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Expert consensus on genetic diagnosis of sudden unexplained death

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Abstract

Sudden unexplained death (SUD) is the sudden death of an individual with no discernible cause despite a thorough investigation, clinical history review, and complete autopsy with histological examination and toxicological analysis. The causes of death in these cases are complex, making their determination challenging and disputes recurring. In recent years, genetic diagnostic evidence has emerged as a new approach for determining the cause of death in such cases. However, guidelines and expert consensus for such investigations remain lacking in China. The present study proposes a guideline to integrate the opinions of domestic experts in forensic medicine and related fields. It provides systematic recommendations for key aspects, including forensic investigation, specimen collection, and laboratory analysis. In addition, it introduces postmortem genetic testing based on high-throughput sequencing, together with a series of recommendations for variant pathogenicity interpretation, to provide supporting evidence for cause-of-death determination in SUDs and to promote standardization of forensic investigations in such cases. The present study also highlights molecular autopsy in SUDs, including contemporary American College of Medical Genetics and Genomics (ACMG)-compatible molecular methodology that provides a clinically meaningful diagnostic yield. Its major value extends beyond postmortem diagnosis and supports family-based preventive genomic medicine.

INTRODUCTION

This article is based on a study first reported in the *Chin J Forensic Med*, 2026, Vol. 41, No. 5: 537-544[1].

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In forensic practice, many sudden deaths remain unexplained even after routine autopsy, histopathological examination, and toxicological analysis. The incidence of sudden unexplained death (SUD) varies across countries and regions, owing to differences in definitions, study populations, statistical methods, and other factors[2]. Internationally, nearly 30% of sudden deaths in young individuals remain unexplained after conventional autopsy[3]. In China, large-scale retrospective autopsy studies from different regions indicate that SUD accounts for 12.1% to 15.1% of all sudden cardiac deaths, with an estimated overall autopsy rate of 3‰-8‰[4-6]. Given the enormous population base, the absolute number of forensic autopsies and SUD cases remains substantial. Most of these cases involve underlying conditions such as inherited arrhythmias or early-stage cardiomyopathies. These conditions may be associated with QT prolongation, QT shortening, Brugada syndrome (BrS)-like electrocardiographic changes, or a history of unexplained syncope, all of which can be identified during life but leave no detectable morphological abnormalities at autopsy. Consequently, the heart typically appears structurally normal on gross examination, and conventional histopathological analysis often fails to reveal a lethal lesion[7-9]. When deaths occur in special settings such as hospitals or custodial facilities, and are accompanied by factors like minor trauma, physical restraint, or psychological stress, the lack of sufficient morphological evidence to establish the cause of death can easily undermine the scientific and professional credibility of the forensic experts' opinion. Consequently, determining the cause of death poses a significant challenge and necessitates seeking additional evidence beyond morphological findings to support the determination.

Genetic evidence may provide an emerging approach to cause-of-death determination in such cases. Recent evidence supports the value of molecular autopsy in SUD, with pathogenic variants detected in a substantial proportion of cases[10], and functional candidate variants identified by extending the analysis to untranslated regions[11]. SUD cases are significantly enriched for pathogenic and likely pathogenic variants compared with populations of similar genetic background[12]. Postmortem genetic testing can identify the pathogenic and likely pathogenic variants associated with sudden death, revealing a genetic susceptibility and thereby quantifying the genetic contribution to death. The advancement of whole exome sequencing (WES) and whole-genome sequencing (WGS) has made genetic testing of postmortem samples technically feasible. Such testing is referred to as postmortem genetic testing or molecular autopsy. In accordance with the American College of Medical Genetics and Genomics/Association for Molecular Pathology (ACMG/AMP) guidelines (https://clinicalgenome.org/working-groups/sequence-variant-interpretation/), the pathogenicity of loss-of-function and gain-of-function variants can be systematically assessed through collaborative analysis by forensic and genetics experts. The definitive identification of death-related genetic variants through the integration of human genetics and functional genomics offers an objective basis for a genetic determination of the cause of death.

In accordance with evidence-based medical standards, the latest domestic and international research findings from the past decade were incorporated. The "Expert consensus on genetic diagnosis of sudden unexplained death" was developed in the present study, proposed as a guideline to follow medicolegal practice in the context of SUD. This consensus aims to standardize forensic autopsy procedures and assessment of such deaths by providing systematic guidance on SUD investigation, pathology specimen collection, forensic laboratory analysis, and genetic diagnosis. The present study is expected to serve as a guideline for forensic and judicial practice in the context of SUD. We hereby present the principles, types of investigation, specimen collection, laboratory and personnel conditions, DNA sequence analyses, interpretation of genetic variants, and the expert report.

CONSENSUS DEVELOPMENT

The Expert Panel was convened to develop a consensus on the genetic diagnosis of SUD. The panel comprised forensic pathology experts in forensic medicine from China. A Steering Committee was established to lead the project, define the aims and topics, and determine the methodology.

The process unfolded in the following phases: (1) a literature review to identify key topics and unmet needs; (2) a kick-off meeting to organize the process and discuss content; (3) drafting of statements based on the literature and expert input; (4) two rounds of online surveys to collect the Expert Panel's opinions and achieve consensus; (5) analysis of results and discussion of conclusions; and (6) manuscript writing.

A systematic literature search was performed in PubMed, Web of Science, China National Knowledge Infrastructure (CNKI), and Wanfang databases for studies published in English or Chinese up to December 2025. Search terms included "sudden unexplained death", "molecular autopsy", "postmortem genetic testing", "variant interpretation", and "forensic pathology". Retrieved articles were screened by title and abstract and considered if they were relevant to the definition, diagnosis, and management of SUD. Available guidelines and consensus statements were also considered.

An evidence- and consensus-based modified Delphi approach was used[13]. Experts rated each statement using a five-point Likert scale: 1 = strongly agree; 2 = agree; 3 = neutral; 4 = disagree; and 5 = strongly disagree. Consensus was predefined as > 75% agreement (scores 1-2) or disagreement (scores 4-5). Consensus was reached in two rounds of consultation. In the first round, panelists answered the online survey and could add open-text comments. An anonymized summary of results was provided before the second round. Statements that did not reach consensus in the first round were rephrased and voted on again. All panelists completed both rounds, resulting in 100% participation. Consensus was ultimately reached for all statements. All responses were analyzed in aggregate while preserving anonymity. The final draft was reviewed and approved by all panel members.

GENERAL PRINCIPLES

Comprehensive principle

A comprehensive investigation, systematic autopsy, specimen collection, and forensic laboratory analysis should be conducted.

Objective principle

Autopsy and forensic laboratory analysis should be performed in accordance with applicable national and ministerial standards in the People's Republic of China[14], including but not limited to Forensic Medicine Guidelines for Classification and Identification of Causes of Death (GA/T 1968-2021); Forensic Medicine General Technical Specifications for Examination of Deaths (GA/T 147-2019); Forensic Medicine Autopsy Specification (SF/Z JD0101002-2015); Forensic Medicine Specifications for Examination of Sudden Deaths (GA/T 170-2019); Forensic Medicine Specifications for Examination of Neonate Cadavers (GA/T 151-2019); Specifications for collection, preservation and delivery of forensic biologic samples (GA/T 1162-2014). Operational procedures and analytical processes should be carried out objectively and accurately, with experimental procedures and parameters being kept stable and consistent, ensuring that results are reproducible and verifiable.

Exclusion principle

This consensus applies to sudden deaths that remain unexplained after routine autopsy, histopathological examination, and toxicological analysis. Its application requires the prior exclusion of known common causes of death, including but not limited to mechanical injury, mechanical asphyxia (including aspiration asphyxia), poisoning by toxic substances or drugs, burn fatality, hypothermia, and electrocution.

FORENSIC INVESTIGATION

A detailed investigation of the circumstances, medical history, and clinical records is crucial for reconstructing the death process. The investigation should include, but not be limited to, the following:

General information

Every effort should be made to collect the following information from family members, relatives, friends, witnesses, medical personnel, or other individuals:

• The deceased's age, sex, ethnicity, place of origin, occupation, education, marital status, economic status, and health condition;

• Lifestyle, sleep patterns, physical activity, recent psychosocial status, and financial or emotional state, which may have contributed to the death;

• Medical history (e.g., surgical history, history of cardiovascular interventional procedures, chronic diseases, mental disorders), family history (e.g., inherited arrhythmias, family history of sudden death), recent history of blood transfusion, bone marrow or organ transplantation, drug abuse history (including drug type, dosage, and duration);

• Clinical symptoms and laboratory findings (e.g., electrocardiographic abnormalities, myocardial enzyme abnormalities, inflammatory markers);

• Previous relevant examination results (e.g., medical imaging, biochemical, and pathological findings).

Detailed forensic investigation

The death process, time of death, place of death, and scene findings should be thoroughly investigated. The investigation should establish the time and place of symptom onset, accompanying symptoms and signs, and whether medical treatment or resuscitation was provided. Particular attention should be directed to any precipitating factors for sudden death.

Scene investigation

Document where the body was found, the body's position and condition, any evidence of a struggle and associated marks or bloodstains, signs of electrocution or burns, the presence of drugs or toxic substances, and the quantity and distribution of vomitus or excreta. Special attention should be given to the body's location and the bloodstain pattern.

Recommendation 1: Antemortem data, including medication history, electrocardiograms, myocardial enzymes, and inflammatory markers, should be systematically collected.

Recommendation 2: The investigation should focus especially on any exposure to potential triggers of sudden deterioration, such as acute emotional stress.

Recommendation 3: The family history should be thoroughly explored, with particular emphasis on significant diseases among relatives.

SPECIMEN COLLECTION

Specimens for toxicological analysis

Essential specimens for toxicological analysis include blood (preferably cardiac or peripheral blood), gastric contents, and urine. Besides, any particular trait at the scene (e.g., remnants of the amount of food or beverage consumed, vegetal remains) should be taken into account. Blood contaminated or diluted by body cavity fluids should be avoided. In cases of prolonged survival after injury, hematomas associated with the trauma, such as epidural or subdural hematomas and hematomas around fractures, should be collected. If gastric contents are unavailable, gastric tissue may serve as a substitute. For cases where urine cannot be obtained due to an empty bladder, bladder washings may be used, or the entire bladder can be submitted as a specimen. Vitreous humor, liver tissue, and bile should be collected whenever feasible. In deaths of uncertain cause, additional specimens such as brain, kidney, and hair may be taken as needed. Leftover food, beverage, or vegetal remains should be maintained in accordance with the cold chain. All specimens should be kept frozen and submitted for analysis as promptly as possible. If the deceased received medical care before death, specimens collected during medical procedures, including blood, urine, cerebrospinal fluid, vomitus, and gastric lavage fluid, should be retrieved in a timely manner for toxicological analysis.

Specimens for molecular analysis

It is recommended that blood and/or tissue specimens be collected and stored, together with detailed clinical information, for potential postmortem genetic diagnosis. Specimen collection and processing should be carried out by a forensic pathologist. Before collection, an informed consent form must be signed by the commissioning party or a family member, and a specimen collection record form should be completed to document the entire processing procedure.

Molecular specimen collection

To obtain high-quality genomic DNA, well-preserved tissue is preferred, followed by tissue without evident degradation. It is recommended that whole blood and at least two types of tissue be collected for DNA extraction. For blood collection, ethylenediaminetetraacetic acid (EDTA) anticoagulant tubes (e.g., purple-top tubes) are preferred. Blood samples should be refrigerated at 4 °C immediately after collection, and repeated freeze-thaw cycles should be avoided. For tissue collection, fresh myocardium is preferred, while skeletal muscle, liver, or kidney may also be considered. Each tissue sample should be approximately 1 cm^3 in size and placed in separate tubes to avoid cross-contamination. Specimens should be kept at 4 °C only if genomic DNA is to be extracted within 72 h; otherwise storage at -80 °C is preferred. When fresh tissue is not available, formalin-fixed, paraffin-embedded (FFPE) specimens of myocardium or other solid organs can be used. When only an external examination is performed, skin, mucosal tissue, and blood can be collected for genomic DNA extraction.

Molecular specimen storage and transport

Appropriate containers should be used for collection and storage. After collection, each container should be clearly labeled with the sample ID, type, case number, and collection date. Samples must be packaged and transported in compliance with applicable requirements and should be dispatched as promptly as possible. For long-distance transport, refrigeration is required. Specimen quality directly determines the reliability of downstream DNA sequencing. The sample should be evaluated before sequencing, including the extent of necrosis, hemorrhage, and any prior processing that may compromise sequencing, such as decalcification with nitric acid solutions. Genomic DNA should be extracted as soon as possible and stored appropriately. When immediate extraction is not possible, tissue and blood samples should be cryopreserved together, avoiding repeated freeze-thaw cycles. If DNA is to be extracted from formalin-fixed tissue or FFPE samples, the fixative should be free of heavy metal ions, and paraffin should be stored at a temperature below 32 °C.

Recommendation 4: In addition to routine collection of myocardial tissue and blood for genetic analysis, parallel testing of specimens from ectoderm-derived tissues, such as skin and oral mucosa, should be considered when somatic or tissue-specific mosaicism is suspected, in order to exclude such conditions. For decedents with a history of blood transfusion, bone marrow transplantation, or organ transplantation, myocardial tissue should be recommended as the preferred specimen for genetic analysis.

Recommendation 5: Genetic analysis is recommended for decedents with a definite family history of sudden death.

Recommendation 6: When feasible, DNA from the decedent's biological parents should also be collected to identify *de novo* variants.

FORENSIC LABORATORY ANALYSIS

Facility and environment

The laboratory shall operate in accordance with the Specification for the Management of Forensic Biology Identification Laboratory (SF/T 0069-2020) and comply with all applicable requirements of biosafety regulations. The sequencing area must be kept clean and maintained under controlled temperature and humidity conditions, with adequate vibration isolation and appropriate lighting. Environmental temperature and humidity should be monitored according to instrument requirements. Each area should be equipped with dedicated instruments and devices. Single-use consumables must not be shared across different areas. An internal system should be established to document reported gene variants and clinical diagnoses, facilitating the analysis of genotype-sudden death correlations.

Personnel qualifications

Personnel must have a background in forensic medicine and be certified in forensic pathology through professional training and examination, in compliance with the registration and management regulations issued by the Ministry of Justice or the Ministry of Public Security. Data analysts should possess systematic knowledge of human genetics and be proficient in high-throughput sequencing and forensic genetic interpretation. Personnel responsible for variant interpretation should possess sufficient medical knowledge, be familiar with the clinical phenotypes of related diseases, and regularly review the latest guidelines and standards for human genome variant interpretation issued by relevant academic societies. The laboratory shall establish a comprehensive training and assessment system in accordance with Accreditation Criteria for the Competence of Forensic Units (CNAS-CL08). Personnel should undergo regular competency assessments and receive continuing education to ensure their professional competence remains in compliance with accreditation criteria. To facilitate the applicability of this consensus in countries where institutional frameworks differ, such as the United States, experts in DNA testing, pathologists with specialty training in molecular pathology, or other physicians with specialty training in genetics may also be considered to meet the personnel qualification requirements for variant interpretation.

Equipment, reagents, and consumables

In accordance with the Accreditation Criteria for the Competence of Forensic Units (CNAS-CL08), the laboratory should establish and strictly document procedures for the use, maintenance, and verification or calibration of instruments and equipment. Key instruments and equipment should undergo periodic verification or calibration, with satisfactory results documented and retained on file. Their traceability must meet forensic investigation requirements. All reagents must be properly stored under specified conditions and used before their expiration dates. Key consumables, including centrifuge tubes and pipette tips, must be verified to be free of nucleases and polymerase chain reaction (PCR) inhibitors. The use of filter tips is recommended to prevent cross-contamination. Mixing reagent components from different lots is prohibited, as it compromises the reliability and traceability of results and the forensic integrity of the evidence chain.

Testing requirements

The method for genomic DNA extraction should be chosen according to the sample type, and all steps must be performed exactly as specified in the instructions. DNA from fresh samples should undergo quality assessment, with a total DNA input of no less than 200 ng as measured by Qubit fluorometry and an A260/A280 ratio greater than 1.8. For FFPE or decomposed samples, a comprehensive evaluation should be performed, taking into account DNA concentration, integrity, fragment size distribution, and intended fragment length. Electrophoresis should be performed when necessary, and bands should preferably show no obvious degradation. Ideally, a DNA Integrity Number (DIN) ≥ 7 and the fragment-size distribution of a clear high-molecular-weight peak (e.g., > 10 kb) without significant smearing is considered high quality. However, these thresholds serve as general requirements, and the final decision on whether to proceed with library preparation should be comprehensively considered based on the significance of the case. In critical cases, next-generation sequencing (NGS) library preparation should still be attempted even when the DIN value is suboptimal.

Quality control

The laboratory should establish standard operating procedures and record forms that cover the entire workflow. Procedures must be strictly followed; unauthorized modifications are strictly prohibited. Each batch should include at least one positive control (e.g., NA12878) and one negative control (in which DNA is replaced with deionized water). Internal control samples must be processed in parallel with the test samples throughout the entire workflow, including nucleic acid extraction and amplification. The expected genotype or variants must be detected in the positive control, while no signal should be observed in the negative control. If any control fails, a test report shall not be issued. The cause should be promptly investigated, and the test should be repeated when necessary. If the laboratory operates two or more systems (e.g., different instruments or reagents), comparisons must be performed to demonstrate the consistency of results across systems. For manual procedures, inter-operator comparisons should be conducted. Such comparisons should be performed at least once per year and include samples with different variant types [e.g., single nucleotide variants (SNVs), insertions/deletions] as well as negative controls.

Recommendation 7: When high-quality DNA cannot be easily extracted because of the conditions under which the body was preserved, NGS library preparation may still be worth attempting.

Sequence alignment and analysis

The standard analytical workflow includes the following steps: FASTQ processing, sequence alignment, variant calling [generation of Variant Call Format (VCF) files], variant annotation, and interpretation of sequence variants. All bioinformatics software must undergo analytical validation, and the parameters must meet the requirements for result reporting. Whenever software is replaced or updated, validation should be repeated. The versions of all software and reference sequences used in the analytical pipeline must be documented to ensure accuracy and subsequent traceability.

WES, which captures coding regions (the exome) after an enrichment procedure, or WGS, which covers the entire genome, may be chosen as the sequencing strategy. Alternatively, targeted sequencing of high-risk genetic loci for sudden death may be used. A well-recognized reference genome, such as the Genome Reference Consortium human genome (build 38) (GRCh38) assembly, should be employed for alignment. The recommended minimum requirements are as follows: an average sequencing depth of ≥ 50×, genome coverage of ≥ 90% at ≥ 20× depth, and an average read length of 200-500 bp. The analyzed regions should include at a minimum the target gene's consensus coding sequence (CCDS) and 10 bp of upstream and

downstream flanking sequence. During initial consistency screening, the following should be confirmed: accurate sex determination; familial relationships consistent with the genetic data; a contamination level below 3%; and percentages of homozygous, heterozygous, and mosaic variants consistent with those of the other samples in the run. It is important to apply filtering cutoffs; a heterozygous allele fraction of less than 20% is typically considered sequencing noise.

VARIANT NOMENCLATURE, COVERED GENES, AND INTERPRETATION

The rapid advancement of high-throughput sequencing has brought both unprecedented opportunities and challenges to the field of genetic counseling. To support clinicians and genetic counselors, the ACMG/AMP developed guidelines for variant interpretation, which provide a widely recognized framework for assessing variant pathogenicity[15]. Variant pathogenicity and the strength of supporting evidence should be assessed by consulting the ACMG/AMP guidelines and incorporating the refined criteria issued by the Clinical Genome Resource (ClinGen) Sequence Variant Interpretation Working Group and the Association for Clinical Genomic Science (ACGS)[16-21]. These findings should then be integrated with the individual's clinical presentation and familial inheritance pattern, and a comprehensive analysis should be performed to ultimately determine the disease-associated variants.

Variant pathogenicity assessment is based on two principal lines of evidence. One is genetic evidence, which evaluates whether the variant found in the decedent is coincidental. The other is functional or predictive evidence, which examines whether the variant has biological significance. Accordingly, variant interpretation should be conducted collaboratively by a genetics specialist and a forensic expert to integrate autopsy findings, toxicology results, and other relevant information for a comprehensive determination. Notably, the ACMG/AMP interpretation framework is relatively complex and has undergone multiple updates since its initial release in 2015; interpreting variants of uncertain significance still requires a cautious approach. The various annotation resources involved, including population frequency, pathogenicity prediction scores, and disease associations, are scattered across databases, making retrieval extremely cumbersome.

Variant nomenclature

Gene names must follow the gene nomenclature guidelines recommended by the Human Genome Organization (HUGO) (http://www.genenames.org). Transcripts should be selected based on the Matched Annotation from the NCBI and EMBL-EBI (MANE) transcript set (https://www.ncbi.nlm.nih.gov/refseq/MANE/). Variant nomenclature should comply with the recommendations of the Human Genome Variation Society (HGVS) and must include the MANE Select transcript (https://ftp.ncbi.nlm.nih.gov/refseq/MANE/), the coding DNA position, and the nucleotide change, for example, *BRCA1* NM_007294.4:c.5444G>A.

Covered genes and regions

A considerable proportion of SUDs are due to cardiac arrhythmia, making sudden cardiac death (SCD)-related susceptibility genes a focus of special interest[22]. The list of key genes [Table 1] should be consulted. In addition, attention should be directed to other genetic disorders that may lead to sudden death, such as sudden unexpected death in epilepsy (SUDEP), hereditary aortic diseases, and metabolic disorders.

INTERPRETATION OF GENETIC VARIANTS

Variant pathogenicity is determined by the aggregate of multiple lines of evidence. The supporting and exclusionary evidence described in the following sections should not be used in isolation.

Supporting evidence for loss-of-function variants

Variants that directly abolish gene function are considered important genetic findings. Loss-of-function variants are strong evidence for disruption of gene function. These variants include nonsense variants, frameshift variants, canonical splice site variants at the ±1 or ±2 positions, start codon variants, and single-exon or multi-exon deletions.

Table 1. Genes for consideration in postmortem genetic testing for inherited arrhythmia syndromes in sudden unexplained death

Gene	Associated disease	Gene-disease validity	Inheritance	Recommended transcript
ALG10B[28]	LQTS	Limited	AD	NM_001013620.4
CACNA1C[29]	LQTS	Moderate	AD	NM_000719.7
CALM1[30]	LQTS	Definitive	AD	NM_006888.6
	CPVT	Moderate		
CALM2[31]	LQTS	Definitive	AD	NM_001743.6
	CPVT	Moderate		
CALM3[32]	LQTS	Definitive	AD	NM_005184.4
	CPVT	Moderate		
CASQ2[33]	CPVT	Definitive	AR	NM_001232.4
		Moderate	AD	
CAV3[34]	LQTS	Limited	AD	NM_033337.3
KCNE1[35]	LQTS	Limited	AD	NM_000219.6
KCNH2[36]	LQTS	Definitive	AD	NM_000238.4
	SQTS	Definitive		
KCNJ2[37]	LQTS	Limited	AD	NM_000891.3
	SQTS	Moderate		
KCNQ1[38]	LQTS	Definitive	AD	NM_000218.3
	SQTS	Strong		
RYR2[39]	CPVT	Definitive	AD	NM_001035.3
SCN5A[40]	BrS	Definitive	AD	NM_000335.5
	LQTS	Definitive		
SLC4A3[41]	SQTS	Moderate	AD	NM_005070.4
TECRL[42]	CPVT	Definitive	AR	NM_001010874.5
TRDN[43]	LQTS	Strong	AR	NM_001256021.2
	CPVT	Definitive		

Recommended transcripts generally correspond to MANE Select transcripts. For *TRDN*, NM_001256021.2 (MANE Plus Clinical) was selected because it represents the clinically relevant transcript for cardiac disease reporting. AD: Autosomal dominant; AR: autosomal recessive; BrS: Brugada syndrome; CPVT: catecholaminergic polymorphic ventricular tachycardia; LQTS: long QT syndrome; SQTS: short QT syndrome.

1. Classifying such variants as pathogenic or likely pathogenic requires confirmation that loss of function is an established disease mechanism for the gene and that the variant is consistent with the inheritance pattern of the disease.

2. Particular caution should be exercised when interpreting loss-of-function variants located near the 3' end of the transcript, as much of the gene's function may still be preserved.

3. When variants are predicted *in silico* to cause exon skipping without disrupting the reading frame, interpretation should be approached with caution, and additional functional evidence should be obtained before final interpretation.

4. Caution is required when interpreting variants in genes with multiple transcripts; special attention must be given to how many transcript isoforms are affected.

Supporting evidence for missense and other non-truncating variants

SNVs are the most common form of variation in the human genome and are frequently detected in the molecular autopsy of SUD[23,24]. For SNVs that result in a nonsense change, affect a canonical splice site at the ±1 or ±2 positions, or alter the start codon, the guidance in Section "Supporting evidence for loss-of-function variants" should be followed. For SNVs with other consequences, the following guidance applies:

Genetic evidence

1. Caution is warranted when interpreting SNVs that lie at exon-intron boundaries. The use of *in silico* prediction tools, such as SpliceAI, is encouraged to evaluate potential splicing abnormalities[25].

2. When parental DNA is available, *de novo* variant calling can be performed, and the *de novo* variants serve as supporting evidence. It should be noted that confirming legal parentage is not sufficient, as egg donation, surrogacy, and similar situations can lead to non-biological parentage and result in the misclassification of a *de novo* variant.

3. Repeated detection of the same variant in unrelated sudden-death cases, combined with its low frequency in reference populations, may serve as supporting evidence.

4. Co-segregation of a recessive variant with the phenotype in the decedent's family provides supporting evidence.

5. For a recessive disorder, identification of the variant under evaluation in trans with a known pathogenic variant provides supporting evidence for its pathogenicity.

6. For a variant that is absent or rare in large genome databases, such as the 1000 Genomes Project, its identification in the homozygous state serves as supporting evidence. If the variant is heterozygous, interpretation should be approached with caution.

7. When multiple computational predictions [e.g., Rare Exome Variant Ensemble Learner (REVEL), AlphaMissense] indicate a deleterious effect on the gene, this can be considered supporting evidence[26,27]. It should be noted that results from different computational models should be counted as a single piece of evidence.

Functional evidence

1. If a nucleotide variant leads to the same amino acid alteration that has been established as pathogenic, this provides supporting evidence.

2. For ion channel variants, a significant electrophysiological alteration confirmed by manual or automated patch-clamp experiments can be regarded as supporting evidence.

3. A significant reduction in RNA or protein levels confirmed by *in vitro* experiments provides supporting evidence.

4. When a variant is located within a frequently mutated region and/or within a well-characterized functional domain (e.g., an active site of an enzyme), this can be considered supporting evidence.

Table 2. Summary of key recommendations for the genetic investigation of sudden unexplained death

Stage	Recommendation	Key message
Forensic Investigation	1	Collect antemortem clinical data systematically
	2	Evaluate potential triggers of sudden deterioration
	3	Investigate family history thoroughly
Specimen Collection	4	Consider ectoderm-derived tissues when mosaicism is suspected, and prioritize myocardial tissue in decedents with a history of transfusion or transplantation
	5	Perform genetic analysis in decedents with a definite family history of sudden death
	6	Collect parental DNA when feasible to identify *de novo* variants
Forensic Laboratory Analysis	7	Consider NGS library preparation when high-quality DNA cannot be obtained
Interpretation of Genetic Variants	8	Confirm parentage for suspected *de novo* variants
	9	Validate important variants by Sanger sequencing

NGS: Next-generation sequencing.

5. When a new missense variant occurs at the same amino acid position as a previously identified pathogenic missense variant, this can be considered supporting evidence. For example, if Arg156His is an established pathogenic variant, the finding of Arg156Cys serves as supporting evidence.

Exclusionary evidence

Genetic evidence

1. A variant with a frequency greater than 5% in genome databases, such as the 1000 Genomes Project, generally serves as exclusionary evidence. However, for certain recessive disorders or disorders with reduced penetrance, pathogenic variants may occur at relatively high population frequencies, and specific frequency thresholds may be appropriate.

2. When multiple computational predictions (e.g., REVEL, AlphaMissense) indicate a benign effect on the gene, this can be considered exclusionary evidence[26,27]. It should be noted that results from different computational models should be counted as a single piece of evidence.

Functional evidence

When a recessive (homozygous), dominant (heterozygous), or X-linked disorder identified in an adult decedent is expected to severely impair survival, this serves as exclusionary evidence. However, such evidence should not be used in isolation. The interpretation must incorporate penetrance, phenotype, inheritance pattern, clinical history, and autopsy findings.

Recommendation 8: If a variant is suspected to be *de novo*, parentage testing involving the decedent and the presumed parents is highly recommended to confirm both maternity and paternity.

Recommendation 9: Sanger sequencing is necessary to confirm important variants identified by high-throughput sequencing.

The key recommendations of this consensus are summarized in Table 2.

EXPERT APPRAISAL REPORT

Laboratory report

Methodology description

The report should provide a detailed description of the experimental methods employed, the types of variants, any challenges encountered during the procedure, and the inherent limitations of the methods used. The methodology description should include both the library preparation (such as Multiplex PCR, target capture, and whole-genome input) and the nucleic acid detection strategies (such as Sanger sequencing, NGS, microarray analysis, and genotyping). This information will inform the determination of the cause of death and help decide whether further experiments are necessary.

Variant reporting

Variants should be selected for reporting based on Sanger sequencing confirmation and pathogenicity interpretation. The report must include all essential elements, such as structured results, interpretation, references, and methodology. A limitation may also be included regarding common issues encountered during the procedure, including sample decomposition, contamination, or mix-up.

Variant classification

Variants should be described and classified according to the standard nomenclature recommended by the HGVS (https://hgvs-nomenclature.org). The variant description should include gene name, mode of inheritance, nomenclature at both the nucleotide (genomic and transcript) and protein levels, affected exon, and variant classification. For each variant listed in the report, it should be noted whether it has been previously reported in the literature or in population databases. At the end of the report, the references for variant classification should be provided.

Expert report

The expert report should be formulated based on the ACMG/AMP classification and a comprehensive analysis of the case details and clinical data. The expert opinion can be expressed as one of the following categories: supportive, cannot be excluded, or exclusionary.

• Supportive: This indicates that a pathogenic or likely pathogenic variant has been identified, and the functional abnormality of the associated gene is highly correlated with the decedent's clinical phenotype or the mechanism of death. Statement example: the variant *KCNQ1* NM_000218.3:c.1032G>A fulfills the ACMG/AMP criteria PS3, PS4, PM2, PP3, and PP4 and is classified as pathogenic, thereby providing supporting evidence that this variant is the genetic cause of the SUD.

• Cannot be excluded: This indicates that a variant of uncertain significance (VUS) has been identified, but there is currently insufficient evidence to support either its pathogenicity or benignity. Statement example: the variant *KCNQ1* NM_000218.3: c.958C>T fulfills the ACMG/AMP criteria PS4, PM1, PM2, and PP3 and is classified as a VUS; therefore, the risk of sudden death cannot be excluded.

• Exclusionary: No pathogenic or likely pathogenic variants associated with any known deadly disease were identified within the scope of the analysis. Statement example: after ACMG/AMP classification of the genomic variants, no definitive pathogenic or likely pathogenic variant that could explain the death was identified within the scope of the analysis.

CONCLUSION

Advances in genomics and the rapid development of molecular autopsy have provided feasible approaches to addressing the challenges of cause-of-death determination in SUDs.

The present study provides systematic recommendations for the investigation of SUD, covering case investigation, specimen collection and storage, laboratory procedures, sequencing data analysis, and variant interpretation. It particularly emphasizes that experts with both genetic and forensic expertise should assess variant pathogenicity in accordance with the variant interpretation guidelines issued by ACMG/AMP, ClinGen, and ACGS. Important variants should be confirmed by Sanger sequencing. This consensus is expected to be updated dynamically to keep pace with forensic and technological advances, thereby ensuring more reliable scientific evidence for the genetic diagnosis of SUD.

DECLARATIONS

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Authors' contributions

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Availability of data and materials

Not applicable.

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During the preparation of this manuscript, the AI tool DeepSeek (version V3, released 2024-12-26) was used solely for language editing. The tool did not influence the study design, data collection, analysis, interpretation, or the scientific content of the work. All authors take full responsibility for the accuracy, integrity, and final content of the manuscript.

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Conflicts of interest

Cong B is the Editor-in-Chief of *Legal Medicine Research*; Chen T is the Executive Editor-in-Chief of *Legal Medicine Research*; Chen F, Guan D, Guo Y, Huang P, and Liu Q are the Editorial Board Members of *Legal Medicine Research*. None of the above authors are involved in any aspect of the editorial process for this manuscript, including reviewer selection, manuscript handling, and decision-making. The other authors declare that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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