Research Article

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Genetic Susceptibility Profile of the Southern Indian Population of Hyderabad for Coronary Artery Disease

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Abstract

In order to portray the genetic susceptibility profile of the population of Hyderabad a prioritized set of 192 SNPs belonging to the 11q23.3, 9p21.3 and other CAD specific genomic regions were genotyped in a sample of ~500 cases and 500 controls. After data pruning, pair-wise logistic regression analysis of 148 CAD specific SNPs revealed significant association of 18 SNPs. The risk score analysis based on 18 SNPs and the ROC curve yielded an AUC of 0.822 suggesting substantial power of these SNPs to serve as predictive markers for CAD. Comparative analysis revealed certain SNPs belonging to APOA4-APOC3, APOA5-APOA4, BUD13, ZPR1, LPA and SMEK1 genes were associated significantly with the three phenotypic categories of CAD. The current study identified a unique set of SNPs to be associated with CAD and also portrays the distinct patterns of association of certain SNPs with the three major phenotypic sub categories of CAD in the population of Hyderabad.

Keywords: Anatomical categories, atherosclerosis, dyslipidemia, phenotypic categories, Single Nucleotide Polymorphisms.

Introduction

Cardiovascular diseases and CAD (coronary artery disease) in particular is one of the most prevalent non-communicable diseases worldwide, along with diabetes and cancers, causing enormous health and economic burden to the society, which is ever increasing. The present work is comprehension of a major research project on the patterns of genetic susceptibility for CAD in the population of Hyderabad initiated in the year 2011. As part of this project, a prioritized set of 192 SNPs involving a number of genes, saturating almost all the CAD specific genomic regions/loci, viz., 11q23.3, 9p21.3 and others were genotyped in a sample of ~500 cases and 500 controls and the results based on the genomic region specific subsets of the above SNPs reported in multiple publications (Pranavchand and Reddy, 2013, Pranavchand et al. 2016, 2017, Manjula et al. 2020, 2022). In this manuscript, we presented the comprehensive genetic susceptibility profile of the people of Hyderabad based on the results obtained hitherto and focused primarily on the additional insights obtained through further analyses of the pooled data set of 192 SNPs and also assess the degree of conformity of the findings on gene-gene interactions with the results of functional interactions of proteins.

Methodology

As mentioned above, the genotypic and other background data utilized for the present paper were generated as part of an earlier project undertaken by the corresponding author and the detailed information on the methods of sampling of subjects, laboratory analyses and the prioritized set of SNPs etc. were furnished as part of the earlier publications (Pranavchand and Reddy, 2013, Pranavchand et al, 2016, 2017, Manjula et al. 2020, 2022). However, for the sake of convenience of the readership, we furnish here a brief account of the same. A total of 1024 participants were recruited for the project, including 508 CAD cases from the CARE Hospitals in Hyderabad, India and 516 controls aged above 45 years. The DNAs were isolated from all the blood samples using phenol chloroform method and quantified with the help of Thermo Scientific Varioskan™ Flash Multimode Reader using Quant-iT™ PicoGreen1 dsDNA Assay Kit. Further, genotyping was performed using fluidigmnanofluidic SNP genotyping system at Sandor Life Sciences, Hyderabad. Of the total 192 SNPs genotyped, 96 belong to the 11q23.3, 35 to the 9p21.3 regions and remaining 61 to the other CAD specific genomic regions. For the prediction of protein-protein interactions, CAD subtype-wise

list of genes that harbored SNPs with significant association were submitted to the STRING software and interactions of high confidence were collected and analyzed (Franceschini et al. 2016).

Results

We have previously analyzed three subsets of coronary artery disease (CAD) related SNPs belonging to chromosomal regions 11q23.3 and 9p21.3 as well as the other CAD specific genomic regions identified by GWAS in the population of Hyderabad (Pranavchand and Reddy BM 2013, Pranavchand et al 2016, 2017, Manjula et al. 2020, 2022) . For the present study, after data pruning, 43 SNPs were excluded either because of their minor allele frequency of <1% or due to departure from the Hardy Weinberg Equilibrium ($p < 0.001$). In total, 18 out of the remaining 148 SNPs showed significant association with CAD (Table 1) of which 10 showed increased risk and 8 were protective. Further, ten of these 18 SNPs were intronic, 6 intergenic, and one each from exon and UTR regions. On the other hand, logistic regression analysis of pair-wise interactions of 148 SNPs (Table 2) revealed that

- among the SNPs of 11q23.3 region, 18 pair-wise SNP interactions were significant of which, 8 interactions were shown to increase CAD risk while the other 10 confer protective effect,
- among the SNPs of 9p21.3 region, two pair-wise interactions involving rs7865618 of CDKN2B-AS1 gene were found to increase CAD risk,
- interaction of rs7865618 (CDKN2B-AS1) of the 9p21.3 region with that of rs2849165 and rs1263163 (APOA5-APOA4), and rs2187126 (BUD13) of the 11q23.3 region were associated with increased risk of CAD,
- no pair-wise SNP interactions were observed either among the 61 GWAS identified SNPs or between the 61 GWAS identified SNPs and SNPs at 9p21.3 region. However, interaction of GWAS-related rs7582720 (WDR12) with that of rs6589566 (ZPR1) and rs1263163 (APOA5-APOA4) of the 11q23.3 region increased the risk of CAD. On the contrary, interaction of GWAS-related rs7582720 (WDR12) with that of rs10488699 (BUD13) of the 11q23.3 region conferred protective effect for CAD, and
- The rs6589566 (ZPR1) of the 11q23.3 region was observed to be the highly replicated SNP with most number of interactions with different SNPs within the same gene as well as with the SNPs of other genes such as BUD13 and APOA5-APOA4 of the same region.

Table 1: Significant SNPs of 11q23.3, 9p21.3 and other GWAS loci associated with coronary artery syndrome

S.No.	Chromosomal locus/Gene	Location of the SNP	SNP ID	Alleles Minor/ Major	Minor allele frequency		Odds ratio	P value
					cases	controls		
1	11q23.3/ APOA5-APOA4	Intergenic	rs1263171	A/G	0.5	0.43	1.29	0.008
2	11q23.3/ BUD13	Intronic	rs664059	T/C	0.37	0.3	1.34	0.005
3	11q23.3/ BUD13	Intronic	rs10488699	A/G	0.24	0.2	1.28	0.041
4	11q23.3/ ZPR1	Intronic	rs6589566	G/A	0.39	0.25	1.87	$4.58x10^{-09}$
5	11q23.3/ ZPR1	Intronic	rs2075294	T/G	0.08	0.05	1.56	0.023
6	11q23.3/ APOA1	Intronic	rs5072	T/C	0.32	0.37	0.8	0.04
7	11q23.3/ APOA5-APOA4	Intergenic	rs2849165	A/G	0.22	0.36	0.49	$2.48x10^{-10}$
8	11q23.3/ APOA5-APOA4	Intergenic	rs633389	T/C	0.08	0.16	0.47	$2.80x10^{-06}$
9	11q23.3/ APOA5-APOA4	Intergenic	rs1263163	A/G	0.15	0.21	0.69	0.004
10	11q23.3/ APOA5-APOA4	Intergenic	rs7396835	T/C	0.39	0.44	0.81	0.045
11	11q23.3/ BUD13	Intronic	rs17440396	A/G	0.03	0.21	0.13	$8.18x10^{-27}$
12	11q23.3/ BUD13	Intronic	rs2187126	G/A	0.1	0.13	0.74	0.049
13	9p21.3/ CDKN2B-AS1	Exon	rs7865618	G/A	0.5	0.41	1.44	0.0003
14	6/ LPA	Intronic	rs10455872	G/A	0.21	0.007	35.9	$2.83x10^{-44}$
15	2/WDR12	Intronic	rs6725887	C/T	0.416	0.02	8.36	$4.04x10^{-22}$
16	2/SMEK1	Intronic	rs782590	T/C	0.304	0.222	1.53	0.0002
17	16/HERPUD1-CETP	Intergenic	rs173539	T/C	0.037	0.011	3.54	0.002
18	3/MRAS	UTR3	rs9818870	T/C	0.08	0.11	0.7	0.041

Table 2: Significant SNP-SNP interaction effects of 148 SNP variants of 9p21.3, 11q23.3 and other GWAS loci on CAD obtained through pair wise logistic regression

Chromosomal location	SNP Pair		Odds ratio	p- value
	SNP1	SNP2		
Between Chr 2& 11q23.3SNPs	**rs7582720**	**rs6589566**	13.31	$3.31e^{-05}$
		rs1263163	8.530	$7.09e^{-05}$
		rs10488699	0.103	$1.54e^{-05}$
Among9p21.3SNPs	**rs7865618**	rs564398	0.360	$1.43e^{-05}$
		rs615552	0.348	$2.81e^{-06}$
Between 9p21.3& 11q23.3SNPs	**rs7865618**	**rs2849165**	3.291	$1.50e^{-08}$
		rs1263163	3.228	$1.98e^{-05}$
		rs2187126	3.485	$2.60e^{-05}$
Among 11q23.3 SNPs	**rs6589566**	**rs11216129**	2.338	$1.19e^{-05}$
		rs1263149	0.373	$2.03e^{-08}$
		rs11216126	2.222	$2.36e^{-05}$
		rs2075295	2.421	$3.11e^{-07}$
		rs623908	2.456	$3.21e^{-07}$
		rs1263163	4.114	$3.47e^{-07}$
		rs17119975	2.374	$5.46e^{-06}$
		rs2041967	2.433	$5.62e^{-07}$
		rs3741298	0.465	$3.29e^{-05}$
		rs10790162	0.399	$9.34e^{-05}$
		rs918144	0.439	$9.80e^{-07}$
	rs1263163	rs2187126	0.043	$1.40e^{-13}$
		rs2849165	0.058	$2.54e^{-15}$
		rs10488699	0.085	$3.68e^{-13}$
		rs1263171	0.341	$7.66e^{-08}$
	rs633389	rs2187126	0.065	$2.12e^{-10}$
		rs10488699	0.147	$2.49e^{-08}$
	rs2187126	**rs1263171**	2.869	$1.35e^{-05}$

Bold indicates risk SNP combinations

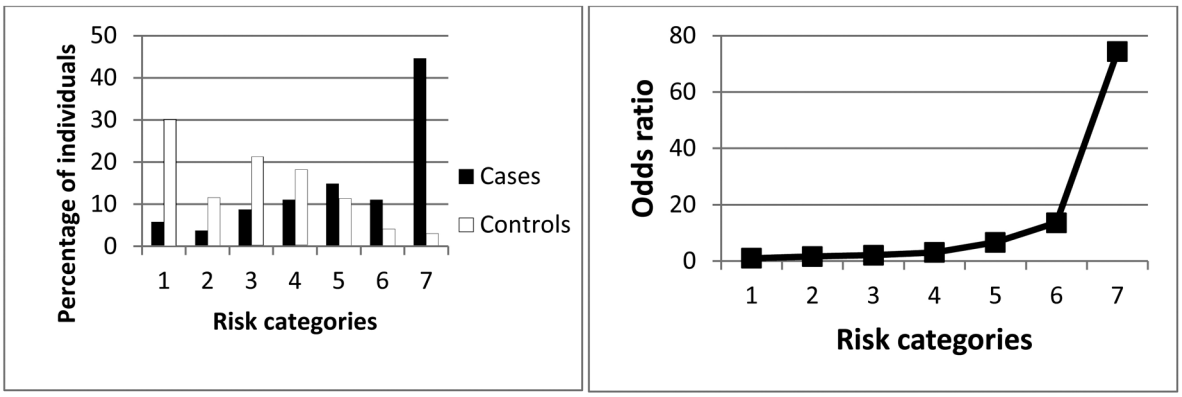
Results based on further analyses of the pooled data set of 192 SNPs.

To determine the combined effect of all the 18 significantly associated SNPs, we have computed the weighted mean proportion of risk alleles by assigning a value of 2 for two risk alleles, 1 for one risk allele and 0 for no risk alleles, with weights as relative log odds ratios of different SNPs. The cumulative risk allele score (CRS) for each individual is obtained by multiplying weighted mean proportion with 18, which is the number of significant SNPs. Further, the individuals with CRS ranging from 1.33 to 24.11 were grouped into 7 risk categories with increasing risk scores and the odds ratios (Table 3).The Z-scores were calculated by taking risk category 1 (risk score 1-9.9) as the reference. The frequency distribution showed an increasing trend in the frequency of cases relative to controls with increasing risk categories (Fig. 1a). Furthermore, an increasing trend in the odds ratios was also observed with increasing risk scores as indicated by the risk categories (Fig. 1b). The ROC curve yielded an area under curve (AUC) as 0.822 (95%CI: 0.791-0.852, $p<0.0001$), which is highly significant indicating that these genetic variants have substantial power to serve as predictive markers for CAD (Fig. 2).

Table 3: Cumulative risk score for CAD associated 18 SNP variants of 9p21.3, 11q23.3 and other CAD specific genomic loci

Risk category	Risk score	Cases (%)	Controls (%)	Odds ratio (95% CI)	Z-score	p value
1	1-9.9	5.83	30.11	Reference	-	-
2	10-10.9	3.79	11.65	1.64 (0.76-3.52)	0.699	0.484
3	11-11.9	8.75	21.32	2.12 (1.14-3.96)	1.272	0.203
4	12-12.9	11.08	18.24	3.08 (1.68-5.64)	1.898	0.057
5	13-13.9	14.87	11.43	**6.67 (3.63-12.2)**	3.21	**0.001**
6	14-14.9	11.08	4.18	**13.6 (6.60-28.0)**	3.564	**0.0004**
7	15-24.9	44.6	3.08	**74.3 (36.1-152)**	5.793	**<0.0001**

Bold indicates significant p value/ odds ratio



(a) (b)

Figure 1: (a) Relative proportion of cases and controls among the risk categories. (b) Plot of odds ratios according to risk categories

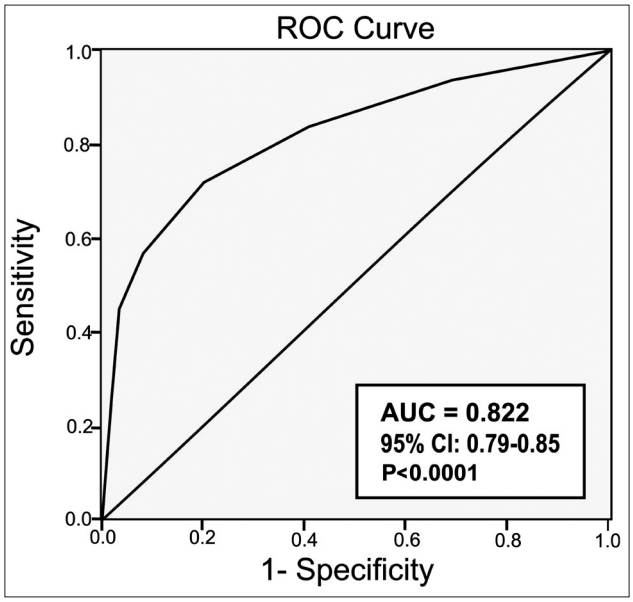


Figure 2: Receiver Operating Characteristic (ROC) curve representing the area under curve (AUC) for the significant SNPs of the pooled cohort.

Patterns of association of the SNPs with anatomical and phenotypic subcategories of CAD.

The association of 148 variants with each of the anatomical [Insignificant, Single vessel disease (SVD), Double vessel disease (DVD) and Triple vessel disease (TVD)], and phenotypic [(Angina, Acute coronary syndrome (ACS) and Myocardial infarction (MI)] categories of CAD was further analyzed. Notably, 40 out of 148 SNPs that belong to 17 genes were associated with one or more of the phenotypic and anatomical categories of CAD (Table 4).The number of SNPs associated varied with the sub-type of CAD: 10 in insignificant, 20 in SVD, 15 in DVD, 18 in TVD, 9 in angina, 14 in ACS and 15 in MI categories. This is interesting as an additional 22 SNPs (apart from the 18 SNPs in the pooled sample of CAD cases) that were not associated in the pooled CAD sample, were associated with the sub-categories of CAD. For example, rs633867 of APOA5-*APOA4* gene was absent from the set of 18 SNPs associated with the pooled analysis, but was found to be associated with SVD and ACS categories. All the SNPs except rs9818870 from the set of 18 SNPs that were significantly associated in the pooled set of CAD were also found to be associated with one or more categories of the CAD. Three SNPs belonging to APOA5-*APOA4* (rs633389), BUD13 (rs17440396) and LPA (rs10455872) genes were found to be associated with all the anatomical and phenotypic categories. Further, to identify the SNP interaction effects on different categories of CAD, pair- wise logistic regression analysis was performed for the 148 SNPs (Table 5). The intergenic SNP variants of *APOA4-APOA5* (rs1263163-rs2849165) were associated with reduced risk for all the anatomical and phenotypic categories of CAD except the MI category. Interaction between the BUD13 and ZPR1 (rs2041967-rs6589566) was shown to significantly increase the risk for SVD (OR=3.147; p=4.04e^{-05}) and ACS (OR=2.769; p= 1.03e^{-05}) categories of CAD. Relatively more number of pair-wise interactions was observed for the phenotypically severe ACS category; the SNP rs6589566 of ZPR1 was found to be interacting with the SNPs of ZPR1, BUD13 and APOA5-*APOA4* of the 11q23.3 region. There were no significant epistatic interactions observed for the MI category.

Table 4: SNPs associated with different clinical categories of CAD with their significant p-value; SNPs in bold are from pooled cohort

				Anatomical categories				Phenotypical categories		
S. NO	**Gene/ Nearby gene**	**SNP**	**Pooled cohort**	**Insignificant**	**Single vessel disease**	**Double vessel disease**	**Triple vessel disease**	**Angina**	**Acute coronary syndrome**	**Myocardial infarction**
1	APOA1	**rs5072**	0.04	-	0.037	-	-	-	-	-
2		rs5081	-	-	0.008	-	-	-	-	0.0203
3		rs632153	-	-	0.02	-	-	-	-	0.035
4	APOA4-APOC3 APOA5-APOA4	rs2849176	-	-	-	-	-	-	-	0.032
5		**rs1263163**	0.004	-	-	0.009	0.004	-	-	1.64×10^{-08}
6		**rs1263171**	0.008	-	-	0.029	0.008	-	-	0.04
7		rs11600380	-	-	-	-	0.03	-	-	-
8		rs2542063	-	-	-	0.047	-	-	-	-
9		rs2727793	-	-	-	0.044	-	-	-	-
10		**rs2849165**	2.48×10^{-10}	3.1×10^{-05}	2.9×10^{-05}	0.0002	0.004	-	1.18×10^{-10}	0.0013
11		rs625524	-	0.006	-	-	0.028	-	-	-
12		**rs633389**	2.80×10^{-06}	0.004	0.033	0.003	0.005	0.0027	0.0054	0.0053
13		rs633867	-	-	0.02	-	-	-	0.015	-
14		**rs7396835**	0.045	-	-	0.042	-	-	-	-
15		rs1263167	-	-	-	-	-	0.037	-	-
16	BUD13	**rs10488699**	0.041	0.04	-	-	0.042	-	-	0.0001
17		**rs17440396**	8.18×10^{-27}	3.5×10^{-10}	1.3×10^{-10}	2.3×10^{-07}	2.1×10^{-06}	1.36×10^{-08}	5.15×10^{-14}	6.89×10^{-08}
18		**rs664059**	0.005	-	0.017	-	0.042	-	0.029	0.008
19		**rs2187126**	0.049	-	-	-	-	-	0.025	-
20	CDKN2A CDKN2B	rs10116277	-	-	-	0.037	-	-	-	-
21		rs1333048	-	-	0.032	-	-	-	-	-
22		rs1333040	-	-	-	0.036	-	-	-	-
23		rs17694493	-	-	0.006	-	-	-	-	-
24		**rs7865618**	0.0003	0.037	0.022	-	0.023	-	0.007	-
25	FADS1	rs174546	-	-	-	-	-	0.041	-	-
26	FTO	rs9940128	-	-	-	-	0.013	-	-	-

27	GALNT2	rs4846922	-	-	-	-	-	-	-	0.0002
28	HERPUD1, CETP	**rs173539**	0.002	-	0.0005	0.017	0.008	-	0.019	5.28×10^{-07}
29		rs247617	-	-	0.036		0.019	-	-	-
30	LPA	**rs10455872**	$2.83x10^{-44}$	1.22×10^{-41}	1.22×10^{-30}	6.20×10^{-26}	4.38×10^{-34}	1.80×10^{-37}	3.06×10^{-47}	2.63×10^{-08}
31		rs7767084	-	-	-	-	-	0.046	-	-
32	LPAL2	rs3127599	-	-	0.035	-	-	-	-	-
33	MTNR1B	rs10830962	-	-	-	-	0.024	-	-	-
34	SMARCA4	rs1122608	-	-	-	-	-	-	0.04	-
35	SMEK1	**rs782590**	0.0002	-	0.03	7.16×10^{-5}	-	0.002	0.014	0.017
36	TWIST1	rs2107595	-	-	0.003	-	-	-	-	-
37	rs2075294	**0.023**		0.012	-	0.048	-	0.049	-	0.026
38		rs3741298	-	0.038	-	-	-	-	-	-
39		**rs6589566**	$4.58x10^{-09}$	1.5×10^{-06}	0.0005	1.9×10^{-05}	0.002	5.63×10^{-07}	3.36×10^{-09}	-
40	WDR12	**rs6725887**	$4.04x10^{-22}$	2.03×10^{-23}	3.09×10^{-11}	2.56×10^{-10}	3.53×10^{-12}	2.44×10^{-20}	1.60×10^{-23}	-
41	MRAS	**rs9818870**	0.041	-	-	-	-	-	-	-

Table 5: Significant SNP-SNP interactions of 148 SNP variants of 9p21.3, 11q23.3 and other GWAS loci with anatomical and phenotypic categories

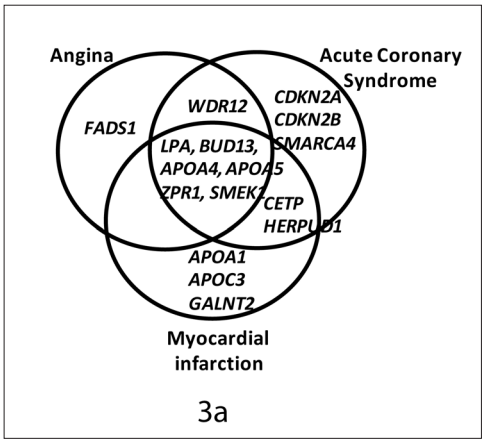
S.No	SNP pair	Associated category	Odds ratio	p value	Chromosomal location	Type of interaction
1	rs1263163-rs2849165	insignificant SVD DVD TVD Angina ACS	0.028 0.035 0.052 0.027 0.031 0.061	$6.20e^{-08}$ $1.21e^{-09}$ $7.03e^{-07}$ $1.90e^{-06}$ $2.09e^{-09}$ $1.09e^{-10}$	chr11-chr11	intergenic variants of APOA5, APOA4 genes
2	rs10488699-rs1263163	SVD	0.065	$4.10e^{-06}$	chr11-chr11	intronic (of BUD13, ZPR1) and intergenic (of APOA5,APOA4) variants
		ACS	0.119	$1.45e^{-07}$		
3	rs2187126-rs633389	SVD	0.075	$5.26e^{-05}$	chr11-chr11	
		ACS	0.052	$1.65e^{-06}$		
4	**rs2041967-rs6589566**	SVD	**3.147**	$4.04e^{-05}$	**chr11-chr11**	
		ACS	**2.769**	$1.03e^{-05}$		
5	rs10488699-rs2187126	Angina	0.106	$5.41e^{-05}$	chr11-chr11	Intronic variants of BUD13 gene
6	rs2187126-rs1263163	SVD Angina ACS	0.014 0.014 0.060	$7.77e^{-05}$ $7.49e^{-05}$ $6.46e^{-08}$	chr11-chr11	Intronic variant of BUD13 and intergenicvariant of APOA5, APOA4 genes

7	rs9349379- rs918144	ACS	0.459	$3.77e^{-05}$	chr6-chr11	PHACTR1-BUD13 CDKN2B AS
8	rs615552-rs7865618		0.302	$6.98e^{-05}$	chr9-chr9	
9	rs180326-rs6589566		0.344	$8.61e^{-05}$	chr11-chr11	
10	rs10488699-rs633389		0.171	$6.59e^{-05}$	chr11-chr11	
11	rs918144-rs6589566		0.393	$4.04e^{-05}$	chr11-chr11	
12	rs1263149-rs6589566		0.349	$7.96e^{-06}$	chr11-chr11	intronic/ intergenic variants of ZPR1/ BUD13/ APOA4&APOA5 genes
13	rs1263163-rs1263171		0.305	$2.10e^{-06}$	chr11-chr11	
14	**rs11216126-rs6589566**		**2.602**	**$8.09e^{-05}$**	**chr11-chr11**	
15	**rs11216129-rs6589566**		**2.717**	**$5.83e^{-05}$**	**chr11-chr11**	
16	**rs2075295-rs6589566**		**3.044**	**$1.46e^{-06}$**	**chr11-chr11**	
17	**rs17119975-rs6589566**		**2.824**	**$2.22e^{-05}$**	**chr11-chr11**	
18	**rs623908-rs6589566**		**2.535**	**$4.62e^{-05}$**	**chr11-chr11**	
19	**rs1942478-rs6589566**		**15.15**	**$9.94e^{-05}$**	**chr11-chr11**	

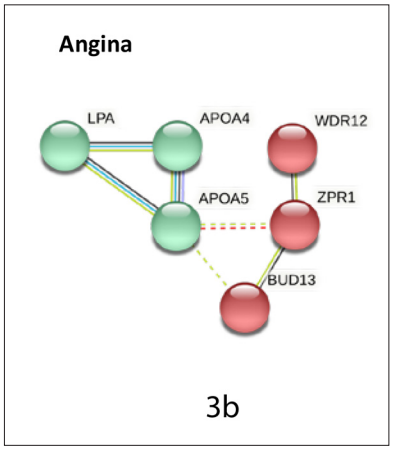
No SNP-SNP interaction was significant in the category- myocardial infarction

Predicted protein-protein interactions by the functional interactions between the genes.

Comparative analysis revealed that at least one SNP belonging to APOA4-APOC3, APOA5-APOA4, BUD13, LPA, SMEK1 and ZPR1 genes were associated significantly with all the three phenotypic categories (Fig. 3a). Further, SNPs of gene WDR12 were associated with both Angina and ACS, and SNPs of genes CETP and HERPUD1 were associated with ACS and MI. Also, certain genes were associated uniquely with a phenotypic category: FADS1 with Angina; CDKN2A, CDKN2B and SMARCA4 with ACS; APOA1, APOC3 and GALNT2 with MI (Fig. 3a). It is however important to interpret the functional implications of SNPs in the context of CAD pathophysiology. Irrespective of the role of SNPs in gene function which needs to be experimentally evaluated, we analyzed predicted protein-protein interactions between the genes that contained SNPs which were implicated in different categories of CAD. Prediction analysis of functional interactions among the genes revealed that protein-protein interactions between LPA, APOA4, APOA5, ZPR1 and BUD13 were present in all the 3 phenotypic categories (Figures 3b, 3c, 3d). Interestingly, the gene WDR12 that was associated with Angina and ACS but not MI, was found to be interacting with the ZPR1 gene (Fig. 3b & c) indicating the potential role of WDR12-ZPR1 protein interactions in the pathogenesis, specifically of Angina and ACS sub-types. The gene CETP that interacts with LPA, APOA1, APOA4, APOA5 and APOC3, was found in ACS and MI but not in the Angina category (Figures 3c & d). Notably, the three genes (CDKN2A, CDKN2B and SMARCA4) that formed a functional interaction network were specifically present in ACS category only (Fig. 3c).



3a



3b

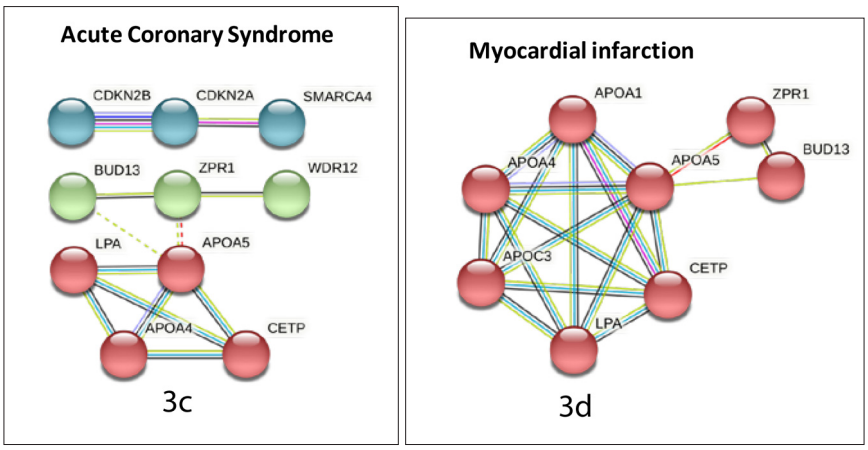


Figure 3: (a) Venn diagram showing the interactions of genes among different phenotypic categories. Networks of predicted protein-protein interactions between genes that contained SNPs of significance for (b) Angina, (c) Acute Coronary Syndrome, and (d) Myocardial Infarction were created using STRING software. The solid lines represent interactions within a cluster while the dashed lines represent inter-cluster interactions. The colored lines represent various types of evidences for the displayed interactions: red line - presence of fusion evidence, green line - neighbourhood evidence, blue line – co-occurrence evidence, purple line - experimental evidence, yellow line – text mining evidence, light blue line - database evidence, and black line – co-expression evidence.

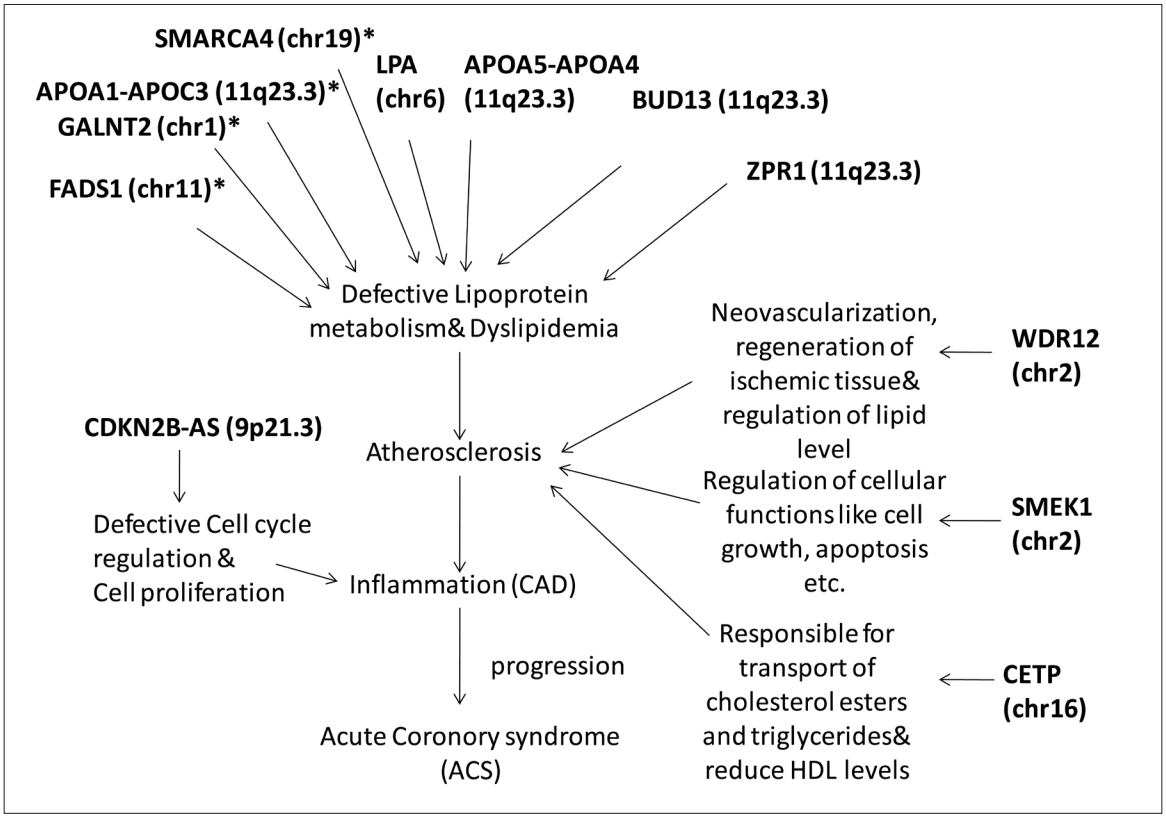
Discussion

The current study presents a comprehensive genetic susceptibility profile of CAD in the population of Hyderabad by considering SNPs from pleiotropic regions: 11q23.3, 9p21.3 and other CAD-specific genomic regions/loci. The importance of 11q23.3 region is evident from the presence of apolipoprotein genes (APOA1, APOC3, APOA4 and APOA5), and regulatory protein coding genes (BUD13 and ZPR1) that play a prominent role in regulating lipoprotein metabolism and cholesterol homeostasis (Karathanasis 1985). Further, the 9p21.3 was found to be involved in several cellular processes like cell-cycle arrest, cell renewal, senescence and apoptosis (Gil and Peters 2006, Popov and Gil 2010). The SNPs from CAD-specific GWAS identified loci were selected from 17 different chromosomes involved in diverse metabolic functions. Specifically, the CAD-associated genes were implicated in various steps during the progression of CAD (Fig 4). The CAD-associated SNPs belonging to APOA5-APOA4, BUD13, ZPR1 and LPA genes were known to be involved in defective lipoprotein metabolism and dyslipidemia consequently resulting in Atherosclerosis. The CAD-specific GWAS genes WDR12 and CETP were known to be involved in defective lipid metabolism (Barter et al. 2003, Zabalza et al. 2015) and SMEK1 in abnormal cell cycle regulation (Duan et al. 2021) which in turn were associated with atherosclerosis (Fig 4). Given the characteristic dyslipidemic feature of Indian populations (Kumar et al. 2014) and clinical heterogeneity of CAD (Luo et al. 2007) it is pertinent to explore the pattern of association of these variants with clinical/phenotypic categories of CAD in order to understand the genetic mechanisms involved in the progression of the disease.

The genes APOA5-APOA4, BUD13 and LPA that are known to be involved in defective lipoprotein metabolism were found to be present in all the phenotypic and anatomical categories, which could suggest their prominent role towards disease progression in the population of Hyderabad (Fig 4). Further, WDR12 was found to be common for Angina and ACS categories. However, the role of WDR12 in the development of CAD needs yet to be established. Similarly, CETP is commonly associated with ACS and MI and this gene was observed to be involved in the transfer of cholesteryl ester from high density lipoprotein (HDL) to other lipoproteins. The CDKN2B-AS of 9p21.3 was known to be involved in defective cell cycle regulation and cell proliferation causing inflammation and might play an important role in the later part of atherosclerosis (Fig 4). The pair-wise logistic regression analysis showed significant interaction of WDR12 with ZPR1 and APOA5-APOA4. Further, rs6589566 (ZPR1) exhibited risk towards CAD in interaction analysis with most of the other genes (Table 5). The significant genes observed in this study are confirmatory with the results of functional interactions of proteins as APOA4-APOA5, ZPR1, BUD13 and LPA are common to all the phenotypic categories suggesting their importance in the development of CAD (Fig 3a). Further, the unique pattern of association of CDKN2A and CDKN2Bgenes in ACS (Fig 3c) as well as APOA1 and APOC3 in MI (Fig 3d) may suggest the distinct role of these genes in specific phenotypic/clinical categories of CAD.

To understand the correlation between the genes that were identified in this study and the development of CAD, we reviewed previous studies related to this multi-step pathogenic process (Fig 4).The genes LPA, APOA5-APOA4, BUD13 and ZPR1 from the pooled cohort as well as the genes SMARCA4, APOA1-APOC3, GALNT2 and FADS1 that were associated with phenotypic categories only, correspond to defective lipoprotein metabolism and dyslipidemia that might lead to atherosclerosis (Fig 4). Furthermore, the gene WDR12 that is involved in deregulation of lipid levels, SMEK1 that is associated with dysfunction of cellular processes, and CETP that is known to contribute to atherogenic lipoprotein profile were previously reported (Popov and Gil 2010, Barter et al. 2003, Zabalza et al. 2015) to be associated with atherosclerosis

(Fig4).The CDKN2B-AS of 9p21.3 was found to be involved in inflammation, which will ultimately lead to CAD (Fig 4).Thus the SNPs found in our study showed significant association to the disease phenotypes, suggesting their functional role in CAD pathogenesis.



*Indicates SNPs specifically associated with clinical/phenotypic categories.

Figure 4: Schematic representation of the model depicting potential multi-step mechanism of CAD pathogenesis involving significant risk conferring genes in the population of Hyderabad.

Conclusion

This study not only identified a set of SNPs that are associated with CAD but also for the first time established distinct patterns of association of certain SNPs with three major phenotypic/clinical sub-categories of CAD in the population of Hyderabad, hence offers an opportunity to develop population-specific diagnostic and prognostic biomarkers for CAD and its phenotypic categories or stages.

Recommendations

Given the distinct population structure and genetic susceptibility patterns of Indians, it is imperative to conduct extensive studies that take into account the ethnic and geographic diversity of the nation in order to derive representative genetic susceptibility profiles of CAD. More population specific research on the subcategories of CAD is necessary in order to understand the genetics of the disease which helps in better diagnosis and treatment of coronary artery disease.

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Authors' Contribution

BMR designed and executed the study and supervised drafting and finalization of the manuscript, IK, RKK and GM analyzed data, interpreted the results and drafted the manuscript in consultation with BMR, PR and SA who were also actively involved earlier in the process of acquiring background data, blood samples and genotyping. BSR participated in the discussions on clinical aspects of the problem and assisted IK in the statistical analysis and drafting of the manuscript. All the authors read and approved the manuscript.

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Declarations

Conflict of interest

There are no conflicts of interest.

Ethical Statement

The study protocol was approved by the Indian Statistical Institute Review Committee for Protection of Research Risks to Humans. Written informed consent of all the participants is obtained as per the guidelines.

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