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Targeted Exome Sequencing in Pre-Hospital Sudden Cardiac Arrest Reveals a High Genetic Diagnostic Yield

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Abstract: Background: The role of genetic testing in sudden cardiac arrest (SCA) among young and middle-aged adults remains incompletely understood, particularly in pre-hospital settings and in individuals with established clinical diagnoses such as coronary artery disease. **Methods:** We performed whole-exome sequencing with targeted analysis in a city-wide cohort of individuals who experienced pre-hospital SCA in Hangzhou, China. Variants were evaluated using a virtual panel of 2151 cardiovascular-related genes. Variant classification followed the American College of Medical Genetics and Genomics guidelines. **Results:** A total of 69 individuals (mean age 38 ± 14.6 years) were included. Pathogenic or likely pathogenic variants were identified in 13 individuals, corresponding to a diagnostic yield of 18.8%. One individual carried two variants. The identified variants involved genes associated with cardiomyopathies, arrhythmia syndromes, metabolic disorders, and lipid metabolism. Genetic findings were observed both in individuals without a clear clinical diagnosis and in those with apparently established causes of cardiac arrest, including coronary artery disease. When rare variants of uncertain significance with supportive evidence were considered, up to 47.8% of individuals carried potentially relevant variants. Broader gene panels identified more variants than narrower panels limited to established sudden cardiac death genes. **Conclusions:** Systematic exome sequencing in young and middle-aged individuals with pre-hospital SCA identifies clinically relevant genetic variants in a substantial proportion of cases. Genetic testing may complement conventional clinical investigation and may contribute to molecular autopsy and family-based risk assessment in selected patients.

Keywords: sudden cardiac arrest; pre-hospital; exome sequencing; molecular autopsy; diagnostic yield

1. Introduction

Sudden cardiac arrest (SCA) is a condition in which the heart stops beating, causing a cessation of blood flow to the brain and vital organs. The incidence varies across different regions or countries. The incidence of cardiovascular diseases in China was 41.8 per 100,000 individuals in 2005 [1,2], doubling to 95.7 per 100,000 by 2020 [3]. Comparable rates ranging from approximately 50 to 100 cases per 100,000 individuals have been reported in Europe and the United States [4,5]. Among these conditions, coronary artery disease (CAD) is the most prevalent, leading to a rise in sudden cardiac death (SCD), which represents a substantial healthcare burden. According to recent estimates, SCD accounts for over 750,000 deaths annually in China [3]. Despite ongoing public health efforts, such as the distribution of automatic external defibrillators (AEDs) in public spaces, effective preventative policies and measures remain limited.

In contrast to elderly populations where acquired cardiovascular disease predominates, genetic factors are increasingly recognized as major contributors to SCA in younger individuals. Over the past two decades, advances in molecular genetics and next-generation sequencing (NGS) technologies have enabled the identification of numerous genes associated with inherited arrhythmia syndromes and cardiomyopathies, including long-QT syndrome, Brugada syndrome, and hypertrophic cardiomyopathy [6–10]. Identification of pathogenic variants in these genes not only provides a molecular diagnosis for affected individuals but also enables cascade screening among relatives who may carry the same variant and face a significantly elevated risk of sudden death [11,12]. Early recognition of such hereditary conditions therefore has substantial implications for clinical management and preventive strategies.

Current clinical guidelines generally recommend genetic testing for patients with suspected inherited cardiac disorders or for cases of sudden death that remain unexplained after thorough clinical investigation or autopsy [13–16]. While this approach has proven valuable for diagnosing classical channelopathies and cardiomyopathies, it may overlook a substantial subset of patients in whom SCA occurs in the presence of an apparently established clinical cause, such as coronary heart disease (CHD). In these individuals, clinical evaluation often stops once a structural or ischemic explanation is identified, and genetic investigation is rarely pursued. Emerging evidence, however, suggests that genetic variants may contribute to susceptibility even in patients with apparently acquired cardiovascular disease, including premature coronary artery disease and arrhythmia-associated sudden death [17–20] Consequently, restricting genetic testing only to unexplained cases may underestimate the overall genetic contribution to SCA.

Another major challenge in the investigation of sudden death is the limited availability of traditional autopsy. Autopsy remains the gold standard for distinguishing cardiac from non-cardiac causes of sudden death, such as toxicological or pulmonary etiologies [21,22], However, in many regions—including parts of China—autopsy rates remain relatively low due to cultural considerations, logistical barriers, and resource limitations. This diagnostic gap is particularly problematic for out-of-hospital cardiac arrest cases, where the majority of deaths occur before patients reach medical facilities. Under such circumstances, genetic testing has emerged as a complementary approach often referred to as a "molecular autopsy," capable of identifying pathogenic variants that may explain otherwise unexplained deaths and inform risk assessment for surviving relatives [19,23]. Recent studies using whole-exome sequencing (WES) or targeted sequencing have demonstrated the value of genomic approaches in sudden death investigations, although most previous studies have focused primarily on hospital-based cohorts or unexplained deaths [17–19].

Despite these advances, the genetic landscape of pre-hospital SCA—particularly among young and middle-aged individuals with diverse clinical backgrounds—remains insufficiently characterized. Most existing genomic studies have focused on narrowly defined patient groups or on cases lacking an identifiable cause after autopsy. As a result, little is known about the diagnostic yield and clinical implications of systematic genomic analysis in large, population-based cohorts of pre-hospital SCA patients that include both unexplained cases and those with established clinical etiologies.

To address this knowledge gap, the Hangzhou Emergency Center of Zhejiang Province initiated a comprehensive study integrating emergency medical data with genomic analysis between 2020 and 2023. In this study, we performed exome sequencing with targeted analysis in a city-wide cohort of individuals who experienced pre-hospital SCA. Importantly, our cohort deliberately included cases with known clinical diagnoses as well as those lacking autopsy confirmation. The primary objective of this study was to evaluate the diagnostic yield of systematic genomic analysis in this under-studied population and to explore the potential clinical implications of identified variants for both patients and their at-risk relatives. By examining the genetic architecture of pre-hospital SCA in a real-world emergency medical setting, our findings aim to provide evidence that may inform future strategies for genetic testing and guideline development in sudden cardiac arrest.

2. Materials and Methods

2.1. Study Population

This study included both retrospective (2020–2022) and prospective (2022–2023) analyses of pre-hospital sudden cardiac arrest (SCA) cases managed by the Hangzhou Emergency Center of Zhejiang Province, China. Individuals aged ≤ 60 years who experienced SCA were eligible for inclusion. The upper age limit was chosen to focus on early-onset cardiovascular events while still capturing cases of premature coronary artery disease.

Although the primary focus of this study was young and middle-aged adults, a small number of pediatric cases were also included in order to capture the full spectrum of early-onset sudden cardiac arrest. In the final cohort of 69 individuals, three cases were infants or children (age < 18 years).

Cases with clear non-cardiac causes of cardiac arrest, including trauma, suicide, drowning, or other external causes, were excluded from the analysis. Clinical information, including electrocardiograms, hospital diagnostic records, and imaging results, was obtained from the participating hospitals. When available, forensic autopsy findings were provided by the Zhejiang University Forensic Identification Center according to the Chinese National Forensic Autopsy Guidelines (SF/Z JD0101002-201).

2.2. Diagnostic Assessment

Emergency medical data for out-of-hospital cardiac arrest (OHCA) cases were obtained from the Hangzhou Emergency Medical Service (EMS) system. Clinical diagnoses for SCA patients and surviving individuals were determined by cardiologists and emergency physicians at the treating hospitals based on clinical records, electrocardiographic findings, imaging studies, and laboratory results.

For deceased individuals who underwent forensic investigation, autopsy conclusions were determined by certified forensic pathologists at the Zhejiang University Forensic Identification Center. When autopsy data were unavailable, the cause of death was classified based on available clinical and emergency records. Cases lacking sufficient clinical or pathological evidence for a specific diagnosis were categorized as “unexplained”.

2.3. Exome Sequencing

Genomic DNA was extracted from peripheral whole-blood samples using the Qiagen DNeasy Blood & Tissue Kit (Qiagen, Germany) according to the manufacturer’s instructions. Sequencing libraries were prepared using the Rapid Plus DNA Library Preparation Kit for Illumina (ABclonal Biotechnology Co., Ltd., Wuhan, China).

For library preparation, 200 ng of genomic DNA was fragmented by sonication to generate DNA fragments of approximately 200–250 bp. After end repair and A-tailing, paired-end sequencing adapters were ligated to the DNA fragments. The adapter-ligated fragments were purified and amplified by six cycles of PCR.

Exonic regions were captured using the NadPrep® Hybrid Capture NEXome XP Panel (Nano Digm Bio-Tech, Nanjing, China). Approximately 500 ng of the purified library was hybridized with capture probes for 16 h in a multiplex reaction. Following hybridization, captured fragments were washed, eluted, and amplified with six additional PCR cycles.

Sequencing was performed on an Illumina NovaSeq platform (Illumina Inc., San Diego, CA, USA) with 150-bp paired-end reads. For each sample, at least 10 Gb of raw sequencing data were generated. The mean sequencing depth across the exome exceeded 100×, and gene-level coverage metrics are provided in Supplementary Table S1.

2.4. Bioinformatic Analysis

Raw sequencing reads (FASTQ files) were processed using fastp for quality filtering and trimming. Sequence quality was evaluated using FastQC. Clean reads were aligned to the human reference genome (hg19) using the Burrows–Wheeler Aligner (BWA). Duplicate reads were marked using Samblaster, and sorted BAM files were generated for downstream analysis.

Variant calling was performed using GATK HaplotypeCaller (version 4.2.0.0). Variant call format (VCF) files were subsequently annotated using ANNOVAR together with additional annotation tools including Variant Effect Predictor (VEP) and snpEff.

Variants were filtered using standard quality metrics. For single nucleotide variants (SNVs), filters included QD < 2.0, FS > 60.0, MQ < 40.0, SOR > 3, MQRankSum < −12.5, ReadPosRankSum < −8.0, and depth thresholds. For insertion/deletion variants (INDELs), filtering criteria included QD < 2.0, FS > 200.0, and ReadPosRankSum < −20. Variants with insufficient read depth (DP < 5) were excluded.

Population allele frequencies were evaluated using multiple databases, including the 1000 Genomes Project (East Asian population), gnomAD exomes (East Asian population), and a local exome sequencing database.

Variants with allele frequency greater than 0.01% in public databases or greater than 0.05% in the local database were excluded from further analysis.

2.5. Virtual Gene Panel and Variant Interpretation

We created a custom virtual panel of 2151 genes associated with cardiovascular disease. The panel integrated three major sources:

(1) A set of 222 genes derived from Genomics England PanelApp consensus panels related to sudden cardiac death (SUD) and syndromic cardiomyopathy (SCM) [24,25] (Supplementary Table S2). Genes classified as 'Green' (diagnostic-grade) or 'Amber' (borderline) were included.
(2) Fifty-seven genes curated by the ClinGen Cardiovascular Clinical Domain Working Group (CDWG) with moderate, strong, or definitive evidence of disease association (Supplementary Table S3).
(3) A list of genes derived from the OMIM database's Human Phenotype Ontology (HPO), associated with phenotypes such as 'arrhythmia', 'cardiomyopathy', 'mitral valve prolapse', 'ventricular fibrillation', 'coronary artery disease', 'atrial fibrillation', 'cardiac arrhythmias', and 'tachycardia' (Supplementary Table S4).

Variants identified in PanelApp and ClinGen gene sets were categorized as primary findings, whereas variants in genes derived from OMIM/HPO were considered secondary findings. Variant interpretation followed the standards and guidelines of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology (ACMG/AMP) [26]. Variant classification was assisted by CardioClassifier [27] and the Franklin interpretation platform. Gene-specific inheritance patterns were considered according to OMIM and ClinGen disease models.

Computational prediction tools including SIFT, PolyPhen-2, and REVEL were used to evaluate the functional impact of missense variants [28,29]. For certain VUS where clinical symptoms aligned closely with gene function, AlphaMissense from DeepMind was used for further pathogenicity prediction [30,31]. Importantly, AlphaMissense predictions were used only as supportive computational evidence and were not used as independent criteria for variant classification. The detailed prediction results are provided in Supplementary Table S5.

2.6. Evaluation of Variants of Uncertain Significance

Variants classified as variants of uncertain significance (VUS) according to ACMG/AMP criteria were further evaluated when additional supporting evidence was present. VUS were highlighted for further interpretation when they met the following criteria:

1. Extremely low population frequency (minor allele frequency < 0.0001);
2. Predicted deleterious by multiple computational tools (SIFT, PolyPhen-2, REVEL, and AlphaMissense);
3. Located in genes with established cardiovascular disease association;
4. Clinical phenotype consistent with the reported gene–disease relationship.

These variants were discussed as variants with supportive evidence but were not considered diagnostic findings. In several cases, additional segregation analysis in family members could potentially allow reclassification of these variants according to ACMG guidelines; however, family validation was not always feasible because relatives of deceased individuals frequently declined genetic testing.

2.7. Sanger Sequencing Validation

All pathogenic or likely pathogenic variants identified through exome sequencing were validated by Sanger sequencing. Selected variants of uncertain significance that met the additional evaluation criteria described above were also validated. Primers were designed using Primer3 software, and PCR amplification products were sequenced using standard capillary electrophoresis methods.

2.8. Main Outcomes and Measures

The primary outcome of this study was the proportion of individuals carrying pathogenic or likely pathogenic variants identified through exome sequencing. Diagnostic yield was defined as the percentage of individuals in the cohort with at least one pathogenic or likely pathogenic variant.

Secondary analyses evaluated the distribution of rare variants across the custom gene panel and compared the yield of the extended gene set with that obtained using narrower virtual panels derived from PanelApp and ClinGen gene lists.

2.9. Statistical Analysis

The proportion of individuals carrying pathogenic or likely pathogenic variants was calculated for the entire cohort. Comparisons of variant detection across different virtual gene panels were performed descriptively because of the limited cohort size. No formal hypothesis testing was performed.

3. Results

3.1. Cohort Characteristics

A total of 69 individuals who experienced pre-hospital sudden cardiac arrest (SCA) were included in the final analysis. Baseline demographic and clinical characteristics of the cohort are summarized in Table 1.

The majority of individuals were male (61/69, 88.4%), and the mean age at the time of cardiac arrest was 38 ± 14.6 years. Twelve individuals (17.4%) survived the cardiac arrest event, whereas the remaining cases were fatal.

Table 1. Baseline Clinical and Demographic Characteristics of the Pre-Hospital Sudden Cardiac Arrest Cohort (N = 69). This table summarizes the baseline demographic and clinical characteristics of the 69 individuals included in the pre-hospital sudden cardiac arrest cohort. Variables include age, sex, cardiovascular risk factors, initial clinical diagnosis, and family history of cardiac disease or sudden death. Continuous variables are presented as mean ± standard deviation, and categorical variables as counts and percentages. Abbreviations: SCA, sudden cardiac arrest; BMI, body mass index; CAD, coronary artery disease; HTN, hypertension; DM, diabetes mellitus.

Male, n (%)	61 (88.4%)
Age at arrest (years)	38 ± 14.6
Age range, y	newborn to 60
Survivors, n (%)	12 (17.4%)
Family history of SCD and Cardiac events, n (%)	5 (7.2%)
Diagnostic testing performed prior to WES	
Autopsy	7
Cardiac CT and/or coronary angiography	8
Cardiac color Doppler ultrasound	8
Suspected diagnosis following initial diagnostic testing prior to WES [a]	
Unexplained (including IVF)	57 (82.6%)
LVNC	1
HCM	2
DCM	2
Coronary artery disease	3
Hypokalemia	3
Hypoglycemia	1

[a] Based on the hospital's medical diagnostic records or the forensic pathological autopsy findings.

Prior clinical investigation was limited for most patients. Only 12 individuals had a definitive clinical or pathological diagnosis before genetic testing. These included hypertrophic cardiomyopathy (n = 2), dilated cardiomyopathy (n = 2), coronary artery disease (n = 3), left ventricular non-compaction (n = 1), hypokalemia (n = 3), and hypoglycemia (n = 1) (Table 1). The remaining 57 cases (82.6%) were classified as unexplained based on conventional clinical evaluation.

Autopsy examinations were performed in seven cases, providing pathological confirmation or exclusion of structural cardiac disease. Detailed integration of autopsy findings and genetic results for deceased individuals is summarized in Table 2.

3.2. Identification of Pathogenic or Likely Pathogenic Variants

Using exome sequencing with the custom 2,151-gene virtual panel, a total of 14 pathogenic or likely pathogenic (P/LP) variants were identified in 13 individuals. Based on the number of individuals carrying at least one P/LP variant, the diagnostic yield of genetic testing in this cohort was 18.8% (13/69). Detailed information on these variants and the corresponding clinical characteristics of the carriers is provided in Table 3.

Table 2. Integration of Autopsy Findings and Genetic Results in Deceased Individuals with Pre-Hospital Sudden Cardiac Arrest. This table summarizes autopsy findings together with genetic results for individuals who died following pre-hospital sudden cardiac arrest. Pathological findings are presented alongside genetic variants identified through exome sequencing, including pathogenic or likely pathogenic variants and selected variants of uncertain significance. The table illustrates how genetic findings may complement post-mortem examination in selected cases.

Case ID	Age at SCA	Sex	SCA Scene	Toxicology	Heart Weight, (g)	Autopsy Conclusion	Variants and Conclusion with Genetic Testing
50	41	Male	During the duty process	Neg	269	gross pathology: Short stature, 147 cm; polydactyly with six toes on each foot; no heart abnormalities ventricular histopathology: Negative Forensic evaluation results: Unexplained	p.Tyr121Ter, *GLI1* (LP) associated with short stature and polydactyly c.990+1G>A, *SYNE2* (P) associated with arrhythmias and resulting in SCD
71	46	Male	Occurred during medical treatment	Neg	661	gross pathology: Enlarged heart with a significantly increased weight ventricular histopathology: Myocyte enlargement, nuclear hyperchromatic, severe coronary atherosclerosis present Forensic evaluation results: Acute myocardial infarction on the basis of pre-existing old myocardial infarction	/
72	49	Male	Occurred during medical infusion	Neg	493	gross pathology: ventricular histopathology: HCM Forensic evaluation results: Sudden death due to circulatory and respiratory failure caused by dilated cardiomyopathy with coronary atherosclerosis	NM_201574.3/c.213dup/p.Glu72Ter–*SLC4A3* (LP) lead to SCD
73	22	Female	Occurred while taking a train	Neg	285	gross pathology: Negative ventricular histopathology: Negative Forensic evaluation results: Cause of death unknown, cannot rule out severe hypoglycemia, hyperkalemia, hypokalemia, or other endocrine abnormalities leading to cardiac arrest	NM_001035.3/c.5774T>C/p.Ile1925Thr, *RYR2* (VUS) associated with cyclic vomiting syndrome, vomiting leads to hypoglycemia, then lead to SCD
74	Newborn infant	Male	Died 5 h after being born in the hospital	Neg	18.5	gross pathology: Negative ventricular histopathology: Negative Forensic evaluation results: Death due to acute respiratory failure caused by amniotic fluid aspiration in both lungs	/
75	3-month-old infant	Male	Abnormalities occurred after vaccination, and death occurred during hospital treatment	Neg	68.5	gross pathology: HCM and atrial septal defect ventricular histopathology: Negative Forensic evaluation results: acute heart failure	NM_000256.3/c.2905+1G>A,*MYBPC3* (P) NM_000256.3/c.836del/p.Gly279Valfs Ter21, *MYBPC3* (LP) two P/LP variants lead to severe HCM
76	Newborn infant	Male	A stillbirth occurred during delivery at the hospital	Neg	17.4	gross pathology: Ventricular septum is abnormally thick with a Z-score greater than 2, and the thymus is enlarged at 20.5 g ventricular histopathology: Negative Forensic evaluation results: Death due to acute respiratory failure caused by amniotic fluid aspiration in both lungs	/

Abbreviations: SCA, sudden cardiac arrest; P/LP, pathogenic or likely pathogenic; VUS, variant of uncertain significance.

Table 3. Pathogenic or Likely Pathogenic Variants Identified by Whole-Exome Sequencing and Clinical Characteristics of the Carriers *. This table lists pathogenic or likely pathogenic variants identified through whole-exome sequencing in the study cohort. For each variant, information includes the affected individual, gene, variant type, classification according to ACMG criteria, and relevant clinical phenotype.

Case ID	Gene	RefSeq Transcript	Nucleic Change	Protein Change	Sex	Age at Arrest	Survivor or Not	Zygosity	Diagnosis # Following Genetic Testing (Probable)
12	*ALPK3*	NM_020778.4	c.1206G>A	p.Trp402 *	M	18	NO	Het	HCM (Risk Allele) **
17	*PLN*	NM_002667.4	c.157T>C	p.Ter53Argext *	M	35	NO	Het	DCM, HCM
20	*SCN5A*	NM_198056.2	c.1486_1490del	p.Lys496Ter	M	36	NO	Het	Brugada Syndrome
32	*CYP27A1*	NM_000784.4	c.435G>T	(p.Gly145=)	M	53	NO	Het	Coronary artery disease (Risk Allele) **
46	*SLC22A5*	NM_003060.4	c.497+1G>T	Splicing	M	43	NO	Het	HCM (Risk Allele) **
47	*LDLR*	NM_000527.5	c.682G>C	p.Glu228Gln	M	49	NO	Het	Familial hypercholesterolemia
50	*GLI1*	NM_005269.3	c.363C>A	p.Tyr121Ter	M	41	NO	Het	polydactyly (Definite)
	SYNE2	NM_182914.3	c.990+1G>A	Splicing				Het	Arrhythmia
53	*MIB1*	NM_020774,4	c.2470del	p.Asp824Ilefs Ter2	M	53	NO	Het	Left ventricular noncompaction
54	*LPA*	NM_005577.4	c.3496C>T	p.Gln1166Ter	M	35	NO	Het	Coronary artery disease (Definite)
57	*HMBS*	NM_000190.4	c.730_731del	p.Leu244Alafs Ter6	F	35	Yes	Het	Acute Intermittent Porphyria
70	*VCL*	NM_014000.3	c.3397_3400dup	p.Gln1134Leufs Ter53	M	37	Yes	Het	DCM, HCM
72	*SLC4A3*	NM_201574.3	c.213dup	p.Glu72Ter	M	49	NO	Het	Short QT Syndrome
75	*MYBPC3*	NM_000256.3	c.2905+1G>A	Splicing	M	3-month-old	NO	Het	HCM (Definite)
	MYBPC3	NM_000256.3	c.836del	p.Gly279Valfs Ter21				Het	(Compound Heterozygous)

* Additional details are provided in Supplementary Table S7, including extended clinical data, list of prior publications of the listed variants, and ClinVar entry. ** Het = Heterozygous. Variants in *ALPK3*, *CYP27A1*, and *SLC22A5* are typically recessive; heterozygous findings are classified as potential risk factors or carriers in the absence of a second allele. # The diagnostic conclusions were reached through a collaborative discussion between the attending physician and the genetic counselor.

The identified variants were distributed across multiple genes associated with inherited cardiovascular disorders, including cardiomyopathy, arrhythmia syndromes, and metabolic diseases. Representative genes included *SCN5A*, *PLN*, *MYBPC3*, *VCL*, *SLC4A3*, *LDLR*, and *LPA*. A comprehensive list of rare variants detected in the cohort is provided in Supplementary Table S6, and detailed phenotypic information for individuals carrying P/LP variants is presented in Supplementary Table S7.

The distribution of rare variants detected across the analyzed genes is illustrated in Figure 1, highlighting the broad spectrum of cardiovascular-related genes implicated in this cohort.

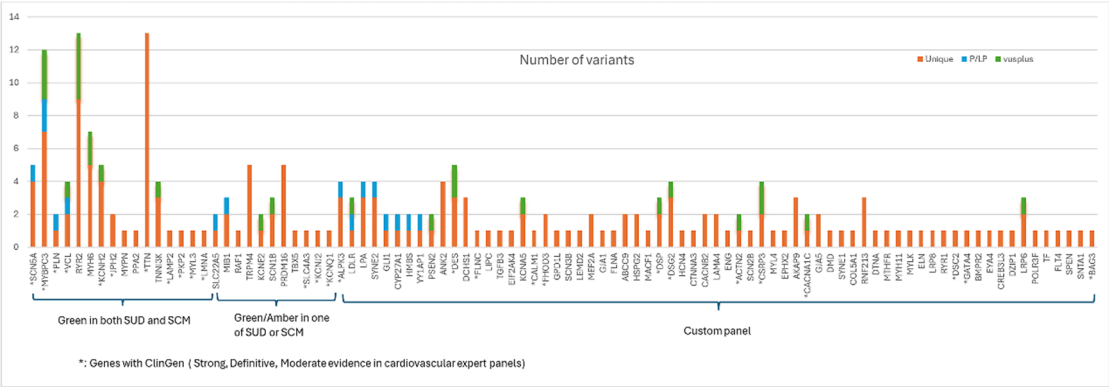

Figure 1. Distribution of Rare Variants Identified by Exome Sequencing in the Study Cohort. Distribution of rare genetic variants identified through whole-exome sequencing in individuals with pre-hospital sudden cardiac arrest. The figure illustrates the genes in which rare variants were detected across the study cohort. Variants include pathogenic or likely pathogenic variants as well as variants of uncertain significance. This overview highlights the genetic heterogeneity of sudden cardiac arrest in young and middle-aged individuals.

3.3. Comparison of Diagnostic Yield across Virtual Gene Panels

To assess the impact of panel design on variant detection, we compared the diagnostic yield obtained using the full 2,151-gene custom panel with that obtained using narrower virtual panels derived from PanelApp and ClinGen gene lists.

Using the extended custom panel, P/LP variants were identified in 18.8% of individuals. When the analysis was restricted to PanelApp genes, the diagnostic yield decreased to approximately 8.7%, and when restricted to ClinGen genes the yield was approximately 7.3%. A comparison of diagnostic yields across these gene panels is shown in Figure 2.

These findings indicate that broader gene panels incorporating additional cardiovascular disease-related genes may improve the likelihood of identifying clinically relevant variants in SCA cohorts.

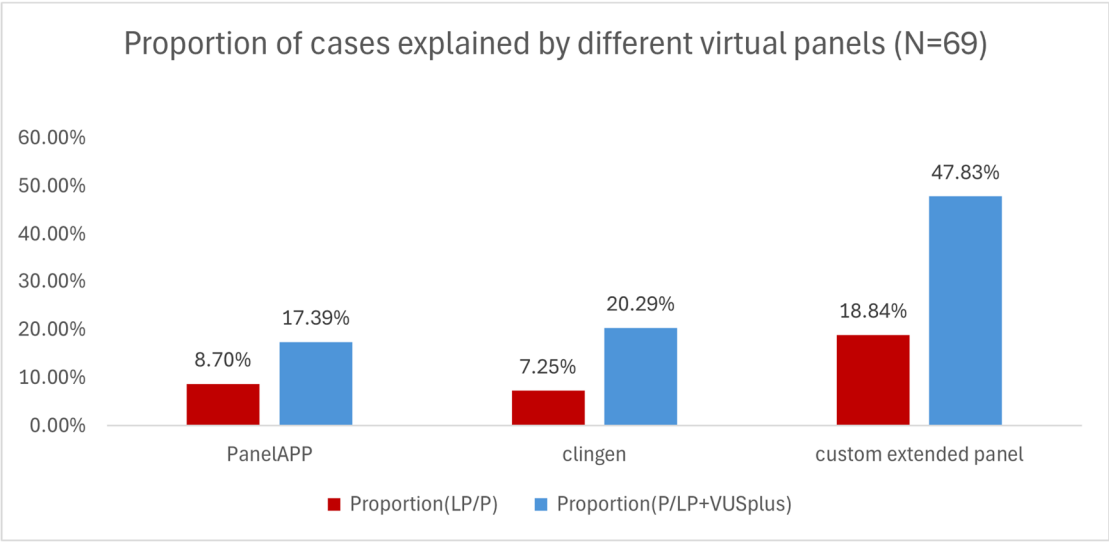

Figure 2. Comparison of Diagnostic Yield Across Different Virtual Gene Panels. Comparison of diagnostic yield obtained using different virtual gene panels applied to the exome sequencing data. The expanded 2,151-gene panel identified pathogenic or likely pathogenic variants in 18.8% of individuals. In comparison, analysis restricted to

narrower panels derived from PanelApp or ClinGen gene lists identified fewer variants. These findings suggest that broader gene panels may increase the likelihood of detecting clinically relevant variants in sudden cardiac arrest cohorts.

3.4. Variants of Uncertain Significance with Supportive Evidence

In addition to P/LP variants, a number of rare variants classified as variants of uncertain significance (VUS) were identified. These variants and their associated clinical information are summarized in Table 4.

Several VUS showed multiple lines of supportive evidence, including extremely low population allele frequency, damaging predictions across multiple computational algorithms, and phenotypic concordance with known gene–disease associations. Computational prediction results for these variants, including AlphaMissense and other in-silico tools, are summarized in Supplementary Table S5.

Although these variants cannot be considered diagnostic findings according to ACMG/AMP criteria, they may represent potential contributors to disease susceptibility. When these VUS with supportive evidence were considered as potentially explanatory variants, up to 33 of the 69 cases (47.8%) carried rare variants that could plausibly contribute to the observed clinical phenotype.

Table 4. Variants of Uncertain Significance Identified in the Study Cohort *. This table lists variants of uncertain significance identified through whole-exome sequencing in the study cohort. For each variant, details include the affected individual, gene, variant type, and available clinical phenotype information.

Case ID	Gene	RefSeq Transcript	Nucleic Change	Protein Change	Sex	Age at Arrest	Survivor or Not	Diagnosis Following Initial Phenotypic Testing (Definite)	Diagnosis # Following Genetic Testing (Probable)
2	*DSP*	NM_004415.3	c.943C>T	p.Arg315Cys	M	31	Yes	Arrhythmias, Hypokalemia	Arrhythmogenic right ventricular dysplasia
3	*DSG2*	NM_001943.4	c.1688T>G	p.Leu563Arg	M	32	Yes	unexplained	Arrhythmias
4	*MYBPC3*	NM_000256.3	c.3343G>A	p.Val1115Ile	M	23	Yes	Left ventricular noncompaction	Left ventricular noncompaction (Definite)
6	*RYR2*	NM_001035.3	c.7151T>A	p.Met2384Lys	M	14	Yes	unexplained	CPVT
15	*ACTN2*	NM_001103.4	c.1831T>G	p.Trp611Gly	M	17	No	unexplained	DCM/HCM
19	*RYR2*	NM_001035.3	c.7328C>G	p.Pro2443Arg	M	46	No	unexplained	CPVT
	DES	NM_001927.4	c.694C>T	p.Leu232Phe	M				
23	*RYR2*	NM_001035.3	c.7328C>G	p.Pro2443Arg	M	38	No	unexplained	CPVT
	MYH6	NM_002471.4	c.2852A>G	p.Glu951Gly	M				
	DES	NM_001927.4	c.694C>T	p.Leu232Phe	M				
25	*PSEN2*	NM_000447.3	c.838A>G	p.Thr280Ala	M	30	No	unexplained	DCM/HCM
26	*TRPM4*	NM_017636.4	c.2987_3014del	p.Glu996fs	M	60	No	unexplained	Progressive familial heart block, type IB
	KCNE2	NM_172201.1	c.79C>T	p.Arg27Cys					
27	*SCN1B*	NM_001037.5	c.566C>T	p.Thr189Met	F	52	No	unexplained	BrS
28	*CSRP3*	NM_003476.5	c.298C>T	p.Arg100Cys	M	50	No	unexplained	DCM/HCM
43	*VCL*	NM_014000.3	c.1676C>A	p.Ala559Asp homo	M	26	No	unexplained	DCM/HCM
44	*CACNA1C*	NM_000719.7	c.512C>T	p.Thr171Met	M	41	Yes	unexplained	BrS or LQTs
45	*MYH6*	NM_002471.4	c.3140G>A	p.Arg1047His	M	37	No	unexplained	DCM/HCM
51	*KCNH2*	NM_000238.4	c.44T>A	p.Leu15Gln	F	19	No	unexplained	LQTs
64	*CSRP3*	NM_003476.5	c.3G>A	p.Met1?	M	54	Yes	unexplained	DCM
65	*MYBPC3*	NM_000256.3	c.2504G>T	p.Arg835Leu	M	49	Yes	unexplained	unexplained
73	*RYR2*	NM_001035.3	c.5774T>C	p.Ile1925Thr	F	22	No	unexplained	vomiting leads to hypoglycemia, then leads to SCD
74	*LRP6*	NM_002336.3	c.4760G>T	p.Cys1587Phe	M	new-born	No	unexplained	unexplained
	TNNI3K	NM_015978.3	c.1153T>C	p.Cys385Arg					

* Additional details are provided in Supplementary Table S5, including extended data, list of prior publications of the listed variants, and ClinVar entry, Computational prediction results (SIFT, Polyphen2, REVEL, Clinpred, AlphaMissense), Briefly emergency history. # The diagnostic conclusions were reached through a collaborative discussion between the attending physician and the genetic counselor.

3.5. Genetic Findings in Selected Clinical Contexts

Genetic testing provided clinically relevant insights in several scenarios. For example, in two individuals whose deaths were attributed to coronary artery disease, pathogenic variants were identified in *LDLR* (Case 47) and *LPA* (Case 54), suggesting a genetic predisposition to premature atherosclerotic disease.

Genetic findings also clarified cases with autopsy data. A three-month-old infant with autopsy-confirmed hypertrophic cardiomyopathy carried compound heterozygous variants in *MYBPC3*, inherited from each parent, providing a definitive molecular diagnosis (Table 2).

In another case classified as unexplained after forensic examination, a pathogenic variant in *SYNE2*, a gene associated with Emery–Dreifuss muscular dystrophy and cardiac arrhythmias, provided a plausible genetic explanation for sudden death.

Clinical characteristics and genetic findings for surviving individuals are summarized in Table 5, whereas combined pathological and genetic findings for deceased individuals are presented in Table 2.

Table 5. Clinical Characteristics and Genetic Findings in Survivors of Pre-Hospital Sudden Cardiac Arrest. This table presents the clinical characteristics and genetic findings of individuals who survived pre-hospital sudden cardiac arrest. Information includes demographic data, clinical presentation, and genetic variants identified through exome sequencing.

Case ID	Sex	Age at SCA	SCA Scene	ECG after Resuscitation	Echocardiogram	* Discharge Diagnosis Results	# Diagnosis Following Genetic Testing (Probable)	Variants
1	M	29	Office	ST segment depression, sinus tachycardia, and left bundle branch block.	Moderate regurgitation of the mitral and tricuspid valves.	Hypokalemia	/	/
2	M	31	Office	Right bundle branch block, and ST segment depression	Moderate regurgitation of the mitral and tricuspid valves.	Arrhythmias, ICD implanted	Arrhythmogenic right ventricular dysplasia	NM_004415.3/c.943C>T/p.Arg315Cys/ *DSP* (VUS)
3	M	32	Bed	/	/	unexplained	Arrhythmogenic right ventricular dysplasia	NM_001943.4/c.1688T>G/p.Leu563Arg/*DSG2* (VUS)
4	M	23	Metro	Ventricular tachycardia and ST-T changes	incomplete ventricular myocardium compaction; mild mitral and tricuspid regurgitation.	Left ventricular noncompaction	Left ventricular noncompaction 10	NM_000256.3/c.3343G>A/p.Val1115Ile/*MYBPC3* (VUS)
6	M	14	Athletic arena	/	/	unexplained	CPVT	NM_001035.3/c.7151T>A/p.Met2384Lys/*RYR2* (VUS)
8	F	25	Shopping mall	ST segment depression and sinus tachycardia	Pulmonary hypertension and mild tricuspid regurgitation	Hypokalemia	/	/
22	M	53	Badminton court	/	/	Hypokalemia	/	/
57	F	35	Public	ST segment depression, sinus tachycardia, and left bundle branch block	Tricuspid valve mild regurgitation	Unexplained, Hypokalemia	Arrhythmia	p.Leu244Alafs Ter6/*HMBS* (LP)
64	M	54	Office	ST segment depression, sinus tachycardia, and right bundle branch block	Ejection fraction (EF) is 62%, with overall left ventricular wall motion being uncoordinated	Partial myocardial infarction.	DCM	NM_003476.5/c.3G>A/p.Met1?/ *CSRP3* (VUS)
65	M	46	Bed	/	/	unexplained	unexplained	NM_000256.3/c.2504G>T/p.Arg835Leu/*MYBPC3* (VUS)
67	M	40	Public	Sinus tachycardia and peaked T waves	Ejection fraction (EF) is 65%, with overall left ventricular wall motion being uncoordinated, suggesting possible cardiomyopathy.	Partial myocardial infarction.	/	/
70	M	37	home	Sinus bradycardia and ST-T changes	Tricuspid valve mild regurgitation	Hypoglycemia	DCM	p.Gln1134Leufs Ter53 / *VCL* (LP)

* The diagnostic results are derived from the clinical summary or discharge report by the primary physician. # The diagnostic conclusions were reached through a collaborative discussion between the attending physician and the genetic counselor. Abbreviations: SCA, sudden cardiac arrest; P/LP, pathogenic or likely pathogenic; VUS, variant of uncertain significance.

4. Discussion

In this study, we performed systematic exome sequencing with targeted analysis in a city-wide cohort of individuals experiencing pre-hospital sudden cardiac arrest (SCA). Our results demonstrate that pathogenic or likely pathogenic variants were identified in 18.8% of individuals, indicating that a substantial proportion of young and middle-aged SCA cases may have an underlying genetic component. Importantly, the use of an expanded gene panel increased the likelihood of detecting clinically relevant variants compared with narrower panels restricted to traditional arrhythmia or cardiomyopathy genes (Figure 2).

4.1. Genetic Contribution to Pre-Hospital Sudden Cardiac Arrest

Most previous genetic studies of sudden cardiac death have focused on unexplained cases or hospital-based cohorts. In contrast, our study evaluated a real-world emergency medical cohort that included both unexplained cases and individuals with established clinical diagnoses. Among the 69 individuals analyzed, conventional clinical investigation identified a clear diagnosis in only a minority of cases, while the majority remained unexplained prior to genetic analysis (Table 1). This observation highlights the diagnostic limitations of traditional clinical evaluation in pre-hospital SCA.

Through exome sequencing, we identified pathogenic or likely pathogenic variants in genes associated with cardiomyopathies, arrhythmia syndromes, and inherited metabolic disorders. Several variants occurred in genes well known to predispose to sudden cardiac death, including *SCN5A*, *PLN*, and *MYBPC3* (Table 3). These findings are consistent with previous genomic studies demonstrating that inherited cardiovascular conditions represent an important cause of sudden cardiac death in younger populations [16–18].

Recent exome sequencing studies have similarly highlighted the contribution of rare genetic variants to sudden cardiac death in younger individuals, further supporting the role of genomic investigation in these patients [32]. Reported diagnostic yields in molecular autopsy or sudden cardiac death sequencing studies typically range from approximately 4% to 20%, depending on cohort characteristics and gene panel design [16–18]. The diagnostic yield observed in our study falls within this range, supporting the validity of our analytical approach.

4.2. Genetic Findings in Individuals with Established Clinical Diagnoses

An important observation from our study is that clinically significant genetic variants were also identified in individuals whose deaths were attributed to apparently established causes such as coronary artery disease. For example, pathogenic variants in *LDLR* and *LPA* were detected in individuals with premature coronary disease. These genes are known to play key roles in lipid metabolism and cardiovascular risk, and their identification suggests that inherited predisposition may contribute to early-onset atherosclerotic disease.

This finding challenges the conventional clinical paradigm in which genetic investigation often stops once a structural or ischemic cause of cardiac arrest has been identified. Our results suggest that, particularly in younger individuals, genetic testing may still provide clinically relevant information even when an apparent cause of death has already been established. Identification of pathogenic variants in genes such as *LDLR* has important clinical implications, as cascade screening and early lipid-lowering therapy are recommended for affected families according to current cardiovascular prevention guidelines [33].

4.3. Interpretation of Variants of Uncertain Significance

In addition to pathogenic variants, a substantial number of rare variants were classified as variants of uncertain significance (VUS). These variants are summarized in Table 4, and detailed computational prediction results are provided in Supplementary Table S5.

Several VUS identified in this cohort demonstrated multiple supportive lines of evidence, including extremely low allele frequency in population databases, damaging predictions across multiple computational algorithms, and phenotypic concordance with known gene–disease relationships. However, according to ACMG/AMP classification guidelines, such variants cannot be considered diagnostic without additional evidence such as segregation data or functional validation.

Interpretation of VUS remains a major challenge in genomic studies of sudden cardiac death, particularly when family data or functional studies are unavailable [34]. In the context of sudden cardiac death, obtaining family segregation data is often difficult because relatives of deceased individuals may be reluctant to undergo genetic testing. As a result, variants that might otherwise be reclassified as likely pathogenic may remain categorized as VUS. Our findings therefore highlight the importance of family-based genetic investigation

following sudden cardiac death events and the need for continued re-evaluation of variants as additional evidence becomes available.

4.4. Implications for Molecular Autopsy and Family Screening

Another important implication of this study relates to the concept of molecular autopsy. Traditional autopsy remains the gold standard for determining the cause of sudden death, yet autopsy rates remain low in many regions due to cultural or logistical barriers [21,22]. In such circumstances, genetic analysis may provide valuable complementary information for determining potential causes of death.

Recent large-scale studies and reviews further support the diagnostic value of molecular autopsy in unexplained sudden death cases and emphasize the growing role of genomic testing in post-mortem investigations [35,36]. In addition, genomic analysis can reveal concealed cardiomyopathies or inherited arrhythmia syndromes that may not be detectable through conventional autopsy alone [19].

In our cohort, genetic testing provided plausible explanations for several cases that remained unexplained after clinical or forensic investigation. For example, identification of compound heterozygous variants in *MYBPC3* in an infant with hypertrophic cardiomyopathy provided a definitive molecular diagnosis (Table 2). A similar case involving compound heterozygous *MYBPC3* variants causing lethal neonatal hypertrophic cardiomyopathy has previously been reported [37], supporting the pathogenic role of such variants in severe early-onset disease.

Beyond determining the cause of death, genetic findings may also have important implications for surviving relatives. Identification of pathogenic variants enables targeted cascade screening and clinical surveillance in family members who may carry the same variant and therefore have an elevated risk of sudden cardiac death.

4.5. Study Limitations

Several limitations of this study should be acknowledged. First, the cohort size was relatively modest, reflecting the challenges of obtaining genetic samples in emergency medical settings. Larger multi-center studies will be necessary to further characterize the genetic architecture of pre-hospital SCA.

Second, our analysis focused primarily on coding variants identified through exome sequencing. As a result, deep intronic variants or structural genomic alterations that may contribute to disease risk could not be evaluated. Future studies using whole-genome sequencing may help address this limitation.

Third, family-based segregation analysis was not available for many variants identified in this study because relatives of deceased individuals frequently declined genetic testing. This limitation likely contributed to the high proportion of variants classified as VUS.

4.6. Clinical and Research Implications

Despite these limitations, our findings provide important insights into the genetic contribution to pre-hospital SCA. The identification of pathogenic variants in nearly one-fifth of individuals suggests that genetic testing may represent a valuable tool in the investigation of sudden cardiac arrest, particularly in younger populations.

Our results support the integration of genetic analysis into multidisciplinary approaches for sudden death investigation, including collaboration among emergency physicians, cardiologists, forensic specialists, and genetic counselors. Expanding genetic testing in selected SCA cases may improve diagnostic accuracy and facilitate preventive strategies for at-risk relatives.

Future studies involving larger cohorts and international collaboration will be essential to refine the role of genomic testing in sudden cardiac arrest and to inform potential updates to clinical guidelines for the management of these patients and their families.

5. Conclusions

In this city-wide cohort of individuals with pre-hospital sudden cardiac arrest, systematic exome sequencing with targeted analysis identified pathogenic or likely pathogenic variants in 18.8% of cases, highlighting a substantial genetic contribution to SCA in young and middle-aged individuals. The use of an expanded gene panel increased the likelihood of detecting clinically relevant variants compared with conventional gene panels. Genetic testing also provided important insights in cases with apparently established clinical diagnoses, including coronary artery disease, and contributed to the identification of inherited cardiovascular conditions. These findings support the potential value of incorporating genomic analysis into the investigation of pre-hospital sudden cardiac arrest and may help guide future strategies for molecular autopsy and family-based risk assessment.

Supplementary Materials

The additional data and information can be downloaded at: https://media.sciltp.com/articles/others/260414142625834 8/iCirculation-25120207-Supplementary-Materials.zip. Table S1: Gene-based coverage data. Table S2: PanelApp SUD and SCM gene lists. Table S3: ClinGen Cardiovascular Clinical Domain Working Group gene list. Table S4: OMIM HPO terms. Table S5: Computational prediction results for VUS (SIFT, PolyPhen-2, REVEL, AlphaMissense). Table S6: Comprehensive list of rare variants identified. Table S7: Detailed phenotypic and genetic information for P/LP carriers.

Author Contributions

Conceptualization, X.H. and M.Q.; Methodology, L.H., J.W. (Jianying Wang) and Y.L.; Software, X.H. and Y.W.; Validation, Q.Z.; Formal Analysis, X.H., L.H., Q.Z. and Y.W.; Investigation, J.W. (Jiangang Wang), J.X., X.C., M.L., S.D., J.H., W.S., J.L., J.W. (Jianying Wang) and Y.L.; Resources, J.W. (Jiangang Wang), J.X., S.D., J.H., W.S. and J.L.; Data Curation, J.W. (Jiangang Wang), J.X., X.C. and M.L.; Writing—Original Draft Preparation, X.H.; Writing—Review & Editing, X.H. and M.Q.; Visualization, L.H. and X.H.; Supervision, M.Q.; Project Administration, X.H. and M.Q.; Funding Acquisition, J.W. (Jiangang Wang). All authors have read and agreed to the published version of the manuscript.

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Informed Consent Statement

The studies involving human participants were reviewed and approved by the Ethics Staff Hangzhou Emergency center (serial number: HZEMC-2020-09).

Data Availability Statement

All summary-level data generated or analyzed during this study are included in this published article and its supplementary information files. The raw exome sequencing data (in VCF format) and detailed de-identified phenotypic data are not publicly available due to patient privacy and confidentiality restrictions inherent in our ethics approval. However, these data can be made available from the corresponding author (M.Q.) upon reasonable request and contingent upon the execution of a formal data sharing agreement. No new custom code or software was generated for this study; all information tools used are publicly available and detailed in the Supplementary Methods. Web resources: OMIM: https://www.omim.org. PanelApp: https://panelapp.genomicsengland.co.uk/. ClinVar: https://www.ncbi.nlm.nih.gov/clinvar/. Franklin Genoox: https://franklin.genoox.com/. cardioclassifier: https://www.cardioclassifier.org/.

Conflicts of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. Dian Diagnostic group, Yikon Genomics Co., Ltd. are independent clinical laboratory (ICL).

Use of AI and AI-Assisted Technologies

During the preparation of this work the author(s) used Deepseek, in order to improve the manuscript's language, readability, and structure for journal submission. After using this service, the author(s) reviewed and edited the content as needed and take full responsibility for the content of the publication.

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