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Molecular Genetics Etiology Research on a Typical Case of Unexplained Sudden Death in Yunnan Based on Whole Exome Sequencing

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Abstract: *Objective*: To explore the etiology of a typical case of unexplained sudden death in Yunnan (referred to as "Yunnan sudden death"), correlate genotypes with phenotypes to identify high-risk populations, and provide a basis for intervention measures. *Methods*: Epidemiological and clinical investigation data, as well as blood biochemical test results, were collected from three cases of Yunnan sudden death. Whole exome sequencing (WES) was performed on blood samples, using GRCh38/HG19 as the reference sequence. Mutation sites were screened based on genetic heart disease-related gene variants and subjected to filtering, annotation, and analysis. Protein function prediction analysis of mutated genes was conducted using SIFT, Mutation Taster, and PolyPhen2 software. *Results*: After screening, harmful and ambiguous mutation sites were retained, involving 18 mutation site information from four genetic heart disease-related genes (DMD, SIDT1, CSRP3, DSG2). Among them, 17 were predicted by software to have harmful, harmful, or pathogenic protein functions. Deceased 2 carried six mutation sites in DMD, deceased 2 and deceased 4 jointly carried nine mutation sites in SIDT1, and deceased 3 carried two mutation sites in CSRP3. *Conclusion*: The causes of death in the three cases of Yunnan sudden death in this study are highly likely related to mutations in genetic heart disease-related genes.

Keywords: Unexplained sudden death in Yunnan; Genetic heart disease; Gene mutation; Whole exome sequencing

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1. Introduction

Unexplained sudden death in Yunnan (referred to as "Yunnan sudden death") is a special type of endemic disease that primarily occurs in some impoverished rural areas in central and western Yunnan Province. The onset time is concentrated from June to September, exhibiting significant temporal, geographical, familial,

and village clustering characteristics, posing a severe threat to the health of the affected population. Since its first occurrence in 1975, a total of 421 cases have been reported as of 2023, but the etiology remains incompletely understood [1]. The cardiac health status of the population in the affected areas is generally poor, with high detection rates of abnormal electrocardiograms and echocardiograms, and diverse types of abnormal changes [2–4]. Meanwhile, there are obvious specificities in gene mutations among different populations, as well as familial and regional clustering phenomena of gene mutations, suggesting a possible genetic predisposition to Yunnan sudden death [2]. Not all genetic heart disease-related pathogenic genes lead to severe phenotypes, and most carriers have normal hearts [5]. Therefore, genetic and clinical evaluations of relatives are necessary to expand the analysis. Based on the characteristics of onset and death, clinical manifestations, abnormal changes in electrocardiograms and myocardial enzymes, autopsy, and pathological histological examination results of Yunnan sudden death, it is speculated that the etiology of some cases of Yunnan sudden death may be related to genetic heart diseases.

To explore the etiological relationship between gene mutations in genetic heart diseases and Yunnan sudden death, this study, building on previous research, utilized whole exome sequencing technology for the first time to detect mutations in pathogenic genes of genetic heart diseases in a family of four who experienced unexplained sudden death in a severely affected area of Yunnan in 2009. The aim was to find associations between genotypes and phenotypes, identify high-risk populations, provide precise health interventions, and thereby improve the health status of the affected population.

2. Objects and methods

2.1. Study objects

From 11:00 on August 3, 2009, to 03:00 on August 4, 2009, four members of a family in HQSGZ Village, Dali Prefecture, suddenly died consecutively within 24 hours and were determined to be cases of "unexplained sudden death in Yunnan" after investigation. This study used the four deceased individuals from this family as study samples and performed whole exome sequencing analysis on the well-preserved heart cavity blood from three autopsied cases.

2.2. Research methods

2.2.1. Epidemiological investigation and pathological analysis

At 18:00 on August 4, a joint investigation team from the Yunnan Provincial Institute for Endemic Disease Control and Prevention, the Chinese Center for Disease Control and Prevention, the Dali Prefecture Center for Disease Control and Prevention, and the Dali Prefecture People's Hospital arrived at the scene to conduct investigations and response measures. With the consent of the deceased's relatives, autopsies were performed on Deceased 2 (approximately 16 hours post-mortem), Deceased 3 (approximately 17 hours post-mortem), and Deceased 4 (approximately 18 hours post-mortem) among the four cases. Specimens such as the heart, cardiac blood, and liver were obtained and sent to Fuwai Hospital in Beijing for pathological examination and testing. The first deceased, deceased 1, had been deceased for 32 hours, so an autopsy was not performed. Instead, a cardiac puncture was performed, but cardiac blood was not obtained; liver tissue was taken for testing.

2.2.2. Ethical approval

This study has obtained informed consent from the control population and the deceased's relatives and has been approved by the Medical Ethics Committee of the Yunnan Provincial Institute for Endemic Disease Control and Prevention (Yunnan Institute for Endemic Disease Medical Ethics [2024] No. 04). Additionally, the "Implementation Rules for the Regulation on the Administration of Human Genetic Resources" has been strictly enforced.

2.3. Experimental testing

2.3.1. Whole exome sequencing process

(1) Sample preparation

Blood samples that were well-preserved and met the sequencing company's requirements were sent to Sangon Biotech (Shanghai) Co., Ltd.

(2) DNA fragmentation

The genomic DNA from the blood was physically fragmented, and the target DNA fragments were recovered.

(3) End repair and A-tailing

The target DNA fragments underwent end repair and were adenylated with deoxyadenosine (A).

(4) Adapter ligation

The adenylated DNA fragments were ligated with sequencing adapters.

(5) Fragment selection and library enrichment

DNA fragments with successfully ligated sequencing adapters at both ends were recovered to construct the pre-capture library, which was then enriched through PCR amplification.

(6) Hybridization capture

The genomic exonym regions were captured using the Agilent SureSelect Human All Exon V6 exome capture chip.

(7) Library amplification

The captured library underwent PCR amplification for enrichment.

(8) Library quality control

The library underwent quality inspection.

(9) Sequencing

High-throughput sequencing was performed on the diluted and pooled library using the Illumina PE150 sequencing platform.

2.3.2. Data processing and quality control

(1) Raw data filtering

Adapter sequences were removed from the reads using Fastp software. Single-end sequencing reads containing more than 40% low-quality bases (quality threshold ≤ 20) or more than 10% undetermined bases were removed. Sliding window quality trimming was performed with a window size of 4 bp, and reads with an average quality below 20 were trimmed.

(2) Data alignment and processing

The clean data was aligned with the reference genome hg19 or hg38. Non-coding region gene mutations and synonymous mutations were removed, and mutation sites unrelated to inherited cardiac ion channel

diseases and cardiomyopathies were excluded. The GATK software was used for sorting, deduplication, and obtaining sequencing depth and coverage.

2.3.3. Bioinformatics analysis

(1) Variant site screening

ANNOVAR annotation software was used to screen for variant sites in genes previously reported to be associated with cardiomyopathies and cardiac ion channel diseases.

(2) Database comparison

Sites with a minor allele frequency ≤ 0.01 were screened from the 1000 Genomes Project, the Single Nucleotide Polymorphism Database, and the National Heart, Lung, and Blood Institute Exome Sequencing Project database. Diversity sites among individuals were removed, and rare variants were retained.

(3) Pathogenicity prediction

Bioinformatics software SIFT and MutationTaster were used to predict the pathogenicity of the screened gene mutation sites. When the results were inconsistent, PolyPhen-2 software was used for further analysis. A mutation was considered harmful if at least two methods predicted it to be harmful.

3. Results

3.1. Epidemiological investigation

3.1.1. This incident was a case of familial clustered sudden death, as detailed in the genetic pedigree chart

By referring to **Figure 1** of the Genetic Pedigree Chart of the Case's Family, following results were recorded.

(1) Sudden death victim 1 (ZLS0)

Female, 55 years old, of Yi ethnicity, a farmer, mother of Victims 2 and 4, mother-in-law of Victim 3. On August 3, 2009, at 6:30 AM, she went to the cornfield to cut pig fodder and returned home at 10:30 AM without any discomfort. As the family was preparing to eat, she suddenly tilted her head back, gasped for breath, then let her head droop, with clear secretions flowing from the corners of her mouth. She died suddenly at home from unknown causes five minutes later.

(2) Sudden death victim 2 (ZLS2)

Female, 29 years old, of Yi ethnicity, the third daughter of Victim 1 and the younger sister of Victim 4. On the morning of August 3, 2009, she delivered breakfast to her father and returned home at 11:30 AM to find her mother (Victim 1) had died suddenly. She fainted five times in grief before waking up. At 10:00 PM, she went to her room to rest. At 1:45 AM on August 4, she woke up, tilted her head back, gasped for breath, let her head droop, and vomited oral secretions. She died after approximately three minutes of emergency treatment.

(3) Sudden death victim 3 (ZZX)

Male, 32 years old, of Yi ethnicity. His mother-in-law and younger sister-in-law (Victim 2) died suddenly one after another. He and his wife (Victim 4) were in grief and fear. At 2:15 AM on August 4, while the couple was resting in the corridor, he suddenly clenched his hands, convulsed, tilted his head back, gasped for breath, let his head droop, and vomited oral secretions. Despite emergency measures such as artificial respiration and chest compressions, he died approximately five minutes later.

(5) Sudden death victim 4 (ZLS1)

Female, 32 years old, of Yi ethnicity. Between August 3 and 4, 2009, her mother, sister, and husband died successively, leaving her deeply saddened and frightened, and she cried continuously. After returning to her room to rest, at 3:00 AM on August 4, she suddenly tilted her head back, gasped for breath, then let her head droop and vomited clear oral secretions. Despite emergency measures such as artificial respiration, she died suddenly at home from unknown causes approximately five minutes later.

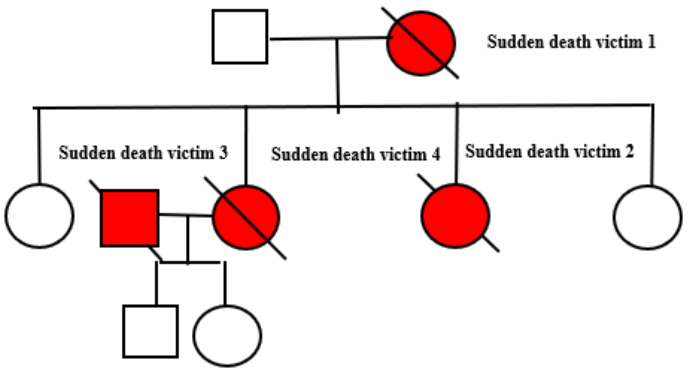

Figure 1. Genetic pedigree chart of the case's family

Note: □ represents male; ○ represents female; ⊠ ⊗ represents cases of sudden death in Yunnan.

3.2. Investigation of related risk factors

The family members enjoyed harmonious relationships and were generally in good health on a daily basis. Within the month preceding the sudden deaths, they had not consumed spoiled, unfamiliar, or cold-mixed foods. Although fresh "Xiaobai Mushrooms" were found in the deceased's home, it could not be confirmed that the incident was related to these mushrooms, and the exact cause of death has not yet been determined.

3.3. Pathological examination results

Beijing Fuwai Hospital conducted pathological examinations on the deceased. The hearts of the three cases showed no abnormalities in shape, size, or color, and no clearly characteristic lethal lesions were identified. Deceased 2: Congenital heart disease with patent foramen ovale; focal lymphocytic myocarditis. Deceased 3: Lymphocytic myocarditis, focal lymphocytic infiltration in the liver, superficial gastritis, acute pulmonary edema, with the main lesions being multifocal necrosis in the heart accompanied by lymphocytic and eosinophilic infiltration. Deceased 4: Eccentric fibrous thickening of the intima of the atrioventricular nodal artery leading to lumen stenosis; focal lymphocytic myocarditis.

3.4. Whole exome sequencing results

3.4.1. Sequencing data quality

The sequencing data quality was good, meeting the requirements for subsequent analysis. 95.85% of the target sites were covered at least 30 times. The central value of the insert size distribution for the samples was around 200 bp, conforming to a normal distribution, as shown in **Table 1**.

Table 1. Summary of whole exome sequencing data

Details	ZLS2	ZZX	ZLS1
Raw data volume	70,907,264	80,338,536	66,222,666
Data volume after QC	69,915,548	79,250,574	65,470,090
Capture rate of exonic regions	99.71%	99.75%	99.52%
Proportion of bases with quality > Q30	90.68%	89.49%	89.60%
Proportion of regions with coverage depth ≥ 10X	95.83%	95.69%	96.02%
Number of reads mapped to reference genome	69,838,081	79,150,888	65,409,298
Ratio of mapped reads to filtered reads	99.71%	99.75%	99.52%
Average sequencing depth of exonic regions	163.12	186.67	157.00

3.4.2. Genetic variation detection

Whole exome sequencing yielded an average of 217,468,466 total reads. After quality control of the results, a total of 23,605 mutation sites were identified, including single nucleotide polymorphisms (SNPs), insertions, and deletions. Among these, there were 22,830 SNPs and 775 INDELs. The vast majority of mutation sites occurred in exonic regions, totaling 23,313 (98.7%), as shown in **Table 2**.

Table 2. Statistics on SNP/InDel variation information in the sample

Sample	Single nucleotide polymorphism	Insertions and deletions	Frameshift deletion	Frameshift insertion	In-frame deletion	In-frame insertion	Missense mutation	Nonsense mutation	Homozygous mutation	Heterozygous mutation	Total mutations
ZLS2	22371	803	117	94	176	153	10729	114	392	402	11495
ZZX	22261	842	138	101	180	148	10548	119	380	462	11343
ZLS1	22632	794	109	89	159	154	10723	106	382	461	11452

3.5. Potential pathogenic gene variations and inherited heart diseases

Using existing databases and software, the variant results were further screened and analyzed to retain harmful mutations and those with uncertain significance. This process identified 18 mutation sites involving four genes associated with inherited heart diseases (DMD, SIDT1, CSRP3, DSG2). Predictions for these 18 variants were made using SIFT, MutationTaster, and PolyPhen2, with 17 mutation sites predicted to be harmful, harmful, or pathogenic. Deceased individual 2 carried six mutation sites in the DMD gene; deceased individuals 2 and 4 (sisters) jointly carried nine mutation sites in the SIDT1 gene; deceased individual 3 carried two mutation sites in the CSRP3 gene. By consulting databases and literature, the heart diseases associated with these mutated genes were identified as long QT syndrome (LQTS), dilated cardiomyopathy (DCM), Duchenne muscular dystrophy (DMD), hypertrophic cardiomyopathy (HCM), and arrhythmogenic right ventricular cardiomyopathy (ARVC). See **Table 3**.

Table 3. Mutation genes carried by the cases

Gene	Mutation site information	Decedent	SIFT	Mutation Taster	Polyphen2	Heart-related diseases
DMD	NM_004011:exon21:c.C3128A:p.S1043Y, NM_004012:exon21:c.C3119A:p.S1040Y, NM_000109:exon49:c.C7127A:p.S2376Y, NM_004006:exon49:c.C7151A:p.S2384Y, NM_004009:exon49:c.C7139A:p.S2380Y, NM_004010:exon49:c.C6782A:p.S2261Y	ZLS2	D	D	D	LQTS, DCM, DMD
SIDT1	NM_001322296:exon21:c.A2023G:p.K675E, NM_001322295:exon22:c.A2125G:p.K709E, NM_001322297:exon22:c.A1756G:p.K586E, NM_001322298:exon22:c.A1666G:p.K556E, NM_001322299:exon22:c.A1453G:p.K485E, NM_001322300:exon22:c.A928G:p.K310E, NM_017699:exon22:c.A2194G:p.K732E, NM_001308350:exon23:c.A2209G:p.K737E, NM_001322294:exon23:c.A2212G:p.K738E	ZLS2, ZLS1	D	D	D	LQTS
CSRP3	NM_001369404:exon2:c.G16A:p.G6R, NM_003476:exon2:c.G16A:p.G6R	ZZX	D	D	D	DCM, HCM
DSG2	NM_001943:exon15:c.C2470T:p.R824C	ZZX	D	P	B	ARVC, DCM, HCM

Note: The prediction results of SIFT are Tolerated (harmless) and Deleterious (harmful); the prediction results of MutationTaster are A, D, N, or P: “A” (“Disease_causing_automatic”), “D” (“Disease_causing”), “N” (“Polymorphism”), and “P” (“Polymorphism_automatic”), with both A and D indicating that the site may be harmful. The prediction results of PolyPhen-2 are Benign (benign), Possibly damaging (possibly pathogenic), and Probably damaging (highly likely pathogenic).

4. Discussion

Since 1984, over 10 similar sudden death incidents have occurred in this region, resulting in nearly 40 deaths, classifying it as a high-risk area for sudden death in Yunnan. In this study, four sudden death victims came from the same family, with the following relationships: 1 was the mother of 2 and 4, 2 and 4 were sisters, and 3 and 4 were husband and wife. The four victims lived with their relatives before death, had no special dietary or lifestyle history, and were previously healthy. They died within 20 hours, with a rapid onset from illness to death, sudden onset and death, and no specific clinical symptoms. This incident exhibited characteristics of familial clustering and cardiogenic sudden death, with victims 1, 2, and 4 being females from two generations of the same family, possibly related to genetic factors.

In recent years, studies have found that the cause of death in some Yunnan sudden death cases may be related to genetic heart disease gene mutations [2,6,7]. On July 21-22, 2015, three Yunnan sudden death cases occurred within 24 hours in a family in Qingshuihe Village, Qingshui Village Committee, Renxing Town, Lufeng City. The victims were the mother, daughter, and granddaughter. Whole-genome resequencing was performed on blood samples from the daughter, granddaughter, and surviving father of the sudden death victims using the BGI research platform. A highly pathogenic mutation was found in the atrial fibrillation-related gene NUP155, which was shared by the daughter and granddaughter but not present in the father [6]. Jia et al. also conducted gene sequencing on immediate family members of 25 Yunnan sudden death cases and found highly pathogenic mutation sites in genes such as SCN5A, IRX4, MYH6, and TTN [7]. Cheng Xue et al. found that the population in the affected area carried 52 site mutations in 36 exons of the ARVC

desmosomal protein gene, with a gene mutation rate of up to 37.1%, approaching the level of clinically reported ARVC patients (39.2%), confirming from a molecular genetics perspective that ARVC is the main cause of some Yunnan sudden deaths [2]. These studies suggest that some Yunnan sudden deaths are related to genetic heart disease gene mutations.

Detecting pathogenic gene variations in genetic heart diseases is an important direction for exploring the causes of unexplained sudden death. Most genetic heart diseases require a comprehensive diagnosis combining clinical and genetic test results. There are hundreds of reported pathogenic genes and thousands of mutation sites for genetic heart diseases, with differences in pathogenic mutations among different populations. Although exons account for less than 2% of the human gene sequence, they contain approximately 85% of known pathogenic variations and have significant advantages in disease gene screening and detection rates [8]. Compared with transcriptome and whole-genome sequencing, whole-exome sequencing covers a smaller genomic range but is more precise, effective, economical, and has high coverage in detecting exon regions, making it the best method for detecting pathogenic gene mutations in genetic heart diseases [9].

4.1. Analysis of gene mutation results

In this study, victim 2 carried six mutation sites in the dystrophin gene (DMD). The prediction results of SIFT, MutationTaster, and PolyPhen-2 for these six variations were all harmful, harmful, and pathogenic, respectively. The DMD gene is one of the largest known human genes, with a high mutation frequency. It is located on the X chromosome at Xp21.2-21.1, spans 2.4 Mb, and encodes a 14 kb mRNA transcript and a 427 kDa dystrophin protein through 79 exons. Due to the special inheritance pattern of the X chromosome, this linkage and co-inheritance phenomenon exhibits different inheritance patterns between males and females. Females carrying DMD generally have no obvious symptoms [10]. It has been reported that DMD gene variations can cause X-linked dilated cardiomyopathy, a unique muscular dystrophy phenotype without any obvious skeletal muscle disease, characterized by preferential involvement of the heart and leading to fatal heart failure [11,12]. When the expression of dystrophin protein encoded by the DMD gene is impaired in the heart, cardiac enlargement and circulatory failure rapidly occur [12]. For patients suspected of having DMD-related genetic heart diseases, gene testing is an important diagnostic method. For DMD-related genetic heart diseases, both muscle and heart aspects need to be comprehensively considered. Drug treatment may include drugs to improve myocardial function (such as angiotensin-converting enzyme inhibitors and β-receptor blockers for treating heart failure) and drugs targeting DMD muscle lesions (such as some gene therapy drugs and exon skipping therapy under development) [13]. In this study, victim 2 was female, had rapid disease progression, slight cardiac lesions, and carried six pathogenic mutation sites in the DMD gene. It is speculated that the cause of death of victim 2 may be related to X-linked dilated cardiomyopathy caused by DMD gene mutations.

In this study, the two sisters, victims 2 and 4, jointly carried nine mutation sites in the SIDT1 gene. It has been reported that this gene is a pathogenic gene associated with long QT syndrome, and mutations in this gene may play a key role in regulating cardiac electrophysiological activity [14,15]. Long QT syndrome is a heart disease characterized by prolonged QT interval on electrocardiogram, which can lead to arrhythmias and even sudden death. The protein encoded by the SIDT1 gene may be involved in regulating ion channels in cardiac cell membranes or interact with other proteins related to cardiac electrophysiology. By studying its intracellular localization, expression level, and interaction network with other proteins, its specific role in the

pathogenesis of long QT syndrome can be revealed [14].

In this study, victim 3 carried two mutation sites in the CSRP3 gene. Huang H et al. studied a family with three generations affected by hypertrophic cardiomyopathy (HCM) and used whole-exome sequencing to sequence the genes of the probands and performed gene mutation validation analysis [16]. This variation was predicted to be pathogenic and would cause protein truncation. At the same time, combined with pathological and imaging examinations, the first case of CSRP3 (p.Arg122*) variation in an HCM family in Asia was confirmed, proving that CSRP3 gene mutations can cause HCM or dilated cardiomyopathy (DCM) [16]. HCM is a common genetic heart disease determined by genes, with mild symptoms and echocardiographic manifestations of symmetric, asymmetric, focal left ventricular hypertrophy or ventricular wall thickening. Patients are at risk of arrhythmias and sudden cardiac death, which is an important and common cause of sudden death in young people [17]. For symptomatic patients, various drug treatments and septal reduction therapy have been proven to relieve symptoms and improve function [18]. Maron B et al. found mutations in cysteine and glycine-rich protein 3 (CSRP3), which encodes muscle LIM protein (MLP) [17]. This protein has complex and diverse functions in myocardial physiology and pathology, including cell fate determination, transcriptional regulation, cell adhesion and movement, signal transduction, and cytoskeletal organization. Sun L et al. found that CSRP3-deficient mice exhibited severe cardiac dysfunction [19]. When CSRP3 was knocked out, mice were born with dilated cardiomyopathy, accompanied by myocardial hypertrophy and heart failure. In this study, victim 3 was a 32-year-old young adult male who died suddenly without specific prodromal symptoms. There was multifocal lymph and eosinophil infiltration in the right ventricle and myocardial necrosis. Through gene testing, two mutation sites in the CSRP3 gene were detected in victim 3. The prediction results of SIFT, MutationTaster, and PolyPhen-2 for these two variations were all harmful, harmful, and pathogenic, respectively. Therefore, it is speculated that his death may be related to dilated cardiomyopathy or hypertrophic cardiomyopathy caused by CSRP3 gene mutations.

In this study, victim 3 also carried one mutation in the desmoglein-2 (DSG2) gene, which is associated with arrhythmogenic right ventricular cardiomyopathy (ARVC). This gene mutation affects the basic structure of the protein, altering the structure and size of glycoproteins, thereby affecting normal myocardial function. Cheng Xue found 10 mutation sites in the DSG2 gene in Yunnan sudden death cases, case relatives, and the population in the affected area [2]. Among them, the c.2470C > T heterozygous missense mutation and c.2930A>G heterozygous missense mutation in exon 15 of the DSG2 gene were only found in Yunnan sudden death cases, while case relatives, the population in the affected area, and the control population did not carry them. ARVC is a genetic cardiomyopathy mainly characterized by the replacement of ventricular myocardial cells with adipose tissue and fibrous tissue, resulting in right ventricular dysfunction and a tendency to develop heart failure, arrhythmias, and sudden death. It is also related to mutations in genes encoding desmosomal proteins [1,20]. Qiu Z et al. conducted animal model studies on genetic cardiomyopathy using DSG2 gene knockout mice and wild-type mice without DSG2 gene knockout as controls [21]. They found that DSG2 gene knockout mice had significantly increased myocardial fibrosis and observed PPARα deficiency. Their studies showed that activating PPARα could improve cardiac fibrosis in patients with arrhythmogenic cardiomyopathy lacking DSG2. ARVC, HCM, and DCM all belong to genetic cardiomyopathies. Gene mutations affect the connections between myocardial cells, making myocardial tissue fragile and prone to arrhythmias, and even leading to sudden death. Therefore, it is speculated that the cause of death of victim 3 may be related to genetic cardiomyopathy.

Gene mutations increase the susceptibility to genetic heart diseases. Although some gene mutations alone are not sufficient to directly cause genetic heart diseases, they increase the risk of an individual developing heart disease. Gene mutations can interact with environmental factors to jointly influence the occurrence and development of genetic heart diseases. In this study, 18 mutation sites in four genes were found in three cases. It is speculated that the four Yunnan sudden death cases may have developed lethal arrhythmias on the basis of gene mutations due to factors such as grief, ultimately leading to cardiac dysfunction and death. This study provides new clues for Yunnan sudden death research. Although the pathogenic mechanism of the mutant genes is currently unclear, in the future, we can construct animal models carrying pathogenic gene mutations, observe changes in their cardiac electrophysiological characteristics, and whether symptoms of genetic heart diseases appear. This will help us better understand the role of pathogenic genes in the body and the impact of their mutations on cardiac function.

4.2. Analysis of intervention measures

For people carrying mutant genes for genetic heart diseases, corresponding intervention measures can be taken [22]. First, long-term follow-up monitoring of patients should be carried out, including arrhythmias, treatment response, and disease progression. Based on this study, whole-exome sequencing and genetic counseling should be conducted on relatives of Yunnan sudden death cases and the population in the affected area to reduce the risk of recurrence in patients with the same gene mutations. At the same time, it can provide a reference for screening high-risk pathogenic mutation genes for sudden death in people in need and detect potential disease risks. Second, behavioral interventions and health education should be carried out. In areas where conditions permit, regular health check-ups mainly consisting of dynamic electrocardiogram, cardiac ultrasound, and magnetic resonance imaging should be conducted to assess cardiac health and prevent sudden death, thereby achieving the goals of disease prevention and control and health promotion.

For relatives of people who died suddenly due to genetic heart gene mutations, measures can be taken from two aspects: medical monitoring and lifestyle intervention [23,24]. In terms of medical monitoring, comprehensive cardiac examinations such as electrocardiogram and echocardiogram should be performed regularly, and the examination frequency should be increased for high-risk relatives. Dynamic electrocardiogram monitoring should be carried out to detect arrhythmias that are easily missed in conventional examinations. Cardiac magnetic resonance imaging should be performed to detect potential heart diseases at an early stage. Gene testing should be actively recommended to determine whether they carry sudden death-related gene mutations, and genetic counseling services should be provided to explain the meaning of the test results and answer family genetic questions. In terms of lifestyle intervention, personalized exercise plans should be formulated according to cardiac conditions to avoid strenuous exercise. A balanced diet is recommended, reducing the intake of high-oil, high-salt, and high-sugar foods, and appropriately increasing foods rich in magnesium and potassium. Stress management techniques such as deep breathing and meditation can be taught, and maintaining good social life and psychological state should be encouraged to reduce psychological stress.

5. Conclusion

This study identifies multiple harmful mutations in genetic heart disease-related genes (DMD, SIDT1, CSRP3, and DSG2) in three cases of Yunnan unexplained sudden death from a single family. These

mutations are predicted to be pathogenic and are associated with conditions such as long QT syndrome, dilated cardiomyopathy, hypertrophic cardiomyopathy, and arrhythmogenic right ventricular cardiomyopathy. The findings suggest that genetic heart disease mutations are highly likely to be the underlying cause of death in these cases. Whole exome sequencing proves to be an effective tool for etiological exploration, and the results highlight the importance of genetic screening, counseling, and long-term cardiac monitoring for at-risk family members and populations in affected areas.

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Disclosure statement

The authors declare no conflict of interest.

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