM patients, CAD patients, and T2DM with CAD patients are presented in Table 2. The mean age among all groups was comparable and showed no statistically significant difference ($p > 0.05$). However, "systolic blood pressure (SBP) and diastolic blood pressure (DBP)" were significantly elevated in "T2DM, CAD, and T2DM with CAD" patients compared to healthy controls ($p < 0.05$). "Fasting blood glucose (FBG), total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C)", HbA1c, and fasting insulin levels were all significantly higher in disease groups compared to controls. HDL-C values were considerably lower across patient groups ($p < 0.05$). The T2DM with CAD group had the most severe metabolic abnormalities of all groups.

**Table 2: Clinical parameters of healthy subjects, T2DM, CAD and T2DM+CAD patient**

| Clinical Parameter | Control Mean ±SD | T2DM Mean ± SD | CAD Mean ± SD | T2DM+ CAD Mean ± SD | p-value (T2DM) | p-value (CAD) | p-value (T2DM +CAD) |
|---|---|---|---|---|---|---|---|
| **AGE (Years)** | 54.85±12.35 | 55.55±13.08 | 54.85±12.34 | 57.23±11.64 | 0.634 | 0.086 | 0.378 |
| **DBP (mmHg)** | 81.17±4.65 | 84.86± 8.06 | 83.25± 4.75 | 83.17±6.19 | <0.05 | <0.05 | <0.05 |
| **SBP (mmHg)** | 121.72± 4.84 | 130.53 ± 14.31 | 123.74± 10.01 | 126 ±11.34 | <0.05 | <0.05 | <0.05 |
| **FBG (mg/dl)** | 93.91± 10.03 | 161.63 ± 49.68 | 94.38 ±12.3 | 166.67 ± 58.0 | <0.05 | 0.72 | <0.05 |
| **TC (mg/dl)** | 177.78 ± 73.98 | 188.01 ± 48.21 | 243.35 ±52.11 | 250.03 ± 42.06 | <0.05 | <0.05 | <0.05 |
| **TG (mg/dl)** | 105.61 ± 54.46 | 170.71 ± 34.02 | 154± 59.64 | 191.76 ± 72.02 | <0.05 | <0.05 | <0.05 |
| **HDL-C (mg/dl)** | 46.44 ± 12.4 | 40.16± 11.30 | 42.77 ± 11.81 | 31.62 ± 14.01 | 0.009 | <0.05 | <0.05 |
| **LDL-C (mg/dl)** | 104.22 ± 78.75 | 123.11 ± 44.27 | 171.51 ± 46.55 | 177.73 ±54.17 | 0.025 | <0.05 | <0.05 |
| **HbA1c (%)** | 4.74 ± 0.96 | 7.72 ± 1.94 | 4.71 ± 0.83 | 7.46 ± 1.82 | <0.05 | 0.77 | <0.05 |
| **Fasting Insulin (IU/ml** | 13.10 ± 10.76 | 19.93 ± 18.96 | 14.78 ± 9.75 | 25.31 ±21.72 | <0.05 | 0.158 | <0.05 |

SD: Standard deviation
p value <0.05 is significant.
T2DM: type 2 diabetes mellitus CAD: coronary artery disease

**Interpretation:** As indicated in Table 2, there was no significant difference between the groups in terms of age ($p>0.05$). SBP and DBP were significantly higher in T2DM and CAD patients than in controls ($p < 0.05$) while significantly raised levels were seen in T2DM + CAD patients than in T2DM patients only. All of these parameters (FBG, TC, TG, HbA1c, and fasting insulin) were significantly elevated in all the illness groups, except for the HDL-C which was significantly reduced. Patients in the T2DM group with CAD group had the most marked metabolic abnormalities.

### 2.2. Genotypic Distribution and allele frequencies of ABCDA1 rs20667115 polymorphism

The genotype and allele frequencies of SNP rs2066715 (V825I) of the ABCA1 gene among healthy controls, T2DM patients, CAD patients, and T2DM with CAD patients are summarized in Table 3. The heterozygous CT genotype was the most common genotype observed in all study groups, followed by CC and TT genotypes. The frequencies of C and T alleles were similar between the control and sick groups. Statistical research indicated no significant relationship between the rs2066715 polymorphism and susceptibility to T2DM, CAD, or T2DM with CAD in allelic, additive, dominant, recessive, or heterozygous genetic models ($p > 0.05$). These findings indicate that the ABCA1 rs2066715 polymorphism may not significantly contribute to the genetic risk of T2DM and CAD in the North Indian population.

**Table 3: Genotypic distribution and allele frequencies of SNP rs2066715 of ABCA1 gene (among healthy subjects, T2DM, CAD and T2DM + CAD patients)**

| SNP rs206 | Healthy subjects | T2DM Patients | Chi square $\chi^2$ | Odd Ratio(OR) | p-value | CAD patients | Chi square $\chi^2$ | Odd Ratio(OR) | p-value | T2DM + CAD patients | Chi square $\chi^2$ | Odd Ratio(OR)(95% | p-value |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|

| 6715 | | | 1 | (95%CI) | | | 2 | (95%CI) | | | 3 | CI) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CC | 51 (34%) | 46 (30.6%) | 0.512 | | 0.774 | 42 (28%) | 1.715 | | 0.424 | 42 (28%) | 2.636 | | 0.268 |
| CT | 74 (49.3%) | 80 (53.3%) | | | | 85 (56.6%) | | | | 88 (58.6%) | | | |
| TT | 25 (16.6%) | 24 (16%) | | | | 23 (15.3%) | | | | 20 (13.3%) | | | |
| **Allelic** | | | | | | | | | | | | | |
| C | 176 (58.6%) | 172 (57.3%) | 0.109 | 0.947 (0.685 – 1.309) | 0.741 | 169 (56.3%) | 0.334 | 0.909 (0.657 – 1.256) | 0.563 | 172 (57.3%) | 0.109 | 0.947 (0.685 – 1.309) | 0.741 |
| T | 124 (41.3%) | 128 (42.6%) | | | | 131(43.6 %) | | | | 128 (42.6%) | | | |
| **Additive** | | | | | | | | | | | | | |
| CC | 51 (34%) | 46 (30.6%) | | | | 42 (28%) | | | | 42 (28%) | | | |
| CT | 74 (49.3%) | 80 (53.3%) | 0.488 | 0.834 (0.502-1.388) | 0.485 | 85 (56.6%) | 1.50 | 0.725 (0.434 – 1.213) | 0.221 | 88 (58.6%) | 1.984 | 0.693 (0.415 - 1.156) | 0.159 |
| TT | 25 (16.6%) | 24 (16%) | 0.032 | 0.940 (0.472 – 1.868) | 0.859 | 23 (15.3%) | 0.796 | 0.735 (0.374 – 1.446) | 0.372 | 20 (13.3%) | 0.006 | 1.029 (0.503 – 2.106) | 0.937 |
| **Dominant (CC vs CT+ TT)** | | | | | | | | | | | | | |
| | 51 (34%) | 46 (30.6%) | 0.381 | 0.859 (0.529-1.394) | 0.537 | 42 (28%) | 1.262 | 0.755 (0.462-1.234) | 0.261 | 42 (28%) | 1.262 | 0.755 (0.462-1.234) | 0.261 |
| **Recessive (TT vs CT+ CC)** | | | | | | | | | | | | | |
| | 25 (16.6%) | 24 (16%) | 0.024 | 0.952 (0.516 – 1.757) | 0.876 | 23 (15.3%) | 0.099 | 1.104 (0.595-2.048) | 0.753 | 20 (13.3%) | 0.654 | 1.30 (0.687 – 2.459) | 0.419 |
| **Heterozygous (CT vs CC+ TT)** | | | | | | | | | | | | | |
| | 74 (49.3%) | 80 (53.3%) | 0.480 | 1.174 (0.746 – 1.847) | 0.488 | 85 (56.6%) | 1.619 | 1.343 (0.852 – 2.116) | 0.203 | 88 (58.6%) | 2.630 | 1.58 (0.924 – 2.30) | 0.105 |
| *p-values are uncorrected. Bonferroni correction for multiple comparisons sets the significance threshold at $p < 0.017$ | | | | | | | | | | | | | |

**Interpretation:** In this present study No significant associations were observed between the genotype and the allele frequencies of the ABCA1 rs2DM polymorphism and T2DM, CAD and T2DM with CAD. The heterozygous CT genotype was the most common genotype in all study group, with CC and TT genotype being the next most common genotype. Likewise, there was no difference in allelic distribution under the additive, dominant, recessive and heterozygous genetic models ($p > 0.05$). The heterozygous genotype (CT) was the most prevalent genotype in all research groups, with the CC and TT genotypes being the next most prevalent. Likewise, allelic distribution and across additive, dominant, recessive and heterozygous genetic models did not differ significantly ($p > 0.05$). These data suggest that the rs2066715 polymorphism might not play a significant genetic contribution to T2DM and CAD susceptibility in the population included in this study in North Indian population.

### 2.3. Association of rs2066715 genotypes with clinical parameters

The association between rs2066715 genotypes and clinical parameters is shown in Table 4. No significant association was observed between genotype distribution and BMI, blood pressure, fasting blood glucose, total cholesterol, LDL-C, HbA1c, or fasting insulin levels across the study groups.

In T2DM, CAD, and T2DM with CAD groups, CT+TT genotype carriers had significantly lower HDL-C levels compared to CC genotype carriers ($p < 0.05$). Triglyceride levels were also considerably higher among CT+TT carriers in the T2DM with CAD group.

These data suggest that the rs2066715 polymorphism may alter lipid metabolism primarily via modulating HDL-C levels.

**Table 4: "Statistical analysis of clinical parameters in association with genotype distribution of SNP V825I of ABCA1 gene" (Control, T2DM, CAD and T2DM+ CAD)**

| Clinical parameters | Control | | | T2DM | | | CAD | | | T2DM + CAD | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CC | CC + TT | p-value | CC | CT + TT | P - value | CC | CC + TT | p-value | CC | CT + TT | P - value |
| BMI ($Kg/m^2$) | 24.5 ± 3.8 | 25.0 ± 3.8 | 0.32 | 26.0 ± 4.1 | 26.6 ± 4.2 | 0.29 | 26.5 ± 4.3 | 27.0 ± 4.3 | 0.28 | 27.0 ± 4.5 | 27.5 ± 4.6 | 0.27 |
| SBP (mmHg) | 132 ± 12.0 | 133.0 ± 12.1 | 0.38 | 136.2 ± 13.4 | 138.0 ± 13.6 | 0.34 | 138.0 ± 13.5 | 139.5 ± 13.8 | 0.33 | 139.0 ± 13.8 | 141.4 ± 14.0 | 0.32 |
| DBP (mmHg) | 82.2 ± 9.1 | 83.2 ± 9.4 | 0.34 | 84.0 ± 9.5 | 85.5 ± 9.7 | 0.31 | 85.1 ± 9.8 | 86.5 ± 9.9 | 0.30 | 86.1 ± 9.9 | 87.5 ± 10.1 | 0.29 |
| FBG (mg/dl) | 98.2 ± 12.5 | 100.5 ±13.0 | 0.30 | 155.3 ± 30.0 | 160.0 ± 32.2 | 0.27 | 110.2 ± 15.0 | 113.0 ± 16.1 | 0.26 | 160.0 ± 31.3 | 165.0 ± 33.3 | 0.25 |
| TC (mg/dl) | 180.6 ± 30.1 | 185.0 ± 32.3 | 0.20 | 190.2 ± 32.2 | 197.4 ± 33.3 | 0.19 | 195.2 ± 33.0 | 202.0 ± 34.3 | 0.18 | 198.0 ± 33.5 | 205.5 ± 35.2 | 0.17 |
| TG (mg/dl) | 150.5 ± 44.0 | 160.0 ± 45.4 | 0.14 | 180.2 ± 50.5 | 195.5 ± 52.0 | 0.11 | 185.2 ± 50.5 | 200.7 ± 53.6 | 0.10 | 190.1 ± 52.2 | 205.0 ± 55.0 | **0.009** |
| HDL-C (mg/dl) | 52.0 ± 12.0 | 47.6 ± 11.5 | 0.23 | 46.0 ± 11.5 | 41.6 ± 10.5 | **0.01** | 44.0 ±10.5 | 39.0 ± 9.5 | **0.01** | 42.0 ± 10.2 | 37.2 ± 9.0 | **0.001** |
| LDL-C (mg/dl) | 105.0 ± 25.8 | 109.2 ± 26.0 | 0.24 | 110.6 ± 27.2 | 115.5 ± 28.0 | 0.22 | 112.0 ± 28.2 | 117.1 ± 29.6 | 0.21 | 114.4 ± 28.5 | 119.0 ± 30.3 | 0.20 |
| HbA1c (%) | 5.5 ± 0.6 | 5.6 ± 0.6 | 0.27 | 7.8 ± 1.2 | 8.0 ± 1.3 | 0.24 | 6.0 ± 0.8 | 6.2 ± 0.8 | 0..23 | 8.0 ± 1.2 | 8.2 ± 1.3 | 0.22 |
| Fasting Insulin (IU/ml) | 5.0 ± 3.0 | 5.3 ± 3.1 | 0.21 | 8.1 ± 4.0 | 8.5 ± 4.2 | 0.20 | 26.5 ± 4.3 | 27.0 ± 4.3 | 0.28 | 27.0 ± 4.5 | 27.5 ± 4.6 | 0.27 |
| Data is presented as mean±standard deviation<br>Bold indicates p<0.05(CC vs CT+TT): statistical significant difference | | | | | | | | | | | | |

**Interpretation:** There were no significant differences in "BMI, SBP, DBP, FBG, TC, LDL-C, HbA1c," and fasting insulin level between CC and CT+TT genotype in all groups "(control, T2DM, CAD, and T2DM with CAD)" as compared to each other ($p > 0.05$) when analyzed with clinical parameters. Among the T2DM, CAD and T2DM with CAD patients, however, the level of HDL-C was significantly reduced in the genotype TT carriers ($p = 0.01$, $p = 0.01$, $p = 0.001$ respectively). Further, the levels of HDL-C was significantly decreased in CT+TT genotype carriers among T2DM ($p = 0.01$), CAD ($p = 0.01$), and T2DM with CAD patients ($p = 0.001$). Moreover, the triglyceride levels were significantly higher in the CT+TT carriers compared to T2DM with CAD ($p = 0.009$). These results suggest that the rs2066715 polymorphism is related to lipids mainly, HDL-C and triglycerides.

### 2.4. Genotypic distribution and allele frequencies of ABCA1 rs2230808 polymorphism

The genotypic and allelic frequencies of SNP rs2230808 (R1587K) of the ABCA1 gene are presented in Table 5. The frequency of the AA genotype and A allele was significantly higher in T2DM, CAD, and T2DM with CAD patients compared to healthy controls.

Significant relationships were found under allelic, additive, and dominant genetic models ($p < 0.05$). The A allele has been linked to an increased risk of T2DM, CAD, and combined T2DM with CAD.

These findings indicate that the rs2230808 polymorphism of the ABCA1 gene may be a significant genetic risk factor for metabolic and cardiovascular illnesses in the North Indian population.

**Table 5: Genotypic distribution and allele frequencies of SNP rs2230808 of ABCA1 gene (among healthy subjects, T2DM, CAD and T2DM + CAD patients)**

| SNP rs2230808 | Healthy subjects | T2DM Patients | Chi square $\chi^2_1$ | Odd Ratio(OR) (95%CI) | p-value | CAD patients | Chi square $\chi^2_2$ | Odd Ratio(OR) (95%CI) | p-value | T2DM + CAD patients | Chi square $\chi^2_3$ | Odd Ratio(OR)(95% CI) | p-value |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| TT | 70 (46.6%) | 40(26.6%) | 13.3 | | 0.001 | 45(30%) | 9.48 | | 0.009 | 42(28%) | 11.91 | | 0.003 |
| TA | 65(43.3%) | 85 (56.6%) | | | | 80 (53.3%) | | | | 82(54.6%) | | | |
| AA | 15 (10%) | 25(16.6%) | | | | 25 (16.6%) | | | | 26 (17.3%) | | | |
| Allelic | | | | | | | | | | | | | |
| T | 205 (68.3%) | 165(55%) | 11.28 | 0.566 (0.406-0.790) | 0.001 | 170 (56.6%) | 8.71 | 0.606 (0.434-0.846) | 0.001 | 166 (55.3%) | 10.74 | 0.574 (0.411 – 0.801) | 0.001 |
| A | 95(63.3%) | 135 (45%) | | | | 130 (43.3%) | | | | 134 (44.6%) | | | |
| Additive | | | | | | | | | | | | | |
| TT | 70 (46.6%) | 40(26.6%) | | | | 45(30%) | | | | 42 (28%) | | | |
| TA | 65(43.3%) | 85 (56.6%) | 10.47 | 0.437 (0264-0.724) | 0.001 | 80 (53.3%) | 6.612 | 0.522 (0.318-0.859) | 0.001 | 82 (54.6%) | 8.514 | 0.476 (0.288 – 0.786) | 0.004 |
| AA | 15 (10%) | 25(16.6%) | 8.16 | 0.343 (0.162 – 0.725) | 0.004 | 28 (18.7%) | 6.544 | 0.386 (0.184-0.810) | 0.001 | 26 (17.3%) | 8.163 | 0.346 (0.165 – 0.727) | 0.004 |
| Dominant (TT vs TA+ AA) | | | | | | | | | | | | | |
| | 70 (46.6%) | 40(26.6%) | 12.91 | 0.416 (0.256 – 0.674) | 0.001 | 45 (30%) | 8.813 | 0.490 (0.395 – 0.788) | 0.003 | 42 (28%) | 11.17 | 0.444 (0.275-0.718) | 0.44 |
| Recessive (AA vs TT+ TA) | | | | | | | | | | | | | |
| | 15 (10%) | 25(16.6%) | 2.88 | 0.556 (0.280-1.102) | 0.08 | 25 (16.6%) | 2.88 | 0.556 (0.280-1.102) | 0.08 | 26 (17.3%) | 3.418 | 0.530 (0.268-1.047) | 0.064 |
| Heterozygous(TA vs AA+ TT) | | | | | | | | | | | | | |
| | 65 (43.3%) | 85 (56.6%) | 5.33 | 0.585 (0.370 – 0.923) | 0.021 | 80 (53.3%) | 3.003 | 0.669 (0.424-1.055) | 0.083 | 82 (54.6%) | 0.855 | 0.634 (0.402-1.00) | 0.050 |
| *p-values are uncorrected. Bonferroni correction for multiple comparisons sets the significance threshold at p < 0.017 | | | | | | | | | | | | | |

**Interpretation:** In the present study genotype and allele frequencies of the ABCA1 rs2230808 polymorphism were significantly associated with T2DM, CAD and T2DM + CAD. The more common genotype was TA+AA genotype and the more common allele was A in sick groups when compared to controls. In both allelic and additive models, significant relationship was found ($p<0.05$) which suggests that the allele may increase the risk of T2DM and CAD. In both allelic and additive models significant relationship ($p<0.05$) was found which suggested that the allele may increase the risk of T2DM and CAD.

### 2.5. Association of rs2230808 genotypes with clinical parameters

The relationship between rs2230808 genotypes and clinical parameters is shown in Table 6. Individuals carrying TA+AA genotypes exhibited significantly higher fasting blood glucose, total cholesterol, triglycerides, LDL-C, HbA1c, BMI, "systolic blood pressure, diastolic blood pressure, and fasting insulin" levels compared to TT genotype carriers.

Also the carriers of TA+AA genotype revealed markedly low level of HDL-C in groups of T2DM, CAD, T2DM+CAD patients. This implies that rs2230808 polymorphism might be associated with dyslipidemia, impaired glucose metabolism and high risk of CAD.

**Table 6: Statistical analysis of clinical parameters in association with genotype distribution of SNP R1587K of ABCA1 gene (Control, T2DM, CAD and T2DM+ CAD)**

| Clinical paramet ers | Control | | | T2DM | | | CAD | | | T2DM + CAD | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | TT | TA + AA | p-value | TT | TA + AA | P - value | TT | TA + AA | p-value | TT | TA + AA | P - value |
| BMI ($Kg/m^2$) | 24.4 ± 2.7 | 25.2 ± 3.0 | 0.032 | 26.2 ± 3.3 | 27.1 ± 3.6 | 0.028 | 26.3 ± 3.1 | 27.5 ± 3.4 | 0.021 | 27.1 ± 3.5 | 28.0 ± 3.8 | 0.019 |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **SBP (mmHg)** | 121.8 ± 6.5 | 124.6 ± 6.9 | 0.026 | 129.1 ±11.4 | 134.8 ±12.6 | 0.019 | 124.3 ± 9.4 | 130.7 ± 10.5 | 0.017 | 126.3 ± 10.1 | 132.1 ± 11.8 | 0.016 |
| **DBP (mmHg)** | 80.3 ± 5.2 | 82.9 ± 6.3 | 0.034 | 84.2 ± 7.4 | 87.5 ± 7.8 | 0.027 | 82.5 ± 6.4 | 85.9 ± 6.9 | 0.024 | 83.6 ± 6.7 | 87.8 ± 7.6 | 0.022 |
| **FBG (mg/dl)** | 94.0 ± 11.2 | 97.8 ± 12.5 | 0.029 | 162.4 ± 45.1 | 173.9 ± 51.6 | **0.008** | 96.5 ± 12.9 | 103.6 ± 14.8 | 0.014 | 166.2 ± 52.7 | 177.5 ± 58.3 | **0.007** |
| **TC (mg/dl)** | 181.5 ± 32.6 | 190.4 ± 35.1 | 0.024 | 191.2 ± 39.7 | 203.4 ± 42.8 | 0.019 | 241.6 ± 46.9 | 255.7 ± 49.3 | **0.001** | 249.1 ± 42.3 | 263.8 ± 45.6 | **0.001** |
| **TG (mg/dl)** | 110.9 ± 42.4 | 126.7 ± 47.3 | 0.021 | 167.8 ± 34.1 | 182.6 ± 38.2 | **0.010** | 155.3 ± 56.8 | 172.5 ± 60.2 | **0.015** | 189.6 ± 60.7 | 205.4 ± 64.2 | **0.009** |
| **HDL-C (mg/dl)** | 48.1 ± 11.4 | 41.8 ± 10.2 | **0.011** | 41.3 ± 10.9 | 35.4 ± 9.6 | **0.005** | 45.1 ± 10.0 | 38.3 ± 8.8 | **0.003** | 34.8 ± 9.6 | 29.4 ± 8.4 | **0.001** |
| **LDL-C (mg/dl)** | 108.7 ± 33.4 | 119.6 ± 36.9 | 0.019 | 122.6 ± 38.9 | 135.9 ± 43.1 | 0.017 | 171.9 ± 58.4 | 186.8 ± 61.3 | **0.001** | 176.4 ± 50.6 | 190.7 ± 53.2 | **0.001** |
| **HbA1c (%)** | 4.8 ± 0.7 | 5.1 ± 0.9 | 0.028 | 7.6 ± 1.7 | 8.2 ± 1.9 | **0.001** | 5.0 ± 0.8 | 5.4 ± 0.9 | 0.018 | 7.4 ± 1.6 | 8.0 ± 1.8 | **0.001** |
| **Fasting Insulin (IU/ml)** | 13.0 ± 8.6 | 15.4 ± 9.3 | 0.031 | 19.8 ± 12.0 | 23.4 ± 13.2 | **0.012** | 14.1 ± 8.7 | 17.0 ± 9.6 | 0.019 | 21.6 ± 14.3 | 25.7 ± 15.2 | **0.011** |
| Data is presented as mean±standard deviation<br>P-values are uncorrected. Bonferroni correction for multiple comparisons sets the significance threshold at $p < 0.017$ | | | | | | | | | | | | |

**Interpretation:** The ABCA1 gene is involved in the regulation of lipid metabolism and cholesterol transport, and polymorphisms of this gene have been associated with dyslipidaemia, insulin resistance and cardiovascular disease. One of these variants is the reverse cholesterol transport (R1587K) that alters the production of HDL-C, which may impact on glucose metabolism, lipid profile and cardiovascular risk. In this study, the association of the genotypes of rs2230808 with biochemical and clinical variables in patients with type 2 diabetes, coronary artery disease, and type 2 diabetes mellitus was therefore, evaluated.

### 2.6. Haplotype analysis of ABCA1 Gene

Haplotype analysis of the rs2066715 and rs2230808 polymorphisms is shown in Table 7. The frequency of the CT haplotype was significantly low in patients with T2DM, CAD and both T2DM and CAD, indicating that it might play a protective effect against the disease. However, CA and TA haplotypes were found more frequently in the disease population, indicating their increased association with susceptibility. The TT haplotype showed no association with any of the diseases studied. Thus, specific ABCA1 haplotypes might affect susceptibility of T2DM and CAD patients.

**Table 7: Haplotype analysis of ABCA1 gene at SNPs rs2066715 and rs2230808**

| **Haplotype** | **Control** | **T2DM** | **Chi square $\chi^2$** | **Odd Ratio (OR) (95% CI)** | **p-value** | **CAD patients** | **Chi square $\chi^2$** | **Odd Ratio (OR) (95% CI)** | **p-value** | **T2DM + CAD** | **Chi square $\chi^2$** | **Odd Ratio (OR) (95% CI)** | **p-value** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **CT*** | 120.27 (40.1%) | 94.60 (31.5%) | 4.77 | 0.688 (0.492 – 0.963) | 0.029 | 95.77 (31.9%) | 4.342 | 0.701 (0.501 – 0.980) | 0.037 | 95.17(31.7%) | 4.560 | 0.694 (0.497 – 0.971) | 0.033 |
| **CA*** | 55.73 (18.6%) | 77.40 (25.8%) | 4.532 | 1.524 (1.033-2,249) | 0.033 | 73.23 (24.4%) | 3.025 | 1.415 (0.956 – 2.096) | 0.082 | 76.83 (25.6 %) | 4.308 | 1.509 (1.022-2.228) | 0.038 |
| **TT*** | 84.73 (28.2%) | 70.40 (23.5%) | 1.786 | 0.779 (0.540 – 1.124) | 0.181 | 74.23 (24.7%) | 0.944 | 0.835 (0.51 – 1.201) | 0.331 | 70.83 (23.6%) | 1.678 | 0.785 (0.544-1.133) | 0.195 |
| **TA*** | 39.27 (13.1%) | 57.60 (19.2%) | 4.138 | 1.578 (1.014 – 2.454) | 0.042 | 56.77 (18.9%) | 3.797 | 1.550 (0.995-2.413) | 0.051 | 57.17 (19.1%) | 3.962 | 1.563 (1.005 – 2.433) | 0.047 |

**Interpretation**: In the present study the CT haplotype of the ABCA1 gene polymorphism was significantly higher in T2DM and CAD patients not compared with T2DM patients, suggesting a protective effect. Otherwise, the CA and TA haplotypes were more prevalent in all illness categories and associated with increased risk of T2DM and T2DM with CAD. There was no significant relationship found for the TT haplotype. The data indicate that specific haplotypes of ABCA1 may have an impact on the susceptibility to metabolic and cardiovascular diseases.

## 3. Discussion

The "ATP-binding cassette transporter A1 (ABCA1)" gene, located on chromosome 9p21, plays a crucial role in lipid metabolism and has been widely investigated for its association with T2DM and CAD. Previous studies have shown that several SNPs within this locus, along with their interactions, are strongly linked to the risk of developing T2DM and CAD across different populations. In the present study, two common genetic variants of the ABCA1 gene - SNP rs2066715 (V825I) and rs2230808 (R1587K)—w re analyzed for their association with T2DM, CAD and T2DM with CAD in the Haryana (north Indian) population. Among these, SNP rs2066715 (V825I) is one of the most frequently studied polymorphisms of ABCA1, though its association with diabetes and cardiovascular disease remains controversial due to inconsistent findings across populations. In this study, there is no significant difference was found in allelic distribution (C vs T) between healthy subjects and T2DM patients ($\chi^2 = 0.109$, OR = 0.947 [95% CI: 0.685–1.309], $p = 0.741$), healthy subjects and CAD patients ($\chi^2 = 0.334$, OR = 0.909 [95% CI: 0.657–1.256], $p = 0.563$) and between healthy subjects and CAD+T2DM patients ($\chi^2 = 0.109$, OR = 0.947 [95% CI: 0.685–1.309], $p = 0.741$). Abd El-Aziz et al., 2014 ; Marvaki et al., 2014; Liu et al., 2015 have explored the relationship between ABCA1 gene polymorphisms and CAD risk [18-19-20]. For instance, studies in Malay and Chinese populations reported a significant association of rs2066715 with cardiovascular disease. Tan et al. (2003) found that rs2066715 might predict an elevated risk of coronary heart disease (CHD) and was significantly linked with CHD in Malays but not in Singaporean Chinese or Indian populations [21]. Conversely, Cyrus et al., 2016; Yin et al. 2012, studied in the Eastern Province of Saudi Arabia, reported no significant correlation between rs2066715 and CAD risk [22-23] demonstrated that while rs2066715 was not associated with BMI, it was related to variations in lipid parameters—specifically, total cholesterol and ApoA1 in individuals of normal weight and LDL-C and ApoB in overweight individuals. However, findings indicate that the ABCA1 SNP rs2230808 (R1587K) polymorphism is significantly associated with altered lipid metabolism and an increased risk of T2DM, C And combined T2DM with CAD We also observed that inT2DM, CAD and CAD+T2DM patients, where the AA genotype and the A allele frequency was significantly higher (p = 0.009), with strong significance in allelic (p = 0.001), additive (p = 0.004), and dominant models (p = 0.003). Hence study suggested that A allele and AA genotype of SNP rs2230808 of the ABCA1 gene, may confer increased risk for T2DM, CAD, and T2DM + CAD both. Lu et al., 2018 has find that the presence of the variant A allele in rs2230808 has been associated with higher total cholesterol levels compared to non-carriers and an elevated risk of both T2DM and CAD [24]. This observation aligns with previous studies and meta-analysis that have consistently linked rs2230808 to adverse lipid profiles, including reduced HDL-C levels and elevated triglycerides and total cholesterol which are key risk factors for CAD Additionally, Haerian et al., 2017 suggest that ABCA1 variants, including rs2230808, may influence glucose metabolism and insulin resistance, contributing to T2DM susceptibility [25]. Overall, these results support the hypothesis that ABCA1 polymorphisms, particularly rs2230808, contribute to cardio metabolic risk through interconnected lipid and glucose regulatory pathways. This highlights the potential of rs2230808 as a genetic marker for identifying individuals at high risk of developing T2DM and its cardiovascular complications.

## 4. Conclusion

Based on these studies it is concluded that in the North Indian population, the polymorphisms of ABCA1 gene (rs2066715

and rs2230808) are found to be associated with increased risk of Type 2 Diabetes Mellitus (T2DM), Coronary Artery Disease (CAD) and T2DM with CAD. The results showed that the rs2230808 polymorphism was highly associated with increased susceptibility to T2DM, CAD and the simultaneous presence of both T2DM and CAD. Poor lipid profile, sub-optimal glycaemic regulation and increased cardiovascular risk were associated with the A allele and the TA+AA genotypes. In contrast, no significant association was found between the rs2066715 polymorphism and disease susceptibility, and only a moderate association with HDL-C and triglycerides. Specific ABCA1 haplotypes were suggested to be associated with disease susceptibility or protection by haplotype research. The results in this study indicate that the ABCA1 rs2230808 may be an important genetic marker of metabolic/cardiovascular disorders in the North Indians. Further large-scale, multicentric studies are needed to validate these results and to study the functional role of ABCA1 polymorphisms in disease.

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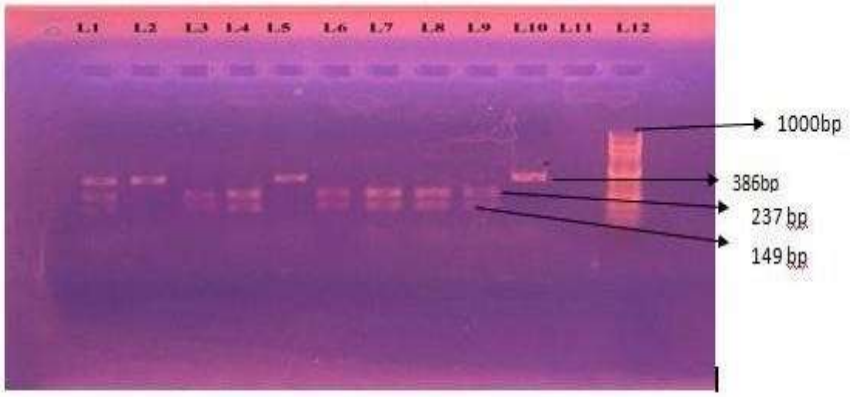


**Figure 1: RFLP analysis of ABCA1 gene at SNP rs2260715 (386bp). Lane 12 shows 1000 bp ladder, lanes 1-11 shows bands after restriction digestion.**

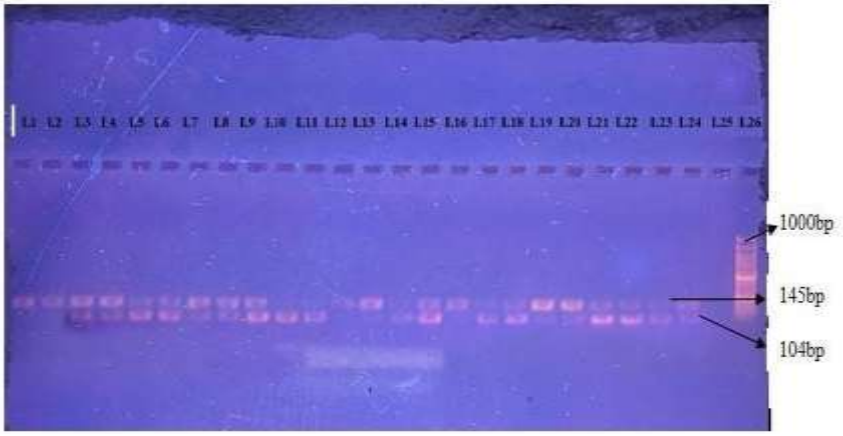


**Figure 2: RFLP analysis of ABCA1 gene at SNP rs2230808 (145bp). Lane 26shows 1000 bp ladder, lanes 1-25 shows bands after restriction digestion.**