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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.

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 2007[3–7] identified the 9p21.3 chromosomal region as being strongly associated with CAD and myocardial infarction (MI). This region has also been associated with stroke,[8,9] aortic,[10] abdominal,[11] intracranial aneurysms,[10] and several types of cancers.[12–14]

The chromosomal region 9p21.3 has been strongly associated with CAD in Caucasian, Italian,[15] US Hispanic,[16] Chinese,[17] Japanese,[18] Korean,[19] Asian Indian,[20] and Pakistani[21] 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 ($p16^{INK4A}$ and $p14^{ARF}$ from *CDKN2A* and $p15^{INK4B}$ from *CDKN2B*) that cause arrest of cell cycle in the G1 phase.[22,23] Further

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Table 1
SNPs studied in the 9p21.3 region.

S. No.	SNP ID	Chromosomal position[a]	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	T- 0.45
6	rs10757274 (A/G)	22096056	CDKN2B-AS1	Intron variant	G- 0.404
7	rs10757278 (A/G)	22124478	Near CDKN2B-AS1	Intron variant	G- 0.408
8	rs10757283 (C/T)	22134173	Near CDKN2B-AS1	Intron variant	T- 0.497
9	rs10811661 (T/C)	22134095	Near CDKN2B-AS1	Intron variant	C- 0.176
10	rs1333040 (T/C)	22083405	CDKN2B-AS1	Intron variant	C- 0.383
11	rs1333042 (G/A)	22103814	CDKN2B-AS1	Intron variant	A- 0.321
12	rs1333045 (T/C)	22119196	CDKN2B-AS1	Intron variant	C- 0.498
13	rs1333048 (A/C)	22125348	Near CDKN2B-AS1	Intron variant	C- 0.442
14	rs1333049 (G/C)	22125504	Near CDKN2B-AS1	Intron variant	C- 0.418
15	rs16905599 (G/A)	22069145	CDKN2B-AS1	Intron variant	A- 0.190
16	rs2383206 (A/G)	22115027	CDKN2B-AS1	Intron variant	G- 0.487
17	rs2383207 (G/A)	22115960	CDKN2B-AS1	Intron variant	A- 0.310
18	rs2383208 (A/G)	22132077	Near CDKN2B-AS1	Intron variant	G- 0.210
19	rs2811712 (A/G)	21998036	CDKN2B-AS1	Intron variant	G- 0.160
20	rs2891169 (A/G)	22131826	Near CDKN2B-AS1	Intron variant	G- 0.493
21	rs3731239 (A/G)	21974219	CDKN2A	Intron variant	G- 0.175
22	rs4977574 (A/G)	22098575	CDKN2B-AS1	Intron variant	G- 0.395
23	rs4977756 (A/G)	22068653	CDKN2B-AS1	Intron variant	G- 0.288
24	rs564398 (T/C)	22029548	CDKN2B-AS1	Intron variant, nc transcript variant	C- 0.184
25	rs615552 (T/C)	22026078	CDKN2B-AS1	Intron variant	C- 0.195
26	rs6475606 (T/C)	22081851	CDKN2B-AS1	Intron variant	C-0.322
27	rs7023329 (A/G)	21816529	MTAP	Intron variant	G- 0.449
28	rs7865618 (A/G)	22031006	CDKN2B-AS1	Intron variant	G- 0.188
29	rs944797 (T/C)	22115287	CDKN2B-AS1	Intron variant	C- 0.487
30	rs9632884 (C/G)	22072302	CDKN2B-AS1	Intron variant	G- 0.304

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

away is another gene *MTAP* that encodes methylthioadenosine phosphorylase, that is involved in the polyamine pathway (salvage of adenine and methionine).[24] 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[25–27] and circular isoforms.[27]

The association of the 9p21.3 risk locus with CAD is believed to be mediated via *ANRIL*.[28–30] Genetic variants associated with CAD are located within intronic and 3′ flanking sequences of *ANRIL*. *ANRIL* is known to be involved in the regulation of *CDKN2A*, *CDKN2B*,[25,30–34] possibly *MTAP*, and several other genes involved in the cardiovascular pathway.[35] Repression of *CDKN2A* and *CDKN2B* is known to cause smooth muscle cell proliferation that occurs at the coronary artery wall during the initial stages of atherosclerosis.[36]

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	1×10^{-6}
Percentage of males/females	36.6/63.4	81.2/18.8	$<1 \times 10^{-7}$
Height in cms (mean ± SD)	157.61 ± 10	163.49 ± 8.1	3×10^{-3}
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	8×10^{-4}
Diastolic BP (mean ± SD)	80.8 ± 5.9	76.6 ± 10.7	1×10^{-7}
Percentage of diabetics/normal	15.8/84.2	50.5/49.5	$<1 \times 10^{-7}$
Duration of diabetes in years (mean ± SD)	0.431 ± 1.13	1.50 ± 1.80	$<1 \times 10^{-7}$
Percentage of hypertensives/normal	24.6/75.4	58.7/41.3	$<1 \times 10^{-7}$
Duration of hypertension in years (mean ± SD)	0.76 ± 1.55	1.74 ± 1.82	$<1 \times 10^{-6}$
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	$<1 \times 10^{-7}$
No. of cigarettes per day (mean ± SD)	0.13 ± 0.92	4.21 ± 7.30	$<1 \times 10^{-7}$
Percentage of alcoholics (ever)/nonalcoholics	9.5/90.5	39.5/60.5	$<1 \times 10^{-7}$
Number of pegs of alcohol (mean ± SD)	1.85 ± 1.10	1.01 ± 1.40	$<1 \times 10^{-7}$
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	$<1 \times 10^{-7}$
Percentage of individuals with regular fruit intake/rare fruit intake	75/25	56.9/43.1	2×10^{-6}
Percentage of individuals with regular physical exercise/no physical exercise	78.3/21.7	45.5/54.5	$<1 \times 10^{-7}$

SD, standard deviation; BMI, body mass index; BP, blood pressure.

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/rarely	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.

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 controls include individuals with CAD, stroke, and peripheral artery disease.

Inclusion criteria for cases include angiographically documented CAD cases who showed at presentation unstable angina/ST segment elevation myocardial Infarction (STEMI)/Non-ST segment Elevation Myocardial Infarction (NSTEMI), aged 30–85 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.

IFNA21 is a proinflammatory cytokine, and elevated serum IFNA21 levels have been associated with the 9p21 risk locus.[37] To examine the association between 9p21 SNPs and the expression of IFNA21 gene, the serum circulating levels of the cytokine were estimated using sandwich enzyme linked immuno sorbent assay (ELISA) (Wuhan Fine Biotech kit method) in a random subset of the entire sample that included 184 controls and 167 CAD cases. The

Table 4
Association of SNPs with demographic and clinical parameters using multivariate logistic regression analysis.

S. No.	SNP ID/variable[a]	Wild-type genotype /reference value	Risk genotype /OR with 95% CI	*P* value	Heterozygous genotype /OR with 95% CI	*P* value
1.	rs1004638	TT(1)	AA		AT	
	A Fruits intake		0.676 (0.469–0.974)	**0.036**	0.767 (0.576–1.022)	0.070
2.	rs10116277	TT(1)	GG		GT	
	A Rh blood groups		3.394 (0.917–12.566)	0.067	3.137 (1.044–9.429)	**0.042**
	B Food habit		0.329 (0.097–1.115)	0.074		
	C Hypertension				1.930 (0.910–4.096)	0.087
3.	rs1011970	GG(1)	TT		TG	
	A Alcohol consumption		0.294 (0.070–1.235)	0.095		
	B Exercise				0.555 (0.278–1.110)	0.096
4.	rs1063192	AA(1)	GG		GA	
	A Age		0.205 (0.040–1.053)	0.058		
	B Hyperlipidemia				0.638 (0.376–1.084)	0.096
5.	rs10757272	CC(1)	TT		CT	
	A Age				2.703 (0.918–7.958)	0.071
6.	rs10757274	AA(1)	GG		AG	
	A Rh blood groups		0.216 (0.059–0.785)	**0.020**		
	B Fruits intake		1.388 (0.998–1.932)	0.051		
	C Food habit				2.656 (0.971–7.266)	0.057
7.	rs10757578	AA(1)	GG		AG	
	A Rh blood groups		0.354 (0.108–1.157)	0.086		
	B Hyperlipidemia				0.565 (0.307–1.040)	0.067
	C Food habit				2.801 (0.898–8.736)	0.076
8.	rs10811661	TT(1)	CC		CT	
	A Food habit				2.739 (0.920–8.154)	0.070
9.	rs1333040	TT(1)	CC		CT	
	A Age				2.292 (1.034–5.079)	**0.041**
	B Rh blood group				2.665 (0.998–7.117)	**0.050**
	C Food habit		0.282 (0.082–0.975)	**0.045**		
10.	rs1333042	GG(1)	AA		AG	
	A Age				2.461 (1.093–5.544)	**0.030**
	B Rh blood group				3.045 (1.094–8.478)	**0.033**
	C Fruits intake		0.720 (0.488–1.060)	0.096		
11.	rs1333045	TT(1)	CC		CT	
	A Rh blood group		0.287 (0.085–0.972)	**0.045**		
12.	rs1333048	AA(1)	CC		CA	
	A Rh blood groups		0.263 (0.071–0.969)	**0.045**		
	B Food habit		3.385 (1.110–10.324)	**0.032**	3.905 (1.462–10.430)	**0.007**
13.	rs1333049	GG(1)	CC		CG	
	A Food habit		3.206 (1.029–9.991)	**0.045**	3.222 (1.232–8.424)	**0.017**
	B Fruits intake		1.316 (0.951–1.821)	0.097		
14.	rs16905599	GG(1)	AA		AG	
	A Alcohol consumption		0.273 (0.065–1.155)	0.078		
	B Rh blood group				4.052 (1.500–10.948)	**0.006**
	C Hyperlipidemia				1.678 (0.939–2.999)	0.080
15.	rs2383206	AA(1)	GG		AG	
	A Fruits intake		1.365 (0.986–1.891)	0.061	1.424 (1.023–1.981)	**0.036**
16.	rs2383207	GG(1)	AA		AG	
	A Age				2.430 (1.073–5.501)	**0.033**
	B Rh blood group				2.887 (1.041–8.011)	**0.042**
17.	rs2383208	AA(1)	GG		GA	
	A Age		7.737E+136 (7.737E+136 to 7.737E+136)	**0.000**		
	B Food habit				2.349 (0.878–6.282)	0.089
	C Fruit intake				1.285 (0.994–1.660)	0.055
18.	rs2811712	AA(1)	GG		AG	
	A Age		5455280.330 (5455280.330–5455280.330)	**0.000**		
19.	rs2891169	AA(1)	GG		AG	
	A Diabetes		0.241 (0.081–0.719)	**0.011**	0.246 (0.092–0.656)	**0.005**
	B Smoking				0.328 (0.101–1.063)	0.063
	C Hyperlipidemia				0.523 (0.258–1.060)	0.072
20.	rs4977574	AA(1)	GG		GA	
	A Rh blood groups		0.236 (0.065–0.855)	**0.028**		
	B Food habit		2.509 (0.832–7.565)	0.102	2.675 (1.025–6.981)	**0.044**
	C Fruit intake		1.354 (0.980–1.871)	0.066		
21.	rs4977756	AA(1)	GG		GA	
	A Age				2.089 (0.907–4.810)	0.083
	B Duration of CAD				0.432 (0.180–1.037)	0.060
	C Hyperlipidemia				0.603 (0.351–1.038)	0.068
22.	rs615552	TT(1)	CC		CT	
	A Age				2.319 (1.024–5.249)	**0.044**
	B Exercise				0.542 (0.260–1.129)	0.102
	C Duration of CAD				0.331 (0.134–0.816)	**0.016**
	D Hyperlipidemia				0.521 (0.300–0.905)	**0.021**
23.	rs6475606	TT(1)	CC		CT	

Table 4 (*continued*)

S. No.	SNP ID/variable[a]	Wild-type genotype /reference value	Risk genotype /OR with 95% CI	*P* value	Heterozygous genotype /OR with 95% CI	*P* value
	A Age				2.684 (1.150–6.265)	**0.022**
	B Duration of CAD				0.364 (0.148–0.892)	**0.027**
	C Hypertension				1.992 (0.934–4.252)	0.075
	D Rh blood group				3.003 (1.046–8.623)	**0.041**
24.	rs7023329	AA(1)	GG		GA	
	A ABO blood groups				1.383 (1.039–1.840)	**0.026**
	B Hyperlipidemia		6.096 (1.595–23.302)	**0.008**		
25.	rs944797	TT(1)	CC		CT	
	A ABO blood groups				0.711 (0.487–1.039)	0.078
	B Rh blood groups		0.369 (0.112–1.210)	0.100		
	C Food habit		3.066 (0.931–10.100)	0.065		
	D Fruit intake		1.562 (1.123–2.171)	**0.008**	1.443 (1.038–2.005)	**0.029**
26.	rs9632884	CC(1)	GG		GC	
	A Age				2.584 (1.149–5.815)	**0.022**
	B ABO blood groups				0.766 (0.569–1.029)	0.077
	C Fruit intake				0.768 (0.594–0.992)	**0.043**

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

[a] 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.

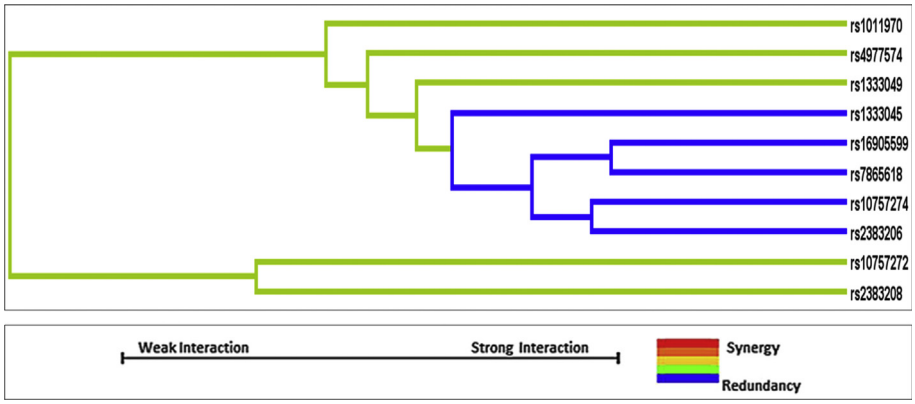

Fig. 1. MDR analysis: SNP–SNP interactions. MDR, multifactor dimensionality reduction; SNP, single-nucleotide polymorphism.

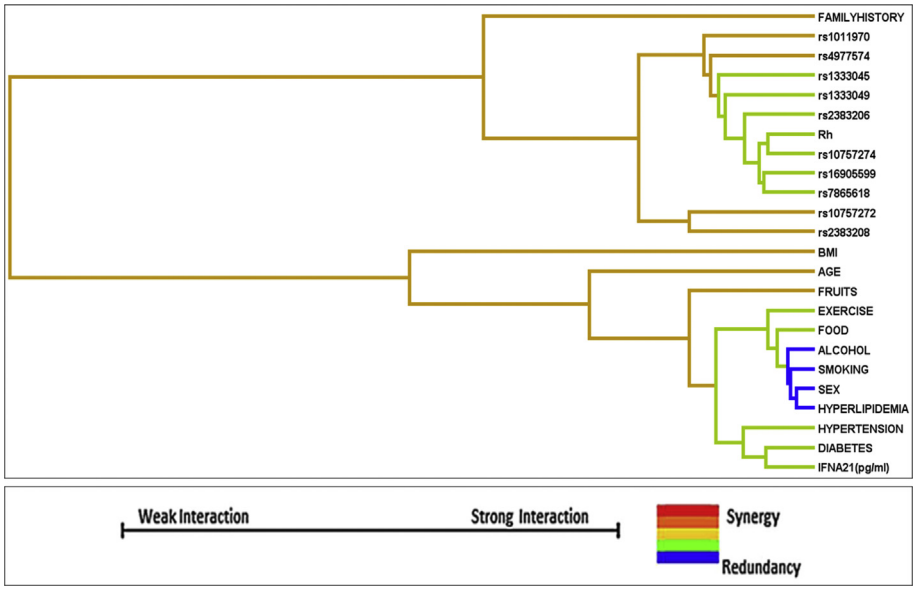

Fig. 2. MDR analysis: SNP–demographic/clinical variable interactions. MDR, multifactor dimensionality reduction; SNP, single-nucleotide polymorphism; BMI, body mass index.

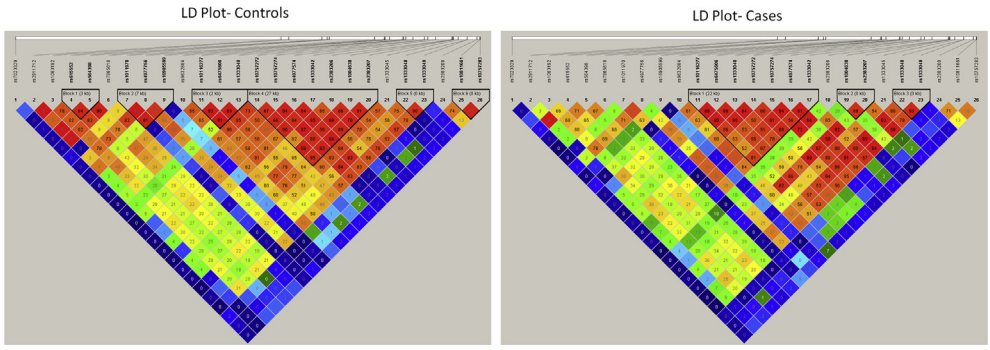

Fig. 3. LD plot for controls and cases. LD, linkage disequilibrium.

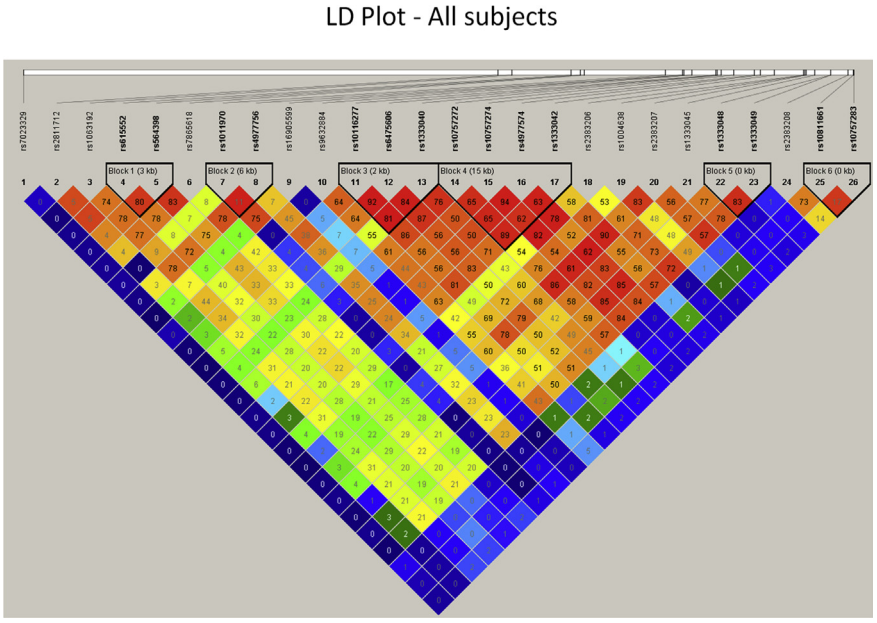

Fig. 4. LD plot for all subjects. LD, linkage disequilibrium.

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[38] (available online), Haploview[39] (available online), and multifactor dimensionality reduction (MDR)[40] 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 etiopathology 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 AG 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. Of 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 IFNA21 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.[4,7]

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.

Key messages

What is already known about this subject?

- The 9p21 risk locus is independent of most traditional risk factors of CAD such as high lipids, hypertension, obesity, and diabetes. The association of SNPs at this locus with CAD has been replicated in many ethnicities. 9p21 is also known to be a predictor of severity of CAD based on the number of vessels involved. The 9p21 risk locus has shown about twofold greater risk in individuals who develop premature CAD (<55 years in men and <65 years in women). This locus mediates its risk at the vessel wall leading to the onset of atherosclerosis in coronary arteries, thereby resulting in CAD. The 9p21 locus may have clinical utility as an early marker for CAD risk.

What does this study add?

- Our study suggests that female gender may be at higher risk of premature CAD. Lifestyle factors and genetic predisposition may have a major role to play in the etiopathology of premature CAD.
- Our study has reported a first ever risk association of SNPs rs1333045 (CC) and rs16905599 (AA) with CAD in an Asian Indian population.
- SNPs rs1333049 (CC) and rs4977574 (GG) have potential as genetic markers to identify individuals at a higher risk of premature CAD.
- Some demographic and clinical variables have shown significant association with 9p21 SNP genotypes.
- SNP rs2383206 is the strongest genetic risk factor for CAD followed by rs16905599 and rs2383208 in the study population.
- SNP rs10757274 (TT and TC) has shown a strong female-specific risk association and rs7865618 (GG) has shown a significant male-specific risk association with CAD in the study population.

How might this impact clinical practice?

This study aimed to identify and understand the unique pattern of risk factors, especially those at the chromosome 9p21.3 locus in our local population. This may contribute to presymptomatic diagnosis in high-risk families and development of preventive/therapeutic strategies most suited to our population in the near future. The 9p21.3 locus also holds good promise for pharmacogenetics research and clinical applications, so that medical treatment could be personalized to suit individuals based on their complex genetic and environmental backgrounds. However, these results require further validation in larger cohorts.

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.ihj.2019.04.005.

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