Original Article

Genetic variants of chromosome 9p21.3 region associated with coronary artery disease and premature coronary artery disease in an Asian Indian population

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ABSTRACT

Introduction: Asian Indians have a propensity for premature, severe, and diffuse coronary artery disease (CAD). Several single-nucleotide polymorphisms (SNPs) in the ‘core CAD’ region of the chromosomal region 9p21.3 are known to be strongly associated with CAD.

Objectives: We aimed to study SNPs in the 9p21.3 region associated with CAD and premature CAD and identify their association with demographic and clinical characteristics in an Asian Indian population.

Methods: SNP genotyping was performed for 30 SNPs of the 9p21.3 region using MassARRAY® technology. Along with demographic and SNP data analysis, we also performed multivariate logistic regression analysis and multifactor dimensionality reduction analysis to study SNP–SNP and SNP–demographic/clinical variable interactions.

Results: Our results suggest that females are at a higher risk of premature CAD. We found that SNPs rs1333045 (CC), rs16905599 (AA), rs2383206 (GG), rs2383208 (AG), and rs4977574 (GG) were significantly associated with premature CAD. When adjusted for covariates/confounders, we found that rs2383206 showed the strongest risk association with CAD followed by rs16905599 and rs2383208. Further, SNPs rs1333049 (CC) and rs4977574 (GG) were found to be exclusively associated with premature CAD cases, suggesting their potential as genetic markers for premature CAD in the local population. Upon gender-based stratification, it was found that rs10757272 (TT and TC) is significantly associated with eightfold to ninefold CAD risk specifically among females. SNP rs7865618 (GG) is significantly associated with more than 2.5-fold CAD risk specifically among males.

Conclusion: Our study suggests that SNPs at the 9p21 risk locus may be used to generate a reliable genetic risk score along with markers at other loci.

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1. Introduction

Coronary artery disease (CAD) has reached epidemic proportions in the Asian Indian population. Asian Indians have a propensity for premature, diffuse, and severe CAD.1-2 Genome-wide association studies starting from 20073-7 identified the 9p21.3 chromosomal region as being strongly associated with CAD and myocardial infarction (MI). This region has also been associated with stroke,8 aortic,9 abdominal,10 intracranial aneurysms,10 and several types of cancers.12-14

The chromosomal region 9p21.3 has been strongly associated with CAD in Caucasian, Italian15 US Hispanic,16 Chinese,17 Japanese,18 Korean,19 Asian Indian,20 and Pakistani21 populations. This region is sparse in genes and also called ‘gene desert’. The nearest genes, about 100 kb upstream of the core CAD region, are two tumor suppressor genes called CDKN2A and CDKN2B that constitute the INK4/ARF locus. This locus encodes cyclin-dependent kinase inhibitors (p16INK4A and p14ARF) from CDKN2A and p15INK4B from CDKN2B that cause arrest of cell cycle in the G1 phase.22,23 Further
away is another gene MTAP that encodes methylthioadenosine phosphorylase, that is involved in the polyamine pathway (salvage of adenine and methionine). Most of the single-nucleotide polymorphisms (SNPs) associated with CAD are present in a 58 Kb region called the ‘core CAD’ region. The core CAD region overlaps CDKN2B and also contains a long noncoding RNA of about 126 kb named ANRIL for antisense noncoding RNA in the INK4 locus, hence also called CDKN2B-AS. ANRIL is known to have several linear and circular isoforms.

Table 1

SNPs studied in the 9p213 region.

| S. No. | SNP ID  | Chromosomal position | Gene view | Functional consequence | Minor allele and global frequency |
|-------|---------|----------------------|-----------|------------------------|----------------------------------|
| 1     | rs1004638 (T/A) | 22115590 | CDKN2B-AS1 | Intron variant | A-0.31 |
| 2     | rs10116277 (T/G) | 22081398 | CDKN2B-AS1 | Intron variant | G-0.323 |
| 3     | rs1011970 (G/T) | 22062135 | CDKN2B-AS1 | Intron variant | T-0.247 |
| 4     | rs1063192 (A/G) | 22003368 | CDKN2B-AS1 | Intron variant, UTR variant 3’ | G-0.205 |
| 5     | rs10757272 (C/T) | 22088261 | CDKN2B-AS1 | Intron variant | C-0.218 |
| 6     | rs10757274 (A/G) | 22096056 | CDKN2B-AS1 | Intron variant | G-0.404 |
| 7     | rs10757278 (A/G) | 22124478 | CDKN2B-AS1 | Intron variant | C-0.408 |
| 8     | rs10757283 (C/T) | 22134173 | CDKN2B-AS1 | Intron variant | T-0.456 |
| 9     | rs10811661 (T/C) | 22134095 | CDKN2B-AS1 | Intron variant | C-0.176 |
| 10    | rs1323940 (T/C) | 22083405 | CDKN2B-AS1 | Intron variant | G-0.312 |
| 11    | rs1333042 (G/A) | 22103814 | CDKN2B-AS1 | Intron variant | A-0.321 |
| 12    | rs1333045 (A/G) | 22119196 | CDKN2B-AS1 | Intron variant | C-0.498 |
| 13    | rs1333048 (A/G) | 22125348 | CDKN2B-AS1 | Intron variant | A-0.442 |
| 14    | rs1333049 (G/A) | 22125504 | CDKN2B-AS1 | Intron variant | C-0.418 |
| 15    | rs1690599 (G/A) | 22069145 | CDKN2B-AS1 | Intron variant | A-0.190 |
| 16    | rs2383206 (A/G) | 22115027 | CDKN2B-AS1 | Intron variant | C-0.195 |
| 17    | rs2383207 (A/G) | 22132277 | CDKN2B-AS1 | Intron variant | G-0.210 |
| 18    | rs2811712 (A/G) | 22083405 | CDKN2B-AS1 | Intron variant | A-0.190 |
| 19    | rs2811960 (A/G) | 22113826 | CDKN2B-AS1 | Intron variant | G-0.493 |
| 20    | rs3123123 (A/G) | 21974219 | CDKN2A | Intron variant | G-0.175 |
| 21    | rs4977574 (A/G) | 22088575 | CDKN2B-AS1 | Intron variant | C-0.487 |
| 22    | rs4977756 (A/G) | 22086853 | CDKN2B-AS1 | Intron variant | G-0.288 |
| 23    | rs654958 (T/C) | 22095458 | CDKN2B-AS1 | Intron variant | A-0.184 |
| 24    | rs615552 (T/C) | 22030078 | CDKN2B-AS1 | Intron variant | T-0.431 |
| 25    | rs647606 (T/C) | 22081851 | CDKN2B-AS1 | Intronic variant | C-0.322 |
| 26    | rs7023329 (A/G) | 21816529 | MTAP | Intron variant | G-0.449 |
| 27    | rs7865818 (A/G) | 22031006 | CDKN2B-AS1 | Intron variant | C-0.188 |
| 28    | rs944797 (T/C) | 22115287 | CDKN2B-AS1 | Intron variant | G-0.487 |
| 29    | rs9632884 (C/G) | 22072302 | CDKN2B-AS1 | Intron variant | G-0.304 |

SNP, single-nucleotide polymorphism.
* Genome build is GRCh38.p12 (taken from dbSNP site).

Table 2

Epidemiological characteristics of the study population.

| Demographic/clinical parameter | Controls | Cases | P value |
|--------------------------------|----------|-------|---------|
| **Age in years (mean ± SD)**  | 51.8 ± 9.8 | 55.9 ± 10.7 | 1 x 10⁻⁶ |
| **Percentage of males/females** | 366/63.4 | 812/18.8 | <1 x 10⁻⁷ |
| **Height in cms (mean ± SD)** | 157.61 ± 10 | 163.49 ± 8.1 | 3 x 10⁻¹ |
| **Weight in Kg (mean ± SD)** | 67.5 ± 11.6 | 67.8 ± 11.7 | 0.603 |
| **BMI (mean ± SD)** | 26.1 ± 4.9 | 25.4 ± 3.8 | 0.044 |
| **Systolic BP (mean ± SD)** | 121.4 ± 6.7 | 125.7 ± 18.1 | 8 x 10⁻⁴ |
| **Diastolic BP (mean ± SD)** | 80.8 ± 5.9 | 76.6 ± 10.7 | 1 x 10⁻¹ |
| **Percentage of diabetes/normal** | 15.8/84.2 | 50.5/49.5 | <1 x 10⁻⁷ |
| **Duration of diabetes in years (mean ± SD)** | 0.431 ± 1.13 | 1.50 ± 1.18 | <1 x 10⁻⁷ |
| **Percentage of hypertensives/normal** | 24.6/75.4 | 58.7/41.3 | <1 x 10⁻⁷ |
| **Duration of hypertension in years (mean ± SD)** | 0.76 ± 1.55 | 1.74 ± 1.82 | <1 x 10⁻⁶ |
| **Percentage having affected first-degree relative/no affected first-degree relative** | 30/70 | 39.4/60.6 | 0.015 |
| **Percentage of cigarette smokers (ever)/nonsmokers** | 4.7/95.3 | 43.6/56.4 | <1 x 10⁻⁷ |
| **No. of cigarettes per day (mean ± SD)** | 0.13 ± 0.92 | 4.21 ± 7.30 | <1 x 10⁻⁷ |
| **Percentage of alcoholics (ever)/nonalcoholics** | 9.5/90.5 | 39.5/60.5 | <1 x 10⁻⁷ |
| **Number of pegs of alcohol (mean ± SD)** | 1.85 ± 1.10 | 1.01 ± 1.40 | <1 x 10⁻⁷ |
| **Number of tea/coffee cups per day (mean ± SD)** | 2.37 ± 1.12 | 2.69 ± 1.86 | 0.021 |
| **Percentage of individuals with vegetarian diet/mixed diet** | 51.9/48.1 | 17.9/82.1 | <1 x 10⁻⁷ |
| **Percentage of individuals with regular fruit intake/rare fruit intake** | 75.25 | 56.9/43.1 | 2 x 10⁻⁶ |
| **Percentage of individuals with regular physical exercise/no physical exercise** | 78.3/21.7 | 45.5/54.5 | <1 x 10⁻⁷ |

SD, standard deviation; BMI, body mass index; BP, blood pressure.
To understand the association of the 9p21 risk locus with CAD and premature CAD, we aimed to study 30 SNPs in this region, their association with demographic, clinical characteristics, and interferon alpha 21 (IFNA21) levels in an Asian Indian population.

2. Materials and methods

The study was conducted in the south Indian state of Telangana, in the twin cities of Hyderabad and Secunderabad. The study population consisted of 661 individuals including 443 controls and 218 angiographically documented CAD cases (recruited from June 2015 to June 2017). The controls were recruited from various parts of the twin cities of Hyderabad and Secunderabad. We have followed the guidelines of the 1975 Declaration of Helsinki in the recruitment of study subjects, sample, and data collection. The CAD cases were recruited from Krishna Institute of Medical Sciences (KIMS), Secunderabad, after the approval of the Ethics Committee of KIMS Foundation and Research Centre. Written informed consent was taken from all subjects prior to sample and data collection.

2.1. Selection criteria of study subjects

Inclusion criteria for controls include healthy individuals from the age of 40 to 85 years.
Exclusion criteria for cases include individuals with liver, kidney, and gastrointestinal disorders and individuals with infectious diseases such as hepatitis, HIV, TB, and so on.

2.2. Methodology

The CAD cases were recruited in consultation with the hospital cardiologist. Peripheral blood sample of 5 ml was taken from each subject and equally dispensed in a vacutainer tube coated with EDTA for plasma and vacutainer tube with clot accelerator for serum separation. DNA extraction from plasma was performed using the kit manufactured by Epicentre (an Illumina company). The DNA samples were estimated qualitatively by electrophoresis on 0.8% agarose gels stained with ethidium bromide, and the bands were viewed in a UV transilluminator. The quantitative estimation of DNA was performed using a Nanodrop Spectrophotometer and taking the ratio of absorbance at 260/280 nm.

The DNA samples were then genotyped for 30 SNPs of the 9p21 region (Table 1). The 9p21 SNPs were chosen based on 2 criteria: (A) extensive literature survey and (B) minor allele frequency (MAF) taken from the dbSNP site of the National Center for Biotechnology Information. We have chosen SNPs having MAF > 0.15. The SNP genotyping was performed by the MassARRAY® technology (Sequenom platform) using the AGENA protocol.

Table 3
Comparison of epidemiological and clinical variables between nonpremature and premature cases.

| S. No. | Variable          | % of Nonpremature cases (n = 109) | % of Premature cases (n = 109) | P value |
|--------|-------------------|-----------------------------------|-------------------------------|---------|
| 1.     | Gender            |                                   |                               |         |
|        | Male              | 86.7                              | 75.2                          | 0.030   |
|        | Female            | 13.3                              | 24.8                          |         |
| 2.     | Diabetes          |                                   |                               |         |
|        | Yes               | 58.4                              | 41.9                          | 0.015   |
|        | No                | 41.6                              | 58.1                          |         |
| 3.     | Hypertension      |                                   |                               |         |
|        | Yes               | 70.8                              | 45.7                          | 0.0002  |
|        | No                | 29.2                              | 54.3                          |         |
| 4.     | Hyperlipidemia    |                                   |                               |         |
|        | Yes               | 10.6                              | 13.3                          | 0.404   |
|        | No                | 55.8                              | 46.7                          |         |
|        | No information    | 33.6                              | 40.0                          |         |
| 5.     | Alcohol           |                                   |                               |         |
|        | Yes               | 36.3                              | 42.9                          | 0.321   |
|        | No                | 63.7                              | 57.1                          |         |
| 6.     | Smoking habit     |                                   |                               |         |
|        | Yes               | 40.7                              | 46.7                          | 0.375   |
|        | No                | 59.3                              | 53.3                          |         |
| 7.     | Exercise          |                                   |                               |         |
|        | Yes               | 49.6                              | 41                            | 0.202   |
|        | No                | 50.4                              | 59                            |         |
| 8.     | Food habit        |                                   |                               |         |
|        | Vegetarian        | 23.9                              | 11.4                          | 0.016   |
|        | Mixed             | 76.1                              | 88.6                          |         |
| 9.     | Fruits intake     |                                   |                               |         |
|        | Daily             | 38.1                              | 37.1                          | 0.592   |
|        | 1–2 times weekly  | 5.3                               | 6.7                           |         |
|        | 3–4 times weekly  | 10.6                              | 16.2                          |         |
|        | Occasionally/carely | 46.0                    | 40.0                          |         |
| 10.    | Family history    |                                   |                               |         |
|        | Yes               | 37.6                              | 41.6                          | 0.597   |
|        | No                | 62.4                              | 58.4                          |         |

To understand the association of the 9p21 risk locus with CAD and premature CAD, we aimed to study 30 SNPs in this region, their association with demographic, clinical characteristics, and interferon alpha 21 (IFNA21) levels in an Asian Indian population.
| S. No. | SNP ID/variable | Wild-type genotype /reference value | Risk genotype /OR with 95% CI | $P$ value | Heterozygous genotype /OR with 95% CI | $P$ value |
|-------|----------------|-------------------------------------|-------------------------------|----------|-------------------------------------|----------|
| 1.    | rs1004638      | AA(1)                               | 0.676 (0.469–0.974)           | 0.036    | 0.767 (0.576–1.022)                | 0.070    |
| 2.    | rs10116277     | TT(1)                               | 3.394 (0.917–12.566)          | 0.067    | 3.137 (1.044–9.429)               | 0.042    |
| 3.    | rs1011970      | TT(1)                               | 0.294 (0.707–1.235)           | 0.095    | 0.555 (0.278–1.110)               | 0.096    |
| 4.    | rs1063192      | AA(1)                               | 0.205 (0.040–1.053)           | 0.058    | 0.638 (0.376–1.084)               | 0.096    |
| 5.    | rs10757272     | TT(1)                               | 13.88 (0.998–1932)            | 0.020    | 2.703 (0.918–7.958)               | 0.071    |
| 6.    | rs10757274     | AA(1)                               | 0.216 (0.059–0.785)           | 0.051    | 2.656 (0.971–7.266)               | 0.057    |
| 7.    | rs10757578     | AA(1)                               | 0.354 (0.108–1.157)           | 0.086    | 0.565 (0.307–1.040)               | 0.067    |
| 8.    | rs10811661     | AA(1)                               | 0.287 (0.085–0.972)           | 0.045    | 2.801 (0.898–8.736)               | 0.076    |
| 9.    | rs1333040      | CC(1)                               | 3.385 (1.110–10.324)          | 0.032    | 3.905 (1.462–10.430)              | 0.007    |
| 10.   | rs1333042      | CC(1)                               | 3.206 (1.029–9.991)           | 0.045    | 3.222 (1.232–8.424)               | 0.017    |
| 11.   | rs1333045      | AA(1)                               | 0.273 (0.065–1.155)           | 0.078    | 4.052 (1.500–10.948)              | 0.006    |
| 12.   | rs1333048      | AA(1)                               | 0.720 (0.488–1.060)           | 0.096    | 1.678 (0.939–2.999)               | 0.080    |
| 13.   | rs1333049      | TT(1)                               | 1.287 (0.857–0.972)           | 0.045    | 1.424 (1.023–1.981)               | 0.036    |
| 14.   | rs1333059      | CC(1)                               | 3.835 (1.110–10.324)          | 0.052    | 2.887 (1.041–8.011)               | 0.042    |
| 15.   | rs1333060      | CC(1)                               | 3.206 (1.029–9.991)           | 0.045    | 3.222 (1.232–8.424)               | 0.017    |
| 16.   | rs1333061      | CC(1)                               | 1.316 (0.951–1.821)           | 0.097    | 4.052 (1.500–10.948)              | 0.006    |
| 17.   | rs1333062      | CC(1)                               | 7.737E+13 to 7.737E+13        | 0.000    | 2.349 (0.878–6.282)               | 0.089    |
| 18.   | rs1333063      | CC(1)                               | 4.052 (1.500–10.948)          | 0.006    | 1.678 (0.939–2.999)               | 0.080    |
| 19.   | rs1333064      | CC(1)                               | 1.285 (0.994–1.660)           | 0.055    | AG                                 |          |
| 20.   | rs1540599      | CC(1)                               | 4.052 (1.500–10.948)          | 0.006    | 1.678 (0.939–2.999)               | 0.080    |
| 21.   | rs1540600      | CC(1)                               | 1.285 (0.994–1.660)           | 0.055    | AG                                 |          |
| 22.   | rs1540601      | CC(1)                               | 4.052 (1.500–10.948)          | 0.006    | 1.678 (0.939–2.999)               | 0.080    |
| 23.   | rs1540602      | CC(1)                               | 1.285 (0.994–1.660)           | 0.055    | AG                                 |          |
| S. No. | SNP ID/variable | Wild-type genotype/reference value | Risk genotype/OR with 95% CI | P value | Heterozygous genotype/OR with 95% CI | P value |
|--------|----------------|-----------------------------------|-------------------------------|---------|-------------------------------------|---------|
| 24.    | A Age          |                                    |                               |         |                                     |         |
|        |                |                                    |                               |         |                                     |         |
|        | B Duration of CAD |                                |                               |         |                                     |         |
|        | C Hypertension |                                    |                               |         |                                     |         |
|        | D Rh blood group |                                |                               |         |                                     |         |
|        | rs7023329 ABO blood groups |      | AA(1) GG | 2.684 (1.150–6.265) | 0.022 | 1.383 (1.039–1.840) | 0.026 |
|        | Hyperlipidemia  |                                    | GA                           |         |                                     |         |
| 25.    | rs944797       |                                    | TT(1) CC                     | 0.008   |                                     |         |
|        | A ABO blood groups |                                |                               |         |                                     |         |
|        | B Hyperlipidemia |                                |                               |         |                                     |         |
|        | rs9632884       |                                    | CC(1) GG                     | 0.008   | 1.443 (1.038–2.005) | 0.029 |
|        | A Age          |                                    |                               |         |                                     |         |
|        | B ABO blood groups |                                |                               |         |                                     |         |
|        | C Fruit intake  |                                    |                               |         |                                     |         |

SNP, single-nucleotide polymorphism; CAD, coronary artery disease; OR, odds ratio; CI, confidence interval.

* The variables included for multivariate logistic regression analysis are age at onset, duration of CAD, diabetes, hypertension, hyperlipidemia, ABO blood groups, Rh blood groups, alcohol habit, smoking habit, physical exercise, food habit, and fruit intake. Only variables for which there is an association or trend towards an association have been shown in the table for each SNP.
IFNA21 gene is located 946,000 base pairs downstream of “core CAD” region in the IFNA gene cluster.

2.3. **Statistical analyses**

Demographic data were analyzed using SPSS software (Statistical Package for Social Sciences, version 21). The SNP data were analyzed using SPSS (version 21), SNPStats (available online), Haploview (available online), and multifactor dimensionality reduction (MDR) software (version 3.2). Two-tailed P values were considered to assess the significance of association in Chi-square tests used in all the statistical analyses. The power of the study was estimated by using online available software M GAS (Genetic Association Study) Power Calculator (version 3) and found to be 0.966.

3. Results and discussion

Demographic data analysis revealed a higher frequency of conventional risk factors such as diabetes, hypertension, hyperlipidemia, smoking, alcohol, nonvegetarian diet, low fruit intake, lack of physical exercise, and family history (affected first-degree relative) in CAD cases as compared to controls (Table 2). We compared demographic and clinical characteristics between premature CAD cases (age at presentation <55 years in men and <65 years in women) and nonpremature CAD cases (Table 3). Analysis revealed that frequency of females and individuals with diabetes, hypertension, and nonvegetarian diet is significantly higher in premature cases as compared to nonpremature cases. Premature cases did not score well with respect to lifestyle habits, except for regular fruit intake. Our results suggest that lifestyle habits and an increased genetic predisposition may play an important role in the etiology of premature CAD.

3.1. **SNP data analysis**

The genotypic and allele frequency distribution of the 30 SNPs among the controls and CAD and premature CAD cases revealed that the CC genotype of rs1333045 (T/C) showed more than 1.4-fold risk for CAD ($P = 0.046$ in the recessive model). We found more than 1.6-fold risk for premature CAD ($P = 0.051$ in the recessive model). Our study is the first to report an association of rs1333045 with CAD risk in an Asian Indian population.

The CC genotype of rs1333049 (G/C), a highly replicated SNP, showed a trend toward risk association with premature
CAD in the study population ($P = 0.061$ in the recessive model).$^{41,42}$

Further, we found that the AA genotype of rs16905599 (G/A) is associated with more than 2.4-fold risk for CAD in the study population ($P = 0.025$ in the codominant model and $P = 0.0069$ in the recessive model). The AA genotype of rs16905599 showed about threefold risk for premature CAD ($P = 0.018$ in the codominant model and $P = 0.0081$ in the recessive model). This is a first ever report of an association of rs16905599 (AA) with CAD indicating its potential as a useful genetic marker for CAD in the local population.

In addition, rs2383206 (A/G) showed robust association with CAD in the study population where the GG genotype of rs2383206 conferred about twofold risk for CAD ($P = 0.0004$ in the codominant model and $P = 0.0001$ in the recessive model)$^{42,43}$ and 2.4-fold risk for Premature CAD ($P = 0.0002$ in Recessive model).

An interesting case of under dominance (risk associated with only heterozygote) was found in case of rs2383208 (A/G) where the GG genotype showed about 1.7-fold risk for premature CAD ($P = 0.021$ in the codominant model and $P = 0.034$ in the overdominant model). We found a similar trend in all CAD cases ($P = 0.051$ in the overdominant model). The GG genotype of rs4977574 (A/G) was also associated with more than 1.7-fold risk for premature CAD ($P = 0.025$ in the recessive model).$^{42}$

Thus, the present study identified SNPs rs1333049 (CC) and rs4977574 (GG)$^{41,42}$ to be associated exclusively with premature CAD in the local population, suggesting that these have the potential to be used as markers to identify asymptomatic individuals at a higher risk of premature CAD. However, these results require further validation in larger cohorts.

We performed association analysis using SNPStats by adjustment for the following covariates: age, gender, diabetes, hypertension, hyperlipidemia, smoking, alcohol, and family history. The SNPs that showed significant risk association despite adjustment for the covariates were rs2383206, rs16905599, and rs2383208. Among the three SNPs, rs2383206 consistently showed the strongest CAD risk association with odds ratio greater than 2 in our study population. Hence, rs2383206, rs16905599, and rs2383208 have the potential to be used as 9p21 markers along with markers at other loci to generate a more reliable genetic risk score (GRS) for CAD in the local population.

We used the Bonferroni correction to reduce the probability of false-positive results (type I errors) and found that only SNP rs2383206 showed significant risk association with CAD ($P < 0.0016$) after doing multiple testing corrections for the 30 9p21 SNPs studied.

Upon gender stratification of the SNP genotyping data, we found that the TT and TC genotypes of SNP rs10757272 (C/T) showed an eightfold to ninefold significant risk association ($P = 0.0032$ in the dominant model and $P = 0.012$ in the codominant model) with CAD specifically among females.

The GG genotype of SNP rs7865618 (A/G) showed more than 2.5-fold risk association ($P = 0.037$ in the recessive model) with CAD specifically among males.

3.2. Multivariate logistic regression, MDR, and Haploview analysis

We performed multivariate logistic regression analysis of 30 9p21 SNPs with 12 demographic/clinical variables (Table 4). Our analysis revealed association of several 9p21 SNPs with demographic and clinical variables such as age, diabetes, hyperlipidemia, RH blood groups, ABO blood groups, food habit, and fruit intake.

Further, we examined SNP–SNP interactions and SNP–demographic/clinical variable interactions using MDR analysis for ten 9p21 SNPs showing good risk association trends with CAD: rs1011970, rs10757272, rs10757274, rs1333045, rs1333049, rs16905599, rs2383206, rs2383208, rs4977574, and rs7865618.

MDR risk prediction model analysis revealed that rs2383206 is the best one-locus model, and rs16905599 with rs2383206 is the best two-locus model in the study population.

With respect to SNP–SNP interactions, MDR analysis revealed a mild interaction between rs10757272 and rs2383208.

SNPs rs1333045, rs16905599, rs7865618, rs10757274, and rs2383206 showed no interaction among themselves. Therefore, these SNPs (showing significant risk association with CAD in our study population) appear to be operating independent of each other (Fig. 1).

With respect to SNP–demographic/clinical variable interactions, a moderate interaction was observed among the variables body mass index, age, and fruit intake, whereas a mild interaction was observed among hypertension, diabetes, and serum IPNA21 levels. Similarly, there is a mild interaction between exercise and food habit. The variables alcohol, smoking, gender, and hyperlipidemia are found to be operating independently (Fig. 2). This corroborates the observation that the 9p21 locus confers CAD risk independent of classic risk factors.$^{4,5,7}$

Linkage disequilibrium (LD) analysis was performed using Haploview software to generate LD plots for controls and cases. Our analysis has revealed six blocks (blocks 1, 2, 3, 4, 5, and 6) of linked SNP variants in controls and three blocks (blocks 1, 2, and 3) in cases (Fig. 3). Similarly, six blocks (blocks 1, 2, 3, 4, 5, and 6) of linked SNP variants were observed for the entire sample (Fig. 4).

One limitation of our study is the limited size of CAD cases. Hence, the results need to be validated in larger CAD cohorts. Our study subjects have been taken from the twin cities of Hyderabad and Secunderabad, irrespective of the geographical region, language, caste group, and community they hail from. Most CAD cases have been taken from KIMS hospital, Secunderabad, which is centrally located and receives patients from different parts of the state and country. Despite these measures, there may be a possibility of a hidden population substructure that has not been considered in this study.

4. Conclusions

Premature CAD cases showed a significantly higher frequency of females, diabetics, hypertensives, and individuals with nonvegetarian food habit as compared to nonpremature cases. SNPs rs1333045 (CC), rs16905599 (AA), rs2383206 (GG), rs2383208 (AG), and rs4977574 (GG) showed significant risk association with premature CAD in our study population. We found rs1333049 (CC) and rs4977574 (GG) to be associated exclusively with premature CAD, suggesting their potential as genetic markers to predict premature CAD in the local population. Of all the 9p21 SNPs that showed significant CAD risk association, rs2383206 emerged as the strongest genetic risk factor followed by rs16905599 and rs2383208. SNP rs10757272 (TT and TC) has shown a robust female-specific risk association, and SNP rs7865618 (GG) has shown significant male-specific risk association with CAD in our study population.

To conclude, results of the present study suggest that SNPs at the 9p21 risk locus may be used to generate a reliable GRS along with markers at other loci. This could be used for presymptomatic diagnosis in high-risk families such that suitable therapeutic measures could be advised to prevent the serious outcomes of acute coronary syndromes.
Author contributions

B.K. has contributed to the design of the work and acquisition of data. D.K.M. has contributed to the substantial revision of the manuscript. N.B. has contributed to the statistical analysis of the data. M.T.A. has contributed to the interpretation of the data and to the preparation of the final draft of the manuscript. All authors have read and approved the submitted version of the manuscript and have agreed to be personally accountable for their own contribution and all queries related to the study.

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Conflicts of interest

All authors have none to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijhj.2019.04.005.

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