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Targeted Resequencing Approach in Identification of Genomic Variants in Individuals with Myocardial Infarction Using Next Generation Sequencing-Based Multigene Custom Designed MI Panel

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ABSTRACT The purpose of this study was to build advanced genetic diagnosis by identifying biomarkers associated with Myocardial Infarction (MI) related disorders and to investigate the genetic predisposition to MI and genes causative. For this investigation and identification, the researchers have chosen genes most predominant in the Indian population towards cardiovascular disorders and designed a customised panel comprising a set of selected genes implicated in cardiovascular health, atherosclerosis, and thrombosis, which is used for further sequencing and screening of the subjects involved in the study. The researchers observed mutations in genes CELSR2, MRPS6 and APOB show clear involvement in MI and cardiovascular related disorders and identified as the most repetitive across all the subjects. This comprehensive analysis from cases to controls can contribute to valuable insights into developing precision medicine strategies focused on examining the individual genes and variants included with MI to elucidate their specific contributions to MI-related conditions and developing Polygenic risk scores.

INTRODUCTION

Myocardial infarction (MI) in common refers to necrosis of heart muscles causing sudden deprivation of oxygen and blood supply to arteries, making a serious medical emergency if not diagnosed and treated at early stages. MI has emerged as a complex disease from the interaction between several genetic and environmental factors (Boffa and Fazio 2024). Genetic risk factors for MI and coronary artery disease (CAD) and genetic risk varies across different ethnic groups based on the diverse range of populations, ethnicity, and lifestyle habits (Khera et al. 2023). Understanding the genetic basis of CVD not only informs risk prediction but also opens avenues for targeted therapeutic interventions and personalised medicine approaches. Iincorporating genetic information into risk assessment models, healthcare providers can better identify individuals at higher risk for MI, leading to more customised prevention and treatment strategies. Integration of polygenic risk scores with clinical risk factors represents a significant advancement in predicting myocardial infarction risk (Huang et al. 2023). Understanding the genetic basis of CVD not only informs risk prediction but also opens avenues for targeted therapeutic interventions and personalised medicine approaches by adjusting medication dosages based on genetic profiles can enhance efficacy and reduce adverse effects on MI patients (Williams and Jacobson 2022). In recent years, Genome-Wide Association Studies (GWAS) have emerged as a powerful tool for investigating the genetic underpinnings of complex diseases, including cardiovascular conditions (Walsh et al. 2023; Mauersberger et al. 2021). Recent GWAS have identified a multitude of genetic variants associated with various cardiovascular phenotypes, shedding light on the intricate interplay between genetic factors and CVD risk (Tcheandjieu et al. 2022). Notably, studies such as the CARDIOGRAMplusC4D Consortium have contributed substantially to the understanding of the genetic architecture of coronary artery disease (CAD) (Aragam and Natarajan 2020). However, despite these advancements, there remains a need for more com-

prehensive investigations into the polygenic nature of CVDs and the potential interactions between genetic variants and environmental factors (Wang et al. 2023).

Objectives of the Study

The main aim and objective of this study is to understand the underlying gene polymorphisms involved in myocardial infarction using the targeted resequencing approach with a customised MI screening panel for NGS analysis:

1. Targeted exon sequencing of reported genes using custom designed MI screening panel among cases and controls.
2. Massively parallel sequencing data from subjects will be combined with the data from controls to validate and identify the specific variants.
3. Identifying the most predominant expressed genetic variants among subjects screened and reporting the novel variants, which may be used as gene markers for early recognition of the condition.

To achieve the objectives, candidate genes were chosen for their acknowledged role from GWAS, and in this present article, the researchers describe the results of the analysis of single-nucleotide polymorphisms (SNPs) drawn from 17 candidate genes in 48 coronary artery disease (CAD) cases.,

METHODOLOGY

In the present study, the researchers focused on around 50 samples from the cardiology department of SVIMS (Sri Venkateswara Institute of Medical Sciences). A 17-gene customised MI panel was designed with the most significant genes associated with coronary artery disease (CAD and CVD) for molecular investigation. Genes selected for the present study and used for screening the control and subject group include CELSR2, MTHFR, MIA3, MYBPH, CXCL12, C12orf43, HNF1A, APOE, WDR12, APOB, KCNE2, MRPS6, SLC5A3, MRAS, PHACTR1, MTAP, and CDKN2B.

Rationale for Gene Selection

The genes included in the customised panel were selected based on a thorough review of existing literature and databases, aiming to encompass key pathways and mechanisms associated with Myocardial Infarction. Considerations for inclusion were as follows:

1. Previous Associations: Genes with established associations with MI, supported by robust evidence from genetic studies and meta-analyses, were prioritised.
2. Pathway Involvement: The researchers ensured the representation of genes involved in crucial pathways such as lipid metabolism, coagulation, endothelial function, and inflammation, as these pathways play integral roles in the pathogenesis of MI.
3. Population Relevance: Genes with variations that show significant relevance in diverse populations were favoured to enhance the panel's applicability across different ethnic groups.

By combining these genes into a comprehensive panel, the researchers aimed to create a tool that provides a nuanced understanding of the genetic factors contributing to Myocardial Infarction, paving the way for more targeted and personalised approaches to risk assessment and prevention.

Materials and Methods

A total of 48 patients aged above 18 years that were diagnosed with acute coronary syndromes and were admitted to the Department of Cardiology, Sri Venkateswara Institute of Medical Sciences, Tirupati, having acute coronary syndromes including ST-Elevation myocardial infarction (STEMI), Non-ST-Elevation myocardial infarction (NSTEMI) and unstable angina, were recruited for this study. Plasma lipid profiles including total cholesterol, high density lipoprotein (HDL), low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), and Triglycerides were measured before inclusion into the study group. Fasting lipid profiles (total cholesterol, HDL-cholesterol, triglycerides) were estimated using commercial kits on DXC600 Beckman autoanalyser. To validate the findings from the present study the researchers also recruited around 22 healthy controls aged above 18 years representing 11 female and 11 male samples without having any symptoms of CVD related disorders. The study obtained clear ethical approval and clearance from the institute, specifically from the Institutional Ethics Committee, with reference number 844(a) dated 23/04/2019.

After obtaining written informed consent from the participants, 5 ml of blood was collected using vacutainers coated with EDTA. DNA extraction was carried out according to the instructions provided by the manufacturer, utilising the PureLink Genomic DNA Isolation Kit from Thermo Fisher Scientific. Following the manufacturer's protocol, approximately 100 ng of genomic DNA was used to construct DNA libraries using the Illumina Ampliseq Library preparation kit. The constructed libraries were quantified using the High Sensitivity Genomic DNA Assay on the Qubit 3.0 platform from Thermo Fisher Scientific. The Illumina Miseq platform was employed for sequencing, utilising the MicroV2 300 cycles kit. Sequencing procedures were conducted following the manufacturer's guidelines. Around 1.8 GB of total data generated after sequencing and raw files were processed for fastq conversion and data analysis.

RESULTS

The researchers describe 21 SNPs at different loci among seven genes from 17 genes screened towards 47 samples and identified CELSR2, WDR12, MTHFR, MIA3, and APOB as the most repetitive across all the subjects (Fig. 1). Also, notably, HNF1A and SLC5A3 genes showed their significant presence, and similarly, APOE, MRAS, and CDKN2B showed weak association with MI. A total of 48 individuals (29 male and 19 female) were included in the study and overall, 244 variations were identified in Table 1. Alterations were observed in 31 samples (20 male and 11 female) against a total of 48 samples showing a 65 percent mutation rate and 96 SNP changes identified overall from all the 17 genes targeted for sequencing (Fig. 2).

Table 1: Showing mutation summary for 47 samples sequenced (total of 244 variants were detected in 47 samples analysed targeting 15 genes)

ID	*Summary*	*Mean*	*Median*
NCBI_Build	NA	NA	NA
Center	NA	NA	NA
Samples	47	NA	NA
nGenes	15	NA	NA
Frame_Shift_Del	25	0.532	0
Frame_Shift_Ins	4	0.085	0
Missense_Mutation	209	4.447	4
Nonsense_Mutation	4	0.085	0
Splice_Site	2	0.043	0
Total	244	5.191	5

Data Analysis

Data analysis was conducted using custom built-in pipelines designed specifically for this panel on GRCh38-hg38 assembly. All the variants were annotated and filtered before processing with adapter trimming, duplicate percentage, and GC content. Analysis was carried out using the GRCh38-hg38 pipeline for targeted genes and further variant prioritisation was based on quality scores, depth, and coverage of sequenced samples. All the samples sequenced were primarily filtered using the sequencing quality Q30 score with greater than 80 percent, having a depth of more than 75 percent with 99 percent coverage by converting bcl files to fastq using the bcl2fastq conversion application of Illumina. For data analysis and variant interpretation, online tools such as Varminer and Franklin Genoox were used, one sample failed the QC and only 47 samples were selected for data analyses and reporting for this study.

DISCUSSION

The present study identified several susceptibility variants for CAD and/or MI in the selected

Table 2: Showing mutation summary from CELSR2 gene

SID	*Chr*	*Ref*	*Gene*	*Variant*	*exon*	*txChange*	*AAchange*
43	chr1	T	CELSR2	FS_Del	exon23	c.7189delT	p.F2397Sfs*19
32	chr1	T	CELSR2	FS_Del	exon23	c.7189delT	p.F2397Sfs*19
33	chr1	T	CELSR2	FS_Del	exon23	c.7189delT	p.F2397Sfs*19
5	chr1	T	CELSR2	FS_Del	exon23	c.7189delT	p.F2397Sfs*19
44	chr1	T	CELSR2	FS_Del	exon23	c.7189delT	p.F2397Sfs*19
6	chr1	A	CELSR2	FS_Del	exon 1	c.1490delA	p.D497Vfs*24
27	chr1	T	CELSR2	FS_Del	exon23	c.7189delT	p.F2397Sfs*19
19	chr1	T	CELSR2	FS_Del	exon23	c.7189delT	p.F2397Sfs*19

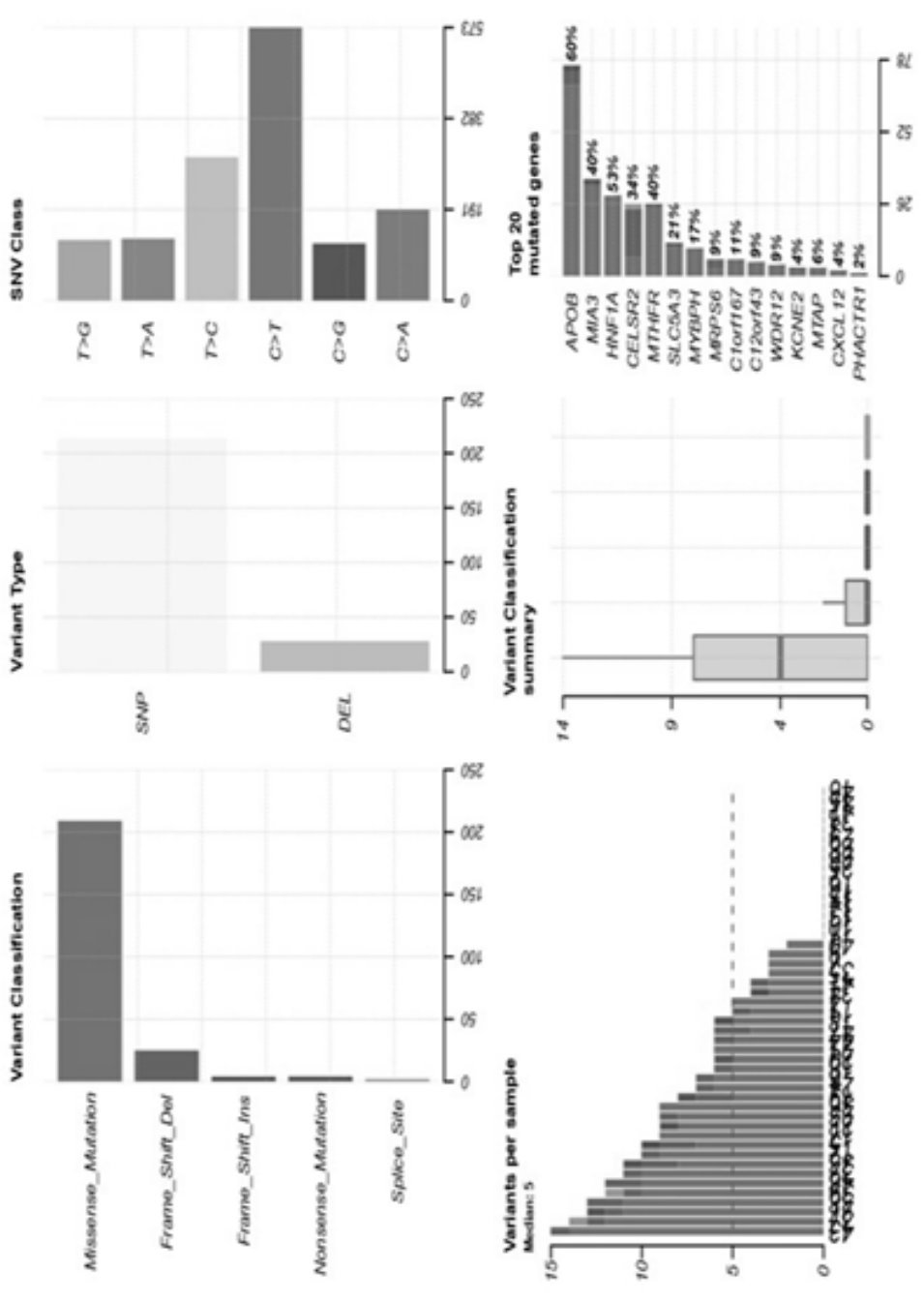

Fig. 1. Showing variant type and classification from all altered samples (the percentage of alterations in the targeted Genomic regions and variant classification with respect to nucleotide change)

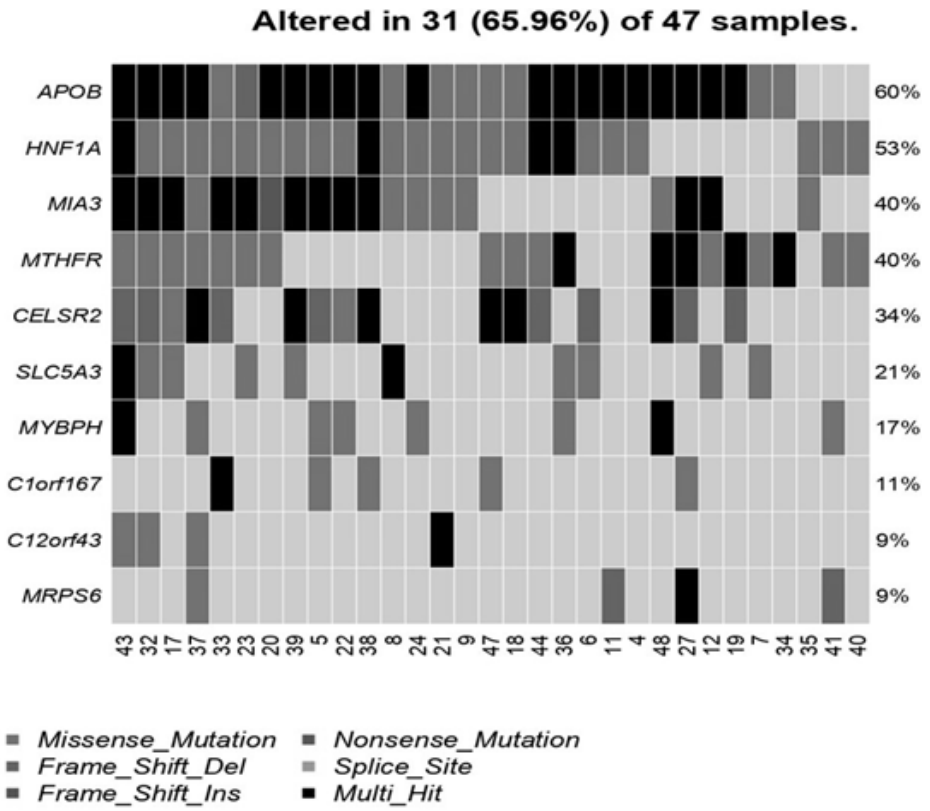

Fig. 2. Showing list of genes and type of variants from all altered samples (around 65% SNV are identified in 47 samples sequenced with different mutation types)

population. These include four genes, namely, CELSR2, WDR12, MIA3, and APOB, that exhibited the most conspicuous association with CAD and MI. By combining these findings with clinical manifestations, one can aim to create risk scores that provide a nuanced understanding of the genetic factors contributing to Myocardial Infarction, paving the way for more targeted and personalised approaches to risk assessment and prevention. CELSR2 has a strong connection in the pathogenesis of MI condition and expresses strong evident frameshift deletion in the majority of the subjects. The association of CELSR2 and mutations in this gene have previously been reported in altering levels of LDL-cholesterol affecting patients with Acute Coronary Syndrome, cardiovascular disease, and congenital heart defects. Based on OMIM and disease database reports, CELSR2 variation can be classified as a likely pathogenic variant. Studies, including the research by Samani et al. in 2008, have highlighted the involvement of CELSR2 genes on chromosome 1 in CAD, primarily through the enhancement of plasma low-density lipoprotein (LDL) levels via cholesterol metabolism (Samani et al. 2008).

Frameshift Deletion in CELSR2

A heterozygous 0 base pair deletion in Exon 23 of the CELSR2 gene that results in a frameshift and premature truncation of the protein XX amino acids downstream to codon 2397 (p.Phe2397SerfsTer19; ENST 00000271332.4) was detected in Table 2. The observed variant has previously been reported in patients affected with Acute Coronary Syndrome Atrial Fibrillation (disease), cardiovascular disease, Cardiovascular

Diseases, congenital heart defects, Coronary Arteriosclerosis, Coronary Artery Disease, coronary heart disease, heart failure, LDL-cholesterol levels, association with Myocardial Infarction disease (OMIM 604265) and lies in the PF00002:7 transmembrane receptor (Secretin family) domain of the CELSR2 protein (PF00002).

Alterations in APOB Gene

Apolipoprotein B (ApoB) and Apolipoprotein A1 are among the emerging markers for acute coronary syndrome (ACS), alterations or elevated in triglyceride levels are associated with increased cardiovascular risk (James and Appunni 2019; Nguyen et al. 2013). A heterozygous frameshift deletion in Exon 26 of the APOB gene on chr.2 is identified, the observed variant has not been reported in the 1000 genomes, gnomAD (v3.1), gnomdAD (v2), topmed, and ClinVar databases (Table 3). Hypercholesterolemia, familial, 2 (OMIM 144010) Hypobetalipoproteinemia (OMIM 615558) is caused by mutations in the APOB gene (OMIM 107730). Acute Chest Syndrome, Acute Coronary Syndrome, and Acute Myocardial Infarction are seen due to altered APOB levels and altered triglyceride levels.

Novel Frameshift Mutation in MRPS6

Interestingly, the researchers identified a Novel frameshift mutation in MRPS6 (mitochondrial ribosomal protein S6) on exon3 of chr.21 showing c.189delT, which is not previously reported in any of the online available databases (Table 3). Earlier studies suggest that a C>T substitution on chromosome 21 shows nucleotide variability and is associated with early-onset MI (Zahger et al. 2024). Genome-wide association studies and meta-analysis of 14 genome-wide association studies confirmed T as a risk allele, however, the mechanism remains unknown (Kathiresan et al. 2009; Schunkertet et al. 2011).

Variants of Uncertain Significance (VUS)

These are also known to be unclassified variants where exactly their implications in disease causing conditions are not clear. Mutations in the regions of MIA3, WDR12, and PHACTR1 have been identified through genetic testing, but it is not clear how they affect a person's health or function and are mostly considered VUS in nature.

MIA3 is mostly involved in early protein termination, a homozygous Nonsense variant in the MIA3 gene is analysed on NGS data in Exon 13 chr1 that results in the early protein termination c.C1216T, and this is not reported in any available clinical databases (Table 3). The variant has previously been reported in patients affected with CAD.CVD and involved in cholesterol metabolism causing arterial plaques by thinning the walls of coronary arteries (Szpakowicz et al. 2015). An amino acid substitution of Serine for Alanine at codon 384 (p.Ala384Ser; ENST00000261015.5) was detected in Exon 12 of the WDR12 gene (chr2:g.202882755C>A) (Table 3). The observed variant has previously been reported in patients affected with Cardiomyopathy, Dilated Coronary Arteriosclerosis, Coronary Artery Disease, and myocardial infarction primarily by increasing plaque vulnerability and narrowing of coronary arteries, primarily due to the accumulation of fatty deposits within the arterial walls (Wang et al. 2011; Zabalza et al. 2015). Two base pair deletions (AG) were observed in Exon6 of PHACTR1 showing amino acid change as p.R386Efs*8, earlier reports reveal PHACTR1 has been linked to early onset of coronary artery disease and has a strong incidence in multiple genome-wide association studies (Moilanen et al. 2015; Rezvan 2023). The percentage of incidence of this variant is comparatively very low and observed only with one case from 47 samples sequenced.

CONCLUSION

Mutations in genes CELSR2, MRPS6, and APOB show clear involvement in MI and CVD related disorders, showing molecular model studies as a definitive answer for risk assessment for CAD/MI. Variants identified in MTHFR, WDR12, and MIA3 are considered as VUS but where the association with coronary artery diseases is through different mechanisms like as increasing plaque vulnerability and narrowing of coronary arteries, accumulation of fatty deposits within the arterial walls conclude to the incidence of MI. The genetic screening of the targeted gene approach will unravel the potential of preventive medicine using Genomic approaches.

Table 3: Showing mutation summary from all the cases sequenced and analysed and all the data is submitted with NCBI under Bio Project with NCBI

S.No.	*SID*	*Chr*	*Ref*	*Gene*	*Variant class*	*tx*	*exon*	*change*	*AAChange*	*Variant type*	*ref gene*	*SRA*
1	43	chr1	T	CELSR2	Frame_Shift_Del	NM_001408	exon23	c.7189delT	p.F2397Sfs*19	DEL	exonic	SRX24541354
2	32	chr1	T	CELSR2	Frame_Shift_Del	NM_001408	exon23	c.7189delT	p.F2397Sfs*19	DEL	exonic	SRX24541355
3	33	chr1	T	CELSR2	Frame_Shift_Del	NM_001408	exon23	c.7189delT	p.F2397Sfs*19	DEL	exonic	SRX24541366
4	33	chr6	AGP	HACTR1	Frame_Shift_Del	NM_001322311	exon8	c.1154_1155del	p.R386Efs*8	DEL	exonic	SRX24541368
5	23	chr2	T	APOB	Frame_Shift_Del	NM_000384	exon26	c.9466delA	p.T3156Qfs*3	DEL	exonic	SRX24541369
6	5	chr1	T	CELSR2	Frame_Shift_Del	NM_001408	exon23	c.7189delT	p.F2397Sfs*19	DEL	exonic	SRX24541370
7	44	chr1	T	CELSR2	Frame_Shift_Del	NM_001408	exon23	c.7189delT	p.F2397Sfs*19	DEL	exonic	SRX24541371
8	6	chr1	A	CELSR2	Frame_Shift_Del	NM_001408	exon1	c.1490delA	p.D497Vfs*24	DEL	exonic	SRX24541372
9	11	chr21	T	MRPS6	Frame_Shift_Del	NM_032476	exon3	c.189delT	p.F64Sfs*23	DEL	exonic	SRX24541373
10	27	chr1	T	CELSR2	Frame_Shift_Del	NM_001408	exon23	c.7189delT	p.F2397Sfs*19	DEL	exonic	SRX24541374
11	19	chr1	T	CELSR2	Frame_Shift_Del	NM_001408	exon23	c.7189delT	p.F2397Sfs*19	DEL	exonic	SRX24541356
12	41	chr21	G	MRPS6	Frame_Shift_Del	NM_032476	exon1	c.18delG	p.A7Lfs*2	DEL	exonic	SRX24541357
13	20	chr1	C	MIA3	Nonsense_Mutation	NM_001300867	exon13	c.C1216T	p.Q406X	SNP	exonic	SRX24541358
14	8	chr1	A	MIA3	Missense_Mutation	NM_001324062	exon4	c.A2642G	p.E881G	SNP	exonic	SRX24541359
15	24	chr1	A	MIA3	Missense_Mutation	NM_001324062	exon4	c.A2642G	p.E881G	SNP	exonic	SRX24541360
16	21	chr1	A	MIA3	Missense_Mutation	NM_001324062	exon4	c.A2642G	p.E881G	SNP	exonic	SRX24541361
17	9	chr1	A	MIA3	Missense_Mutation	NM_001324062	exon4	c.A2642G	p.E881G	SNP	exonic	SRX24541362
18	48	chr1	C	MIA3	Missense_Mutation	NM_001324062	exon6	c.C3439T	p.L1147F	SNP	exonic	SRX24541363
19	35	chr1	A	MIA3	Missense_Mutation	NM_001324062	exon4	c.A2642G	p.E881G	SNP	exonic	SRX24541364
20	8	chr2	C	WDR12	Missense_Mutation	NM_018256	exon12	c.G1150T	p.A384S	SNP	exonic	SRX24541365
21	35	chr2	G	WDR12	Missense_Mutation	NM_018256	exon12	c.C1151G	p.A384G	SNP	exonic	SRX24541367

RECOMMENDATIONS

The gene polymorphisms from the six potential genes associated with CVD pathogenesis conclude that these variants have significant changes in the allele frequency among the studied population. Further, for better management of CVD and MI-related disorders, preventive screening of these mutations could be helpful for better and early diagnosis of this condition and the development of polygenic risk scores towards cardiac disorders.

AUTHORS' CONTRIBUTIONS

DSB analysed and interpreted the data and is the major contributor in study design, writing and revision of manuscript. BMV and NS supervised the complete study, VT and VV have conceptualised the work design.

AVAILABILITY OF DATA

The data analysed has been represented in the manuscript and the raw data has been submitted to NCBI under the BioProject: PRJNA1111277, the custom designed MI gene panel has been patented and published online with Application No: 202441017059.

ETHICAL APPROVAL

This study involves participation of human subjects and a clear ethical permission is obtained from Institutional Ethics Committee, Sri Venkateswara Institute of Medical Sciences University, Tirupati, India, for carrying out this research, and all participants have clearly informed about the study and taken formal written consent.
IEC with reference number: 844(a) dated 23/04/2019

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