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RESEARCH ARTICLE

Ullah et al: WES in obstructive CAD

Whole-exome sequencing in obstructive coronary artery disease identifies rare and novel variants in cardiac arrhythmia and pulmonary arterial hypertension–associated genes

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ABSTRACT

Coronary artery disease (CAD) represents a complex interplay of genetic, environmental, and lifestyle factors. In this study, we utilized whole-exome sequencing (WES) on 28 patients with obstructive CAD to identify rare variants that may influence clinical outcomes beyond conventional atherosclerotic risk. We examined 74 genes curated from the Genomics England PanelApp, focusing on familial hypercholesterolemia (FH), cardiac arrhythmias (CA), and pulmonary arterial hypertension (PAH), ultimately detecting 8,251 variants. After applying a stringent filtering process with a population maximum allele frequency (PopMax AF) threshold of <0.1%, we identified 68 candidate variants across 23 genes. The majority were associated with CA (47/68, 69%), followed by PAH (12/68, 18%) and FH (9/68, 13%). Notably, 30 variants (44%) were novel, and 18 were categorized as high-impact frameshift mutations. The highest burden of candidate variants was found in the sodium voltage-gated channel alpha subunit 10 (*SCN10A*), followed by the ryanodine receptor 2 (*RYR2*), mitochondrial seryl-tRNA synthetase 2 (*SARS2*), A-kinase anchoring protein 9 (*AKAP9*), and hyperpolarization-activated cyclic nucleotide-gated channel 4 (*HCN4*). Clinical evaluation revealed a pathogenic variant in the low-density lipoprotein receptor (*LDLR*) and likely pathogenic variants in sodium voltage-gated channel alpha subunit 5 (*SCN5A*) and potassium voltage-gated channel subfamily Q member 1 (*KCNQ1*); additionally, nine other variants were predicted to be deleterious, including five novel *SCN10A* variants. Functional annotation using Gene Ontology (GO) and Human Phenotype Ontology (HPO) highlighted mechanisms impacting cardiac structure, electrical conduction, and lipid homeostasis.

Keywords: Whole exome sequencing, coronary artery disease, familial hypercholesterolemia, cardiac arrhythmias, pulmonary arterial hypertension, genetic variations, human phenotype.

INTRODUCTION

Cardiovascular diseases (CVDs), particularly coronary artery disease (CAD), remain a leading cause of global morbidity and mortality, accounting for approximately 17.9 million deaths annually [1]. The pathogenesis of CAD is multifactorial, involving a complex interplay of genetic predisposition, environmental influences, and lifestyle factors such as hypertension, hyperlipidemia, obesity, and type 2 diabetes mellitus (T2DM) [2]. Despite advances in diagnostics and therapeutics, a significant proportion of CAD cases exhibit unexplained genetic susceptibility, suggesting the involvement of rare and novel genetic variants that may contribute to disease progression. Recent advances in next-generation sequencing (NGS), particularly whole-exome sequencing (WES), have revolutionized the identification of disease-associated genetic variants, enabling a deeper understanding of molecular mechanisms underlying complex disorders like CAD [3]. WES provides a high-resolution approach to detect coding-region variants, including pathogenic and likely pathogenic mutations, which may influence disease risk and progression. Previous studies have implicated genes associated with familial hypercholesterolemia (FH), cardiac arrhythmias, and pulmonary arterial hypertension (PAH) in CAD pathogenesis, yet the full spectrum of genetic contributors remains incompletely characterized [4,5].

Our study aimed to identify rare and novel variants with potential clinical significance and characterize their functional impact via gene ontology (GO) and human phenotype ontology (HPO) analyses. We employed whole-exome sequencing in a CAD cohort to systematically analyze 74 genes across three clinically relevant panels (FH, cardiac arrhythmias, and PAH). By integrating genomic discovery with phenotypic annotation, this research study advances the understanding of CAD's genetic architecture and provides a framework for translating WES findings into clinical practice.

MATERIALS AND METHODS

CAD patient cohort

The research followed Helsinki declaration guidelines and received ethical approval from the University of Tabuk Research Ethics committee under the reference number (# UT-

91-23-2020). Twenty-eight patients with confirmed CAD participated in this study. Every participant signed consent papers that were provided to them. We included the patient from the King Fahad Specialist Hospital, Tabuk, KSA who underwent elective angiography for diagnosis of the stable angina. Various clinical tests were performed on the study participants including X-rays as well as exercise stress tests and myocardial perfusion imaging and ambulatory electrocardiography and Holter monitoring and chest echocardiogram and computerized tomography coronary angiography and multigated acquisition scans (MUGA). CAD was defined based on clinical symptoms (stable or unstable angina) and confirmed by invasive coronary angiography. Inclusion required macrovascular disease with ≥50% luminal diameter stenosis in at least one of the three major coronary arteries (LAD, LCx, or RCA). These 28 patients were selected consecutively from a larger registry to ensure a representative sample of symptomatic obstructive CAD. Clinical diagnosis of specific arrhythmias, PAH or FH was not an inclusion criterion, and these phenotypes were not systematically assessed.

Genomic DNA preparation and sequencing

High-quality human genomic DNA served as the source material for this investigation. Library preparation adhered to the protocol detailed in the Twist Human Core Exome 2.0 Kit instruction manual. Sequencing was performed on the Illumina NovaSeq 6000 platform, following the manufacturer's recommended procedures.

Quality control and preprocessing of sequencing data

The raw sequencing read quality was initially assessed using FastQC (v0.11.9) [6] to ensure data reliability. Subsequently, TrimGalore (v0.6.6) was employed to remove sequencing adapters and low-quality bases, yielding high-quality (HQ) reads for subsequent analyses. Cleaned reads were aligned to the human reference genome GRCh38 using the Burrows-Wheeler Aligner (BWA-MEM, v0.7.17). Duplicate reads were marked using Picard Tools (v2.23.8).

Variant calling and annotation

Variant discovery was performed using the Genome Analysis Toolkit (GATK) v4.3 [7], which implements the best practice pipeline. Base quality score recalibration (BQSR) was performed using known indel and SNP sites from the dbSNP and Mills and 1000G gold standard sets. Variant calling was performed per-sample using GATK HaplotypeCaller (v4.1.9.0) in gVCF mode. Samples were then jointly genotyped using GATK GenotypeGVCFs. Initial variant filtering was performed using GATK Variant Quality Score Recalibration (VQSR), with tranche sensitivity set to 99.5% for SNPs and 99.0% for indels. Hard filtering was applied to variants failing VQSR, using thresholds such as QD < 2.0, FS > 60.0, and MQ < 40.0 (for SNPs). The final callset exhibited a Transition-to-Transversion (Ti/Tv) ratio of 1.99 and an average call rate of 99%. Pedigree checks were performed to ensure no unexpected relatedness, and sex checks confirmed the reported gender.

The identified variants underwent comprehensive annotation using a variety of databases and tools to facilitate biological and clinical interpretation. The RefSeq database was utilized for gene identification and variant characterization. Variant annotation was performed using the Ensembl Variant Effect Predictor (VEP), providing detailed information regarding functional and biological consequences [8]. Default VEP annotations were supplemented with several plugins, including dbNSFP [9], CADD[10], and Phenotypes [11], to enhance the quality of annotation. Potential disease associations of variants were investigated using publicly available databases such as OMIM [12], ClinVar [13], and UniProtVar [14]. Population allele frequency data were obtained from the 1000 Genomes Project [15] and gnomAD [16] (including both exome and genome datasets) to effectively distinguish rare variants from common polymorphisms. For functional prediction of mutations, tools integrated within dbNSFP, such as SIFT [17], PolyPhen [18], FATHMM [19], MutationTaster [20], MutationAssessor [21], and PROVEAN [22], were employed. Missense variants were further annotated using CADD scores. The SIFT-indel tool [23] was applied to evaluate the functional impact of InDels. Furthermore, only canonical transcript-dependent consequences were retained in the final VEP-annotated file to ensure consistency and relevance in downstream analyses.

Filtering and classification of variants

To identify clinically relevant variants associated with cardiovascular disease, genes linked to "Cardiac arrhythmias," "Familial hypercholesterolaemia," and "Pulmonary arterial hypertension" were prioritized using the Genomic England Panel App [24]. We utilized these gene panels typically used for FH, Arrhythmias, and PAH to identify potential genetic overlap or secondary risk factors in patients presenting primarily with CAD. Variants within these genes were selected for further investigation.

A multi-step filtering process was implemented to ensure the clinical relevance of the identified variants. Variants exhibiting a population frequency of 1% or less in databases such as gnomAD and the 1000 Genomes Project were retained. The analysis was restricted to protein-coding regions and canonical splice sites, prioritizing variants with "HIGH" or "MODERATE" VEP impact. Given the exploratory nature of this study, variants were further filtered using a stringent popmax Allele Frequency (popmax AF) threshold of < 0.1% across all gnomAD subpopulations to enrich for ultra-rare variants while accounting for potential under-representation of the Middle Eastern population.

A comprehensive annotation strategy was employed to assess the potential clinical implications of the filtered variants. Variants were annotated using clinical databases, including ClinVar and UniProtVar, to leverage existing clinical knowledge. Furthermore, a combination of standalone and ensemble *in silico* prediction tools, encompassing SIFT, PolyPhen-2, FATHMM, MutationTaster, MutationAssessor, CADD, PROVEAN, and SIFT-Indels, was utilized to predict the impact of nonsynonymous variants, indels, and frameshift variants.

Based on the combined information derived from clinical databases and *in silico* predictions, variants were classified into five categories: Benign, Likely Benign, Variant of Unknown Significance (VUS), Likely Pathogenic, and Pathogenic. This classification process prioritized clinical data over *in silico* predictions. In conflicting clinical annotations, *in silico* predictions were consulted to establish a final classification. For variants absent in clinical databases, stringent criteria based on CADD scores (≥20 for deleterious) and *in silico* tool predictions (consensus from at least three tools) were applied to classify them as predicted deleterious. Variants not meeting these criteria were classified as VUS.

Functional annotation of candidate variant genes

To elucidate the functional impact of the identified VUS, pathogenic, and likely pathogenic variants, the g:Profiler webserver [25] was utilized for enrichment analysis. This tool facilitates the systematic exploration of gene sets, identifying overrepresented Gene Ontology (GO) terms. By focusing on Gene Ontology - Biological Process (GO:BP) terms, Reactome Pathways, and Human Phenotype Ontology, the aim was to uncover the specific molecular and cellular processes influenced by these variants and their potential contribution to cardiovascular disease. The g:SCS (Gene set size corrected) method was used for multiple testing correction. Crucially, we note that the results are presented as descriptive functional annotation due to the pre-selected nature of the gene panels, and not as proof of statistical enrichment.

Ethical approval and consent to participate

The study was approved by the institutional ethics committee at the University of Tabuk (Number # UT-91-23-2020). All the participants provided written informed consent before their participation in the study.

RESULTS

CAD cohort characteristics

This study enrolled 28 patients diagnosed with coronary artery disease (CAD), providing a detailed profile of this specific cohort. The median age of participants was 53 years, with an interquartile range (IQR) of 40-63 years, indicating a relatively wide age distribution. The study population exhibited a strong male predominance, with 86% of participants being male. Most participants resided in urban areas (75%) and were unemployed (54%). Educational data was available for 16 participants, revealing that 25% had received formal education, while 32% were uneducated. Analysis of cardiovascular risk factors revealed that 57% of participants were non-smokers, while 43% were current smokers. A substantial proportion of patients presented with established cardiovascular risk factors, including hypertension (54%) and hyperlipidemia (46%). Furthermore, over half of the participants were classified as overweight or obese (54%), and a similar proportion had a diagnosis of diabetes (54%). Regarding angina

presentation, the majority (71%) experienced stable angina, while 29% presented with unstable angina. A history of myocardial infarction (MI) was reported in 36% of the participants, with 14% experiencing ST-elevation myocardial infarction (STEMI), 14% experiencing non-ST-elevation myocardial infarction (NSTEMI), and 3.6% having previously undergone coronary artery bypass grafting (CABG). A familial history of cardiovascular disease was present in 36% of the study population.

Laboratory analysis provided a detailed metabolic and hematologic profile of the coronary artery disease patients. The cohort exhibited a pronounced dyslipidemic pattern characteristic of elevated cardiovascular risk. Cholesterol levels were suboptimal in a significant proportion of patients. Nearly half (43%, n=12) presented with high total cholesterol, with a median level of 215 mg/dl. Only 54% (n=15) maintained levels within the optimal range, while a single patient (3.6%) was borderline. This pattern was particularly marked for low-density lipoprotein cholesterol (LDL-C), the primary atherogenic lipid fraction. The vast majority of patients (89%, n=25) had elevated LDL-C levels, with a median of 142 mg/dl. Optimal LDL-C was observed in only two patients (7.1%). High-density lipoprotein cholesterol (HDL-C), the protective lipid fraction, showed a median level of 45 mg/dl. While half of the cohort (50%, n=14) had optimal HDL-C, a concerning 29% (n=8) displayed levels below the desired range. Triglyceride levels were markedly elevated, with a median of 217 mg/dl. The cohort was evenly split, with precisely 50% of patients exhibiting high triglyceride levels. The C-reactive protein was positive in 32% (n=9) with a median of 2.23 mg/dl. The median hemoglobin level was 14.24 g/dL. A comprehensive summary of the patient characteristics of the study cohort is presented in table 1.

Identified variants in the cohort

Whole-exome sequencing analysis of the study cohort identified variants within 74 genes that overlapped with the pre-defined GenePanel. This analysis generated a total of 8251 variants across the cohort. When categorized by the associated condition, 1020 variants were related to gene panel of Familial hypercholesterolemia, 5632 to Cardiac arrhythmias gene panel, and 1599 to Pulmonary arterial hypertension gene panel.

Analysis of variant distribution by genomic location revealed that the majority of these variants were either intronic (37%) or synonymous (20%). Among the remaining variants, 991 were missense variants (12%), 1197 were located in the 3' UTR, 143 in the 5' UTR, and 18 were frameshift variants. Notably, 180 variants (2%) were classified as novel, as they had not been previously reported in public databases such as dbSNP or COSMIC. Variant Effect Predictor (VEP) impact categorization assigned 21 variants as high impact, 2433 as low impact, 1103 as moderate impact, and 4694 as modifier impact. In this initial, unfiltered variant dataset, the most frequently mutated genes were *RYR2*, followed by *AKAP9*, *CACNA1C*, *LDLR*, and *PCSK9*. The methodology employed in the study to identify the candidate variants is illustrated in Figure 1. A key summary of the identified variants within the study cohort is provided in Figure 2.

Candidate variant detection and classification

After applying initial filters based on population frequency and VEP impact, we refined the dataset further. By using a strict PopMax Allele Frequency (AF) threshold, we significantly limited the investigation's scope. This approach effectively removed common variations and noise, resulting in 68 possible rare variants spread across 23 different genes for further analysis. A notable proportion of these candidates, 30 variants (44%), were novel, meaning they had not been previously documented in public databases. The predominant variant type was missense (n = 42, 62%), followed by frameshift mutations (n = 18, 26%). The remaining variants encompassed inframe indels, stop gain mutations, splice site variants, and other protein-altering variations. To evaluate the potential clinical relevance of these candidate variants, annotations were generated using ClinVar, UniProtVar, and a range of *in silico* prediction tools. This assessment resulted in the classification of the variants into distinct categories. Variants of unknown significance (VUS) constituted the largest group (n = 51; 75%), highlighting the need for further investigation to elucidate their clinical implications. Other classifications included likely benign (LB, n = 4; 6%), benign (B, n = 1; 1.5%), Predicted deleterious (D, n =9; 13%), likely pathogenic (LP, n = 2; 3%), and a single pathogenic variant (P, n = 1; 1.5%). The majority of classified variants were associated with the gene panel of Cardiac arrhythmias (n = 47; 69%), Pulmonary arterial hypertension (n = 12; 18%) and Familial hypercholesterolaemia (n = 9; 13%). The *SCN10A* gene (n = 9) harbored the highest

number of candidate variants, followed by *RYR2*, *SARS2*, *AKAP9*, and *HCN4*. A comprehensive overview of the candidate variants identified in each sample is presented in Figure 3.

Rare and potentially pathogenic variant identification

A detailed analysis of the candidate variants identified 12 variants classified as either pathogenic (n = 1), likely pathogenic (n = 2) or predicted deleterious (n = 9), suggesting a potential role in the development of cardiovascular disease. These variants were observed in 8 individual patients, with each patient carrying at least one such variant. Notably, 11 of these variants were unique to individual patients, while one variant, *TRPM4* (ENST00000252826.10:c.247dup), was recurrent, and identified in two separate patients. In the context of Familial hypercholesterolemia, the *LDLR* variant (rs879254847; ENST00000558518.6:c.1255T>G) was classified as pathogenic in one patient. A comprehensive summary of the unique pathogenic, likely pathogenic and predicted deleterious variants identified within this cohort is provided in Table 2.

Functional characterization of candidate variants

To further investigate the functional implications of the identified pathogenic, likely pathogenic, and VUS variants, a comprehensive functional annotation analysis was conducted using Gene Ontology (GO), Reactome Pathways, and Human Phenotype Ontology (HPO). The GO:BP analysis highlights a concentration of genes involved in cardiac muscle cell action potential ($p = 1.2 \text{ X } 10^{-16}$) and metal ion transport ($p = 4.2 \text{ X } 10^{-9}$), driven by key regulators such as KCNQ1, SCN5A, and KCNH22. Reactome pathway analysis further supported these findings, demonstrating enrichment in pathways related to Cardiac Conduction ($p = 1.6 \times 10^{-7}$), Muscle Contraction ($p = 3.6 \times 10^{-6}$), Phase 3 - Rapid Repolarisation ($p = 1.6 \times 10^{-7}$) and LDL Clearance ($p = 9.7 \times 10^{-4}$).

The functional annotation by HPO provided insights into the potential clinical manifestations associated with these variants. The genes were significantly enriched for phenotypes related to severe cardiac dysfunction, including Cardiac Arrest ($p = 5.7 \times 10^{-15}$), Sudden Cardiac Death ($p = 3.6 \times 10^{-13}$), Prolonged QTc Interval ($p = 6.9 \times 10^{-13}$), and Ventricular Fibrillation ($p = 1.2 \times 10^{-12}$). Functional annotation of the candidate genes confirmed that the identified variants fall into primary functional categories related to

cardiac muscle function, ion transport, and lipid metabolism, which is consistent with the initial selection criteria of the gene panels. Figure 4 provides a visual representation of the aggregated GO terms, Reactome pathways, and HPO terms, illustrating the functional impact of the identified variants. Collectively, these annotations, summarized in Table 3, confirm the high functional relevance of the identified variants in maintaining cardiac rhythm and their potential contribution to severe arrhythmic phenotypes.

DISCUSSION

The third most common cause of death globally is coronary artery disease. It is reported that an estimated 17.8 million people die every year from the disease and those with existing conditions have a poor quality of life. According to global statistics, 315 million incidence of CAD were reported to exist in 2022 [26,27]. The origin of CAD shows a complex interweaving of modifiable and non-modifiable risk factors in its multifactorial etiology. Atherosclerosis often starts in younger individuals and is influenced by a range of factors, with abnormal lipid metabolism being a primary contributor. As people age, the severity and prevalence of CAD tend to rise, particularly in those over the age of 75, who are at a higher risk of experiencing multi-vessel CAD [28]. The median age of the current cohort was 53 years which represents a CAD burden on a relatively younger population. However, it is believed that an optimal management of the modifiable risk factors can mitigate the effects of non-modifiable risk factors in CAD. A preventive program for individuals at 70 years of age who were at higher risk of CVD, demonstrated a substantial risk reduction of 13-20% due to improved hypertension and hypercholesterolemia management [29]. In human biology, variations between sexes stem from a complex interaction among sex chromosomes, sex hormones, and environmental influences. These factors can lead to different activation of molecular mechanisms, ultimately affecting how CAD and atherosclerosis manifest phenotypically. Considering the physiology of sex hormones, estrogens and androgens (estradiol and testosterone) both function *via* estrogen and androgen receptors, which are expressed in both male and female sex and are typically present in all cardiovascular tissues [30]. Estrogen is believed to have a protective role against atherosclerosis, which is considered to account for the lower incidence of cardiovascular disease in premenopausal women,

the risk of which increases after menopause [31]. Further our study shows a higher incidence of CAD in males than in females, highlighting a natural gender-based predisposition to the disease. Traditionally, it has been thought that CAD predominantly impacts men, as both morbidity and mortality rates are higher in men, while women generally experience lower incidence rates [32]. However, it is understood that since women are typically diagnosed with CAD 10 years later than men, this could result in a higher comorbid conditions associated with severity when the disease is diagnosed [33]. Moreover, studies have shown that atherosclerotic plaques in women are typically fibrous and stable, while those in men are often more atheromatous with higher levels of inflammatory cells, calcification, lipids, and hemorrhage [34].

The study shows an established pattern of pathological risk factors like hypertension, hyperlipidemia, obesity, type-2 DM and familial history which were observed in substantial proportion of the CAD cohort. Elevated blood pressure is a significant modifiable risk factor for all forms of CAD. Hypertension is considered among the most critical risk factors in CAD, contributing to 90% of the attributable risk for myocardial infarction in men and 94% in women [35]. The pathophysiological mechanisms through which blood pressure is incorporated as a risk factor for CAD are intricate. This encompass the impact of blood pressure as a physical force that affects the formation of atherosclerotic plaques, as well as the link between pulsatile hemodynamics, arterial stiffness, and coronary perfusion [36]. Treating arterial hypertension has been shown to reduce the risk of coronary events in individuals without CAD [37]. It is believed that the benefits of lowering the blood pressure in patients with existing CAD are greater than those of specific medications. Research suggests that the risk of death in CAD from heart disease caused by narrowed arteries is lower when systolic blood pressure is at 115 mmHg and diastolic blood pressure is at 75 mmHg. Also, lowering systolic blood pressure by 20 mmHg can reduces the risk of mortality from this disease by 33–50% for both men and women of all ages between 40 and 89 years [38]. Higher levels of serum lipids like LDL cholesterol and triglycerides are known to increase the risk of heart disease caused by plaque accumulation in arteries [39]. Sometimes, hyperlipidemias are due to lifestyle, but studies have found that genetic variations also exhibit an important role [40]. About half of people with early-onset CAD have dyslipidemia and a family

history, mostly with high LDL cholesterol and/or triglycerides [41]. An interesting study showed that the lipidomic profiles in hyperlipidemias with a family history closely resembled those found in population-based hyperlipidemias, indicating that the underlying mechanisms are similar and have significant overlap [42]. The study further showed that the risks of CAD are remarkably similar, irrespective of whether hyperlipidemic individuals were sourced from families with a high prevalence of the same hyperlipidemia or from the general population. The development of coronary heart disease is independently associated with obesity, and it is understood that reducing weight improves the risk factors for CHD and is associated with a better prognosis [43,44]. Interestingly there is evidence in literature that obesity that is metabolically healthy is defined by the lack of cardiometabolic risk factors, such as insulin resistance, dyslipidemia, hypertension, and type 2 diabetes. There does not appear to be a higher risk of atherosclerosis in such a phenotype. However according to some research, people who are obese but metabolically healthy may later acquire metabolically unhealthy obesity [45,46]. In addition to diabetes by itself being a significant independent risk factor, several pathologies commonly linked to type 2 diabetes, such as hypertension and dyslipidemia, are well-known risk factors for atherosclerotic cardiovascular disease [47]. Through processes like the production of advanced glycation end products and elevated oxidative stress, hyperglycemia plays a role in the development of cardiovascular disease [48]. We know that the development of coronary artery disease progressing to heart failure is significantly influenced by insulin resistance and hyperglycemia [49]. Managing individual cardiovascular risk factors can help prevent or reduce the onset of CAD in people with diabetes, according to a multitude of evidence [50]. Moreover, significant advantages are observed with indications of enduring benefits when multiple cardiovascular risk factors-glycemic control, blood pressure management, and lipid regulation-are managed simultaneously [51]. A family history of CAD is a well-recognized cardiovascular risk factor that is gaining importance in the stratification of patients' cardiovascular risk, moving beyond its traditional role as a modifier of disease [52]. It is widely recognized that first-degree relatives of individuals affected by CAD, particularly siblings, have a significantly higher risk of developing the disease at a younger age [53]. Recent guidelines published by the CCS and ESC now include family

history of CAD as a relevant criterion for calculating risk factor-weighted clinical likelihood (RF-CL) during pre-test assessments, highlighting the significance of this medical history [54].

Genetic variability is recognized to influence cellular mechanisms leading to susceptibility outcomes that can either elevate or reduce the risk of various complex diseases such as CAD [55]. Whole-Exome Sequencing, a technique employed for genetic correlation studies, can assist in detecting molecular abnormalities associated with the relevant pathology [56]. Next-generation whole exome sequencing in our cohort has identified a total of 8251 variants in 74 genes across the cohort. These were categorized within gene panels for familial hypercholesterolaemia, cardiac arrhythmias and pulmonary arterial hypertension. From these, 180 variants (2%) were classified as novel and 21 variants were assigned as high impact in VEP. Post filtering variants in genes associated with Cardiac arrhythmias (n = 47; 69%) accounted for the majority of identified candidates, followed by those in pulmonary arterial hypertension (n = 12; 18%) and familial hypercholesterolemia (n = 9; 13%).While our cohort consisted of patients with primary coronary artery disease, we identified a significant burden of variants in genes canonically associated with cardiac arrhythmias (e.g., SCN10A, ANK2) and pulmonary hypertension. It is important to note that the presence of these variants does not necessarily imply an active clinical diagnosis of arrhythmia or PAH in these patients. Instead, these findings may represent a latent genetic susceptibility. In the context of ischemic heart disease, such variants could act as distinct risk modifiers, potentially lowering the threshold for arrhythmias under ischemic stress or influencing long-term prognosis. Carrying a hidden 'pro-arrhythmic' genetic burden, such as in *SCN5A* or *KCNQ1*, may lower the threshold for ventricular fibrillation during an ischemic event. This might help explain the high risk of sudden cardiac death in certain groups of CAD patients. Likewise, PAH-associated variants may confer a genetic predisposition to harmful vascular changes. These changes can affect how the coronary vasculature reacts to long-term ischemia. This highlights the value of multi-panel WES in uncovering 'silent' genetic risks that single-phenotype screening might fail to recognize.

Evidence of molecular etiology have demonstrated that microstructural abnormalities (e.g., partial or complete lack of structures, fatty and/or fibrous replacement of normal

tissues, calcification) or functional abnormalities of the action potential may be the primary cause of arrhythmogenic diseases [57]. Diagnostic tachycardias or bradycardias may be caused by a genetic predisposition to these pathophysiological alterations. In fact, after reports of familial clustering of the most prevalent cardiac arrhythmias, genetic predisposition to cardiac arrhythmias has been confirmed [58]. Serum LDL cholesterol at an elevated level and a higher risk of coronary artery disease are linked to familial hypercholesterolemia, which is regarded as a Mendelian disorder [59]. The disease is caused by pathogenic DNA variations in any of the three associated genes, LDLR, APOB, or PCSK9. Large-scale population research using gene sequencing have found that familial hypercholesterolemia mutations are present in 0.2% to 0.5% of the general population and up to 2% of individuals with early-onset CAD [60]. A pathogenic variant in *LDLR* gene has been identified in this study, which is related to familial hypercholesterolemia. Chronic hypertension can cause myocardial infarction, atrial fibrillation, congestive heart failure, and left ventricular hypertrophy, among other detrimental alterations in the anatomy and physiology of the heart [61]. The identification of many Mendelian types of hypertension has been essential in comprehending the mechanisms that control blood pressure and increases the risk of coronary artery disease [62].

Our analysis found APOB gene harboring the highest number of candidate variants, followed by SCN10A, AKAP9, ANK2, and RYR2. Apolipoprotein B (APOB) is known to play an important role in lipoprotein assembly and secretion, including VLDL and LDL [63]. Higher levels of APOB have been linked to an increased risk of CAD [64]. Such high APOB levels imply a greater number of atherogenic particles in circulation, a known driver of atherosclerosis, consistent with the clinical presentation of our cohort. Several variants in the APOB gene have been studied for their link to CAD and other risk factors. These polymorphisms can affect APOB levels, lipid profiles, and CAD risk. For example, the APOB gene polymorphism c.12669G>A, which results in the amino acid substitution p.Gln4154Lys, has been studied in various populations [65]. A study in the Indian population found that the frequency of the R-(mutant) allele was significantly higher in CAD patients compared to healthy controls [65]. Another study in Mexican patients with CAD found that the frequency of the X+/X+ genotype in Xba I

polymorphism of the APOB gene was significantly higher in CAD patients compared to controls [66]. Also the R3500Q mutation in the APOB gene is responsible for familial defective apolipoprotein B-100, which is associated with increased LDL-C levels and coronary artery calcification. A study in the Old Order Amish population found a high carrier frequency of the R3500Q mutation (12%) and consequently the carriers of the mutation had significantly higher LDL-C levels and a higher odd of having detectable and extensive coronary artery calcification [67]. The SCN10A gene encodes a protein Nav1.8which functions as a voltage-gated sodium channel that regulates nerve and muscle cell excitability [68]. The protein belongs to the voltage-gated sodium channel family, which is responsible for the rapid influx of sodium ions into cells during the rising state of the action potential. Certain common and rare SCN10A variations have been linked to Brugada Syndrome, a hereditary channelopathy caused by genetically programmed loss-of-function in the cardiac sodium channel [69]. An allele, rs6795970 (V1073), has been demonstrated to enhance the incidence of the syndrome by causing electrophysiological abnormalities such as a positive shift in steady-state activation and slower recovery after inactivation [70]. Interestingly our study has identified five novel variants in SCN10A which have been classified as predicted deleterious. SCN10A is functionally related to cardiac arrhythmias. These novel variants warrant further investigation to understand their role in modulating cardiovascular risk or susceptibility to arrhythmic events in CAD patients. A-kinase anchoring protein 9 (AKAP9) is a scaffolding protein involved in cellular signaling particularly with cAMP/PKA pathways and has been implicated in various diseases, including cardiovascular conditions [71]. These control the intensity, duration, and compartmentalization of nucleotide-dependent signaling, thereby establishing local cAMP pools [72]. Several members of the AKAP families are expressed in the cardiovascular system, where they direct important processes such as endothelial barrier function and excitation-contraction coupling, maintaining the homeostatic functioning of the heart and vasculature [72]. AKAP9 has been identified as a candidate gene in arrhythmogenic diseases with certain genetic variations shown to have potential risk for SCN5A-negative Brugada Syndrome [73] and dilated cardiomyopathy [74]. The ANK2 gene encodes ankyrin-B protein which functions as a necessary factor for accurate membrane protein targeting in the cardiac

tissues [75]. Through its adapter function Ankyrin-B drives proper localization of essential cardiac cell components including ion channels together with transporters receptors and signaling molecules thus maintaining normal heart rhythms and performance [75]. The human ANK2 R990Q, E1425G and V1516D as well as R1788W variants lead to ankyrin-B loss-of-function which causes 'Ankyrin-B syndrome. The disorder manifests through bradycardia and heart rate variability combined with conduction block and atrial fibrillation while also resulting in QT interval prolongation and potentially lethal catecholaminergic polymorphic ventricular tachycardia and sudden cardiac death [76]. A predicted deleterious variant was identified in the ANK2 gene, a well-established susceptibility gene for cardiac arrhythmias. The RYR2 gene encodes the sarcoplasmatic reticulum cardiac ryanodine receptor/calcium release channel RyR2 which functions to maintain intracellular calcium levels and control cardiac excitation–contraction coupling [77]. Research shows that pathogenic RYR2 mutations mainly appear as missense variants (86–92 %) and RyR2 exhibits poor tolerance towards genetic variants that trigger loss of function defects [78]. The functional characterization of the identified variants in our study demonstrated various possible impairments that may affect cardiac structural and functional efficiency. These include aberrant cardiac muscle cell action potential, actin-mediated cell contraction, cardiac conduction, phase 3 - rapid repolarization which may predispose individuals to clinical manifestations such as ventricular fibrillation, arrhythmia, cardiac arrest and sudden cardiac death. Moreover, likely pathogenic variants have been reported in our cohort for *SCN5A* and *KCNQ1* genes. The SCN5A gene encodes the principal voltage-gated sodium channel responsible for cardiac action potential initiation and propagation. Earlier, pathogenic variants in SCN5A were exclusively linked to primary electrical disorders, or channelopathies. Contemporary evidence, however, establishes a significant association between SCN5A dysfunction and the development of structural cardiomyopathies [79]. Furthermore, it has been reported that characterization of KCNQ1 variants improves the risk stratification in patients with cardiac arrhythmias [79].

Limitations

While our study provides valuable insights into the genetic landscape of this CAD cohort, several limitations must be considered. First, the study cohort was limited to 28 patients. While this size is sufficient for an exploratory descriptive analysis of rare-variant burden, it precludes establishing formal genotype-phenotype associations or calculating statistical significance for disease risk. Furthermore, the absence of a locally sequenced healthy control group is also a key primary limitation. To mitigate this, we utilized the gnomAD global database as a reference for population allele frequencies. However, we acknowledge that gnomAD may not fully represent the specific genetic background of Middle Eastern populations, potentially influencing our assessment of variant rarity. Additionally, the variants identified as 'predicted deleterious' or 'VUS' were classified based on *in silico* predictions and existing database evidence. In the absence of in vitro functional assays or segregation analysis within families, the precise biological impact of these novel variants on protein function remains inferred. Finally, our analysis was restricted to 74 genes across three specific panels (FH, CA, and PAH). Consequently, we may have overlooked other relevant genetic factors contributing to CAD that lie outside these pre-defined pathways.

CONCLUSION

In conclusion, our study utilized Whole Exome Sequencing to uncover a significant burden of rare genetic variants within a cohort of 28 patients with Coronary Artery Disease. By applying a stringent population-based filter (PopMax AF < 0.1%), we identified 68 high-confidence candidate variants across 23 genes associated with lipid metabolism, cardiac rhythm, and vascular function. The identification of a pathogenic *LDLR* variant and likely pathogenic variants in *SCN5A* and *KCNQ1* underscores the presence of clinically actionable genetic drivers that may exacerbate CAD progression or increase the risk of secondary complications like arrhythmias. Furthermore, the discovery of nine predicted deleterious variants, including five novel mutations in the *SCN10A* gene, highlights the potential role of latent genetic modifiers in under-represented populations. These findings demonstrate that genomic screening can reveal underlying predispositions

that standard clinical assessments may overlook, moving us closer to a more personalized approach in managing cardiovascular risk.

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TABLES AND FIGURES WITH LEGENDS

Table 1. Clinical characteristics of the study cohort (*n* =28)

Biomarker	***n* (%)**	**Median [IQR]***
Cholesterol		215.46 [150-280] (mg/dl)
Borderline	1 (3.6%)	
High	12 (43%)	
Optimal	15 (54%)	
LDL-C		142.32 [110-205] (mg/dl)
Borderline	1 (3.6%)	
High	25 (89%)	
Optimal	2 (7.1%)	
HDL-C		45.67 [35-62] (mg/dl)
Abnormal	8 (29%)	
Optimal	14 (50%)	
Unknown	6 (21%)	
Triglycerides		217.21 [120-457] (mg/dl)
High	14 (50%)	
Optimal	14 (50%)	
C-reactive protein		2.23 [0.6-4.5] (mg/dl)
Unknown	7 (25%)	
Negative	12 (43%)	

Positive	9 (32%)	
Hemoglobin		14.24 [9.5- 17.9] (g/dl)
Low	1(3.57%)	
Optimal	27(96.42%)	

*IQR: Interquartile Range of the measured parameter in the cohort.

Table 2. Key pathogenic, likely pathogenic, and predicted deleterious variants identified in genes associated with the genomic england genepanelapp for cardiac arrhythmias, familial hypercholesterolemia, and pulmonary arterial hypertension

Gene	Gene App Panel [2]	Variant ID	Variant	Variant type	ClinVar [1]	UniProtVar [1]	In silico prediction [1]	Interpreted classification [1]
ANK2	CA	COSV100003794	ENST00000357077.9:c.2693G>T:p.Ser898Ile	Missense Variant	-	-	D	D
KCNQ1	CA	rs199472737	ENST00000155840.12:c.877C>T:p.Arg293Cys	Missense Variant	US	LP/P	D	LP
LDLR	FH	rs879254847	ENST00000558518.6:c.1255T>G:p.Tyr419Asp	Missense Variant	P/LP	-	D	P
SCN10A	CA	Novel	ENST00000449082.3:c.1200T>A:p.Tyr400Ter	Stop Gained	-	-	D	D
SCN10A	CA	Novel	ENST00000449082.3:c.4473C>G:p.Ile1491Met	Missense Variant	-	-	D	D
SCN10A	CA	Novel	ENST00000449082.3:c.4467C>G:p.Asn1	Missense Variant	-	-	D	D

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SCN10A	CA	Novel	ENST00000449082.3:c.4458C>G:p.Ile1486Met	Missense Variant	-	-	D	D
SCN10A	CA	Novel	ENST00000449082.3:c.4453C>A:p.Leu1485Ile	Missense Variant	-	-	D	D
SCN10A	CA	rs770288343	ENST00000449082.3:c.5339C>T:p.Pro1780Leu	Missense Variant	-	-	D	D
SCN5A	CA	rs199473124	ENST00000423572.7:c.1700T>A:p.Leu567Gln	Missense Variant	US	LP/P	N	LP
TRPM4	CA	rs754625848	ENST00000252826.10:c.247dup:p.Ala83GlyfsTer13	Frameshift Variant	-	-	D	D

Abbreviations: [1]D: Predicted deleterious; N: Neutral; P: Pathogenic; LP: Likely pathogenic; VUS: Variant of unknown significance; [2]CA: Cardiac arrhythmias; FH: Familial hypercholesterolaemia.

Table 3. Descriptive functional enrichment of candidate genes across GO, reactome, and HPO databases

Term category *	Enriched term	Term ID	p_{adj} (g:SCS)	Enriched genes
GO:BP	cardiac muscle cell action potential	GO:0086001	1.2×10^{-16}	KCNQ1,SCN10A,HCN4,SCN5A,KCNE1,RYR2,ANK2,TRPM4,KCNH2,AKAP9
GO:BP	metal ion transport	GO:0030001	4.2×10^{-9}	KCNQ1,SCN10A,HCN4,SCN5A,KCNE1,PCSK9,RYR2,ANK2,SLC22A5,TRPM4,KCNH2,AKAP9
GO:BP	response to purine-containing compound	GO:0014074	6.3×10^{-6}	KCNQ1,HCN4,KCNE1,RYR2,TRPM4,AKAP9
GO:BP	chemical homeostasis	GO:0048878	2.9×10^{-4}	KCNQ1,GDF2,LDLR,APOB,PCSK9,RYR2,ANK2,TRPM4,KCNH2
GO:BP	circulatory system development	GO:0072359	6.4×10^{-4}	KCNQ1,GDF2,HCN4,LDLR,APOB,SCN5A,RYR2,ANK2,ENG
REAC	Cardiac conduction	REAC:R-HSA-5576891	1.6×10^{-7}	KCNQ1,SCN10A,SCN5A,KCNE1,RYR2,KCNH2,AKAP9
REAC	Phase 3 - rapid repolarization	REAC:R-HSA-5576890	1.6×10^{-7}	KCNQ1,KCNE1,KCNH2,AKAP9
REAC	Muscle contraction	REAC:R-HSA-397014	3.6×10^{-6}	KCNQ1,SCN10A,SCN5A,KCNE1,RYR2,KCNH2,AKAP9
REAC	Phase 2 - plateau phase	REAC:R-HSA-	4.6×10^{-4}	KCNQ1,KCNE1,AKAP9

		5576893		
REAC	LDL clearance	REAC:R-HSA-8964038	9.7×10^{-4}	LDLR,APOB,PCSK9
HP	Cardiac arrest	HP:0001695	5.7×10^{-15}	KCNQ1,SCN10A,HCN4,LDLR,APOB,SCN5A,KCNE1,PCSK9,RYR2,ANK2,TRPM4,KCNH2,AKAP9,TANGO2
HP	Sudden cardiac death	HP:0001645	3.6×10^{-13}	KCNQ1,SCN10A,HCN4,LDLR,APOB,SCN5A,KCNE1,PCSK9,RYR2,ANK2,KCNH2,AKAP9
HP	Prolonged QTc interval	HP:0005184	6.9×10^{-13}	KCNQ1,SCN10A,HCN4,SCN5A,KCNE1,ANK2,KCNH2,AKAP9,TANGO2
HP	Ventricular fibrillation	HP:0001663	1.2×10^{-12}	KCNQ1,SCN10A,HCN4,SCN5A,KCNE1,RYR2,TRPM4,KCNH2,AKAP9,TANGO2
HP	Prolonged QT interval	HP:0001657	2.1×10^{-12}	KCNQ1,SCN10A,HCN4,SCN5A,KCNE1,ANK2,TRPM4,KCNH2,AKAP9,TANGO2

Results are ranked using the g:SCS multiple-testing correction. Abbreviations: *GO: Gene ontology; BP: Biological process; REAC: Reactome; HP: Human phenotype.

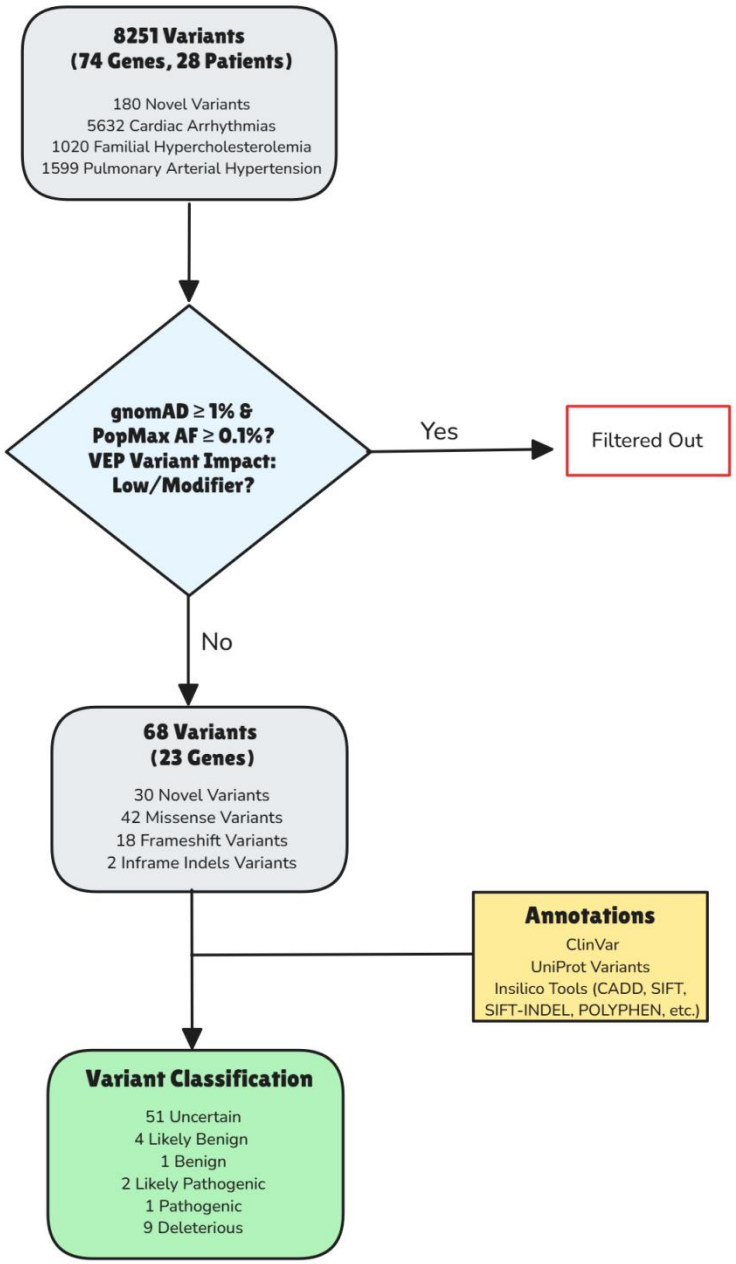


Figure 1. Workflow illustrating the steps involved in the identification, filtering, and classification of variants associated with cardiac arrhythmias, familial hypercholesterolemia, and pulmonary arterial hypertension through whole-exome sequencing

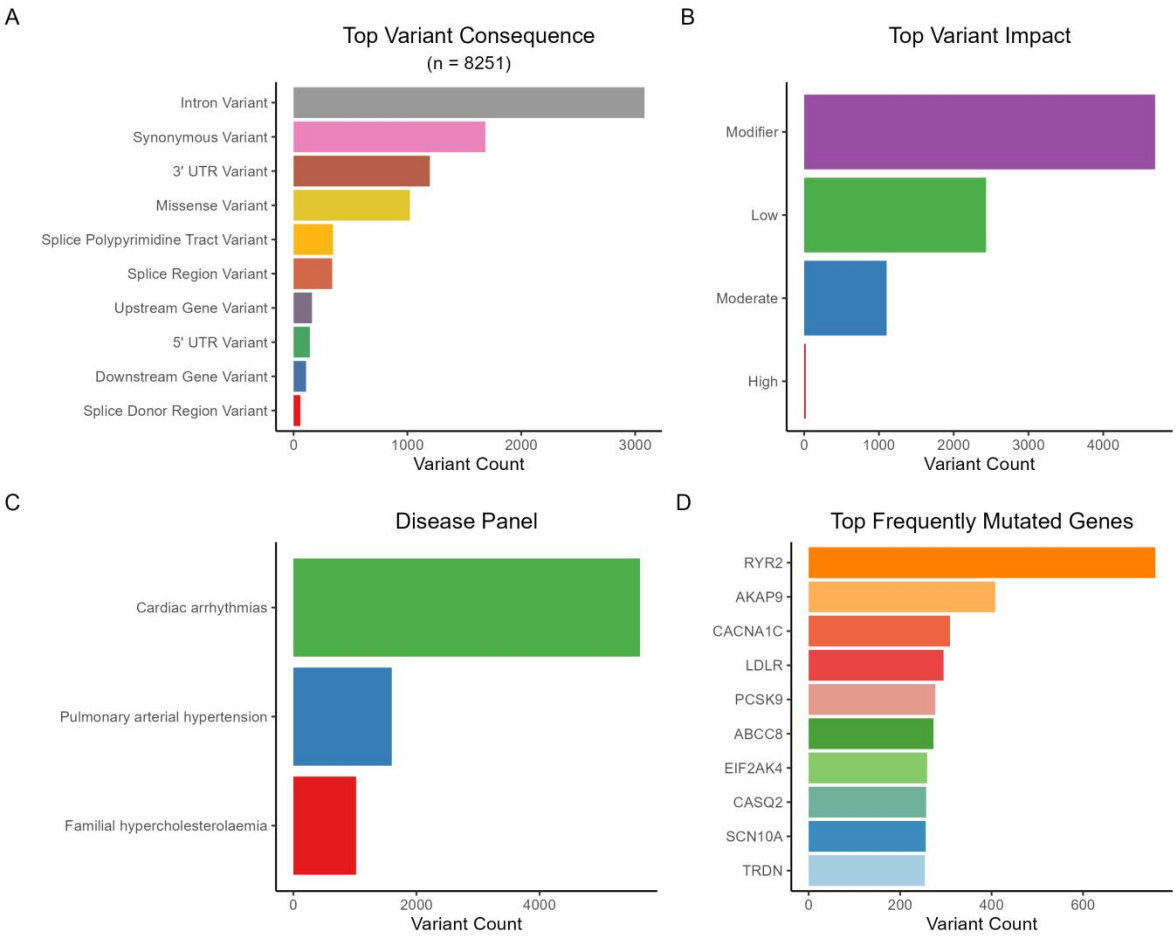


Figure 2. Summary of panel-overlapping variants identified by WES in the obstructive CAD cohort. (A) Top VEP consequence categories across 8,251 variants within 74 PanelApp-curated genes. **(B)** VEP impact distribution (modifier, low, moderate, high). **(C)** Variant counts by disease panel (CA, PAH, FH). **(D)** Top frequently mutated genes in the unfiltered dataset (including RYR2, AKAP9, CACNA1C, LDLR, and PCSK9). **Abbreviations:** CA: Cardiac arrhythmias; CAD: Coronary artery disease; FH: Familial hypercholesterolaemia; PAH: Pulmonary arterial hypertension; PanelApp: Genomics England PanelApp; VEP: Variant Effect Predictor; WES: Whole-exome sequencing.

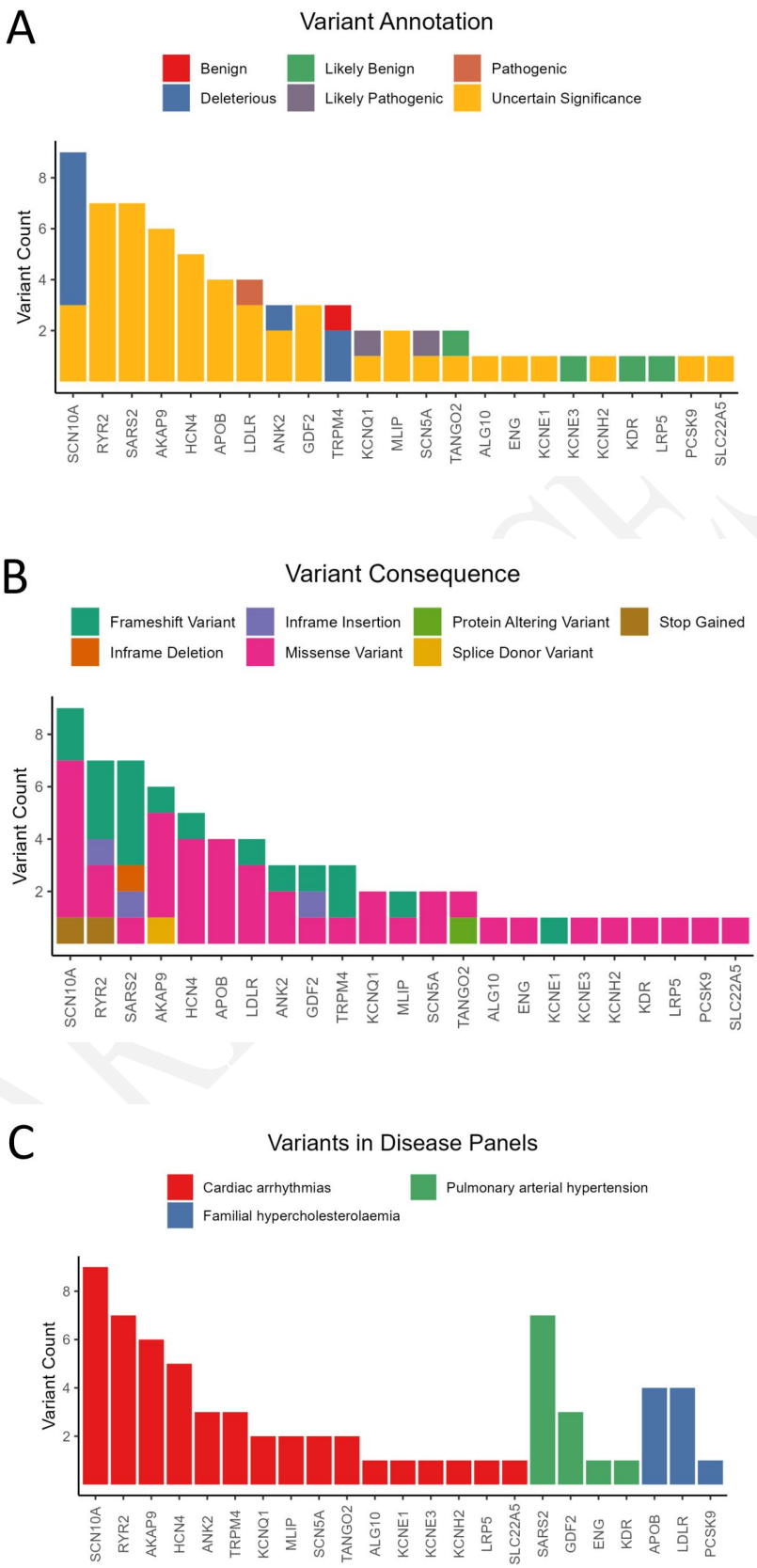
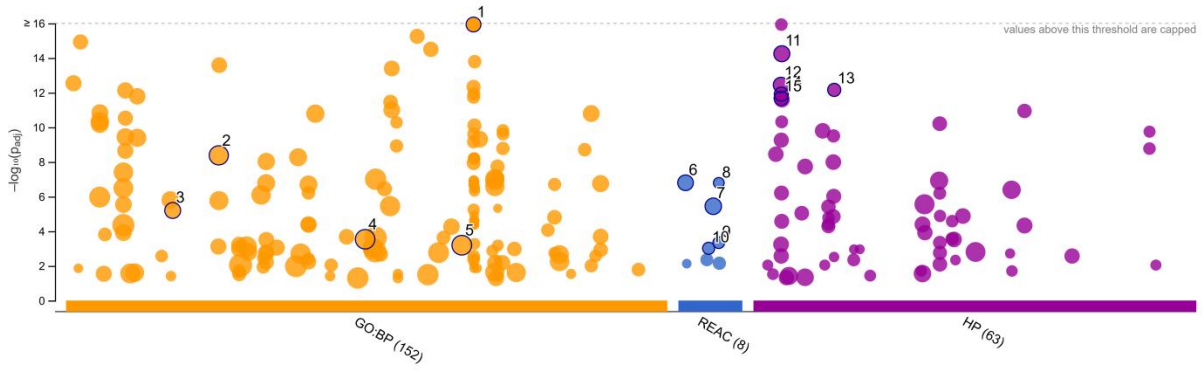

A
Variant Annotation
Benign
Likely Benign
Pathogenic
Deleterious
Likely Pathogenic
Uncertain Significance
Variant Count
8
6
4
2
SCN10A
RYR2
SARS2
AKAP9
HCN4
APOB
LDLR
ANK2
GDF2
TRPM4
KCNQ1
MLIP
SCN5A
TANGO2
ALG10
ENG
KCNE1
KCNE3
KCNH2
KDR
LRP5
PCSK9
SLC22A5
B
Variant Consequence
Frameshift Variant
Inframe Insertion
Protein Altering Variant
Stop Gained
Inframe Deletion
Missense Variant
Splice Donor Variant
Variant Count
8
6
4
2
SCN10A
RYR2
SARS2
AKAP9
HCN4
APOB
LDLR
ANK2
GDF2
TRPM4
KCNQ1
MLIP
SCN5A
TANGO2
ALG10
ENG
KCNE1
KCNE3
KCNH2
KDR
LRP5
PCSK9
SLC22A5
C
Variants in Disease Panels
Cardiac arrhythmias
Pulmonary arterial hypertension
Familial hypercholesterolaemia
Variant Count
8
6
4
2
SCN10A
RYR2
AKAP9
HCN4
ANK2
TRPM4
KCNQ1
MLIP
SCN5A
TANGO2
ALG10
KCNE1
KCNE3
KCNH2
LRP5
SLC22A5
SARS2
GDF2
ENG
KDR
APOB
LDLR
PCSK9

Figure 3. Candidate variant classification and distribution after rare-variant filtering. Following PopMax AF <0.1% and VEP impact filtering of PanelApp FH/CA/PAH genes in WES data from a CAD cohort (*n* = 28), 68 candidate variants across 23 genes were retained (30/68 novel). **(A)** Stacked per-gene counts by final classification (B, LB, VUS, D, LP, P; overall: VUS 51/68, D 9/68, LB 4/68, B 1/68, LP 2/68, P 1/68) based on ClinVar/UniProtVar evidence and in silico predictions. **(B)** Stacked per-gene counts by consequence category, dominated by missense (42/68) and frameshift (18/68) variants. **(C)** Candidate variants mapped to PanelApp panels (CA 47/68; PAH 12/68; FH 9/68); genes are ordered by total candidate count (SCN10A highest). **Abbreviations:** B: Benign; CA: Cardiac arrhythmias; CAD: Coronary artery disease; ClinVar: Clinical Variation database; D: Predicted deleterious; FH: Familial hypercholesterolaemia; LB: Likely benign; LP: Likely pathogenic; P: Pathogenic; PAH: Pulmonary arterial hypertension; PanelApp: Genomics England PanelApp; PopMax AF: Population maximum allele frequency; UniProtVar: UniProt variant annotations; VEP: Variant effect predictor; VUS: Variant of uncertain significance; WES: Whole-exome sequencing.



ID	Source	Term ID	Term Name	p_{adj} (query_1)
1	GO:BP	GO:0086001	cardiac muscle cell action potential	1.169×10^{-16}
2	GO:BP	GO:0030001	metal ion transport	4.165×10^{-9}
3	GO:BP	GO:0014074	response to purine-containing compound	6.331×10^{-6}
4	GO:BP	GO:0048878	chemical homeostasis	2.900×10^{-4}
5	GO:BP	GO:0072359	circulatory system development	6.407×10^{-4}
6	REAC	REAC:R-HSA-55...	Cardiac conduction	1.594×10^{-7}
7	REAC	REAC:R-HSA-39...	Muscle contraction	3.612×10^{-6}
8	REAC	REAC:R-HSA-55...	Phase 3 - rapid repolarisation	1.619×10^{-7}
9	REAC	REAC:R-HSA-55...	Phase 2 - plateau phase	4.562×10^{-4}
10	REAC	REAC:R-HSA-89...	LDL clearance	9.678×10^{-4}
11	HP	HP:0001695	Cardiac arrest	5.667×10^{-15}
12	HP	HP:0001645	Sudden cardiac death	3.593×10^{-13}
13	HP	HP:0005184	Prolonged QTc interval	6.922×10^{-13}
14	HP	HP:0001663	Ventricular fibrillation	1.221×10^{-12}
15	HP	HP:0001657	Prolonged QT interval	2.143×10^{-12}

Figure 4. GO/REAC/HP functional annotation of candidate genes. g:Profiler Manhattan plot showing GO:BP terms (orange), REAC pathways (blue) and HP terms (purple) for the candidate-gene set, plotted as $-\log_{10}$(padj) (g:SCS). Values >16 are capped. The numbered points correspond to the top terms listed in the table below, including GO:BP signals for cardiac muscle cell action potential and metal ion transport; REAC signals for cardiac conduction, muscle contraction, phase 3—rapid repolarisation and LDL clearance; and HP signals for cardiac arrest, sudden cardiac death, prolonged QTc interval and ventricular fibrillation. **Abbreviations:** GO: Gene Ontology; GO:BP: Gene Ontology—Biological Process; g:SCS: Gene set size–corrected significance threshold; HP: Human Phenotype Ontology (g:Profiler source label); HPO: Human Phenotype Ontology; LDL: Low-density lipoprotein; padj: Adjusted *p* value; REAC: Reactome pathways.