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Molecular Autopsy for Sudden Cardiac Death: Current State and Considerations

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Abstract

Purpose of Review The study of the genetic basis of sudden cardiac death has been impacted by advances in sequencing technology, gene variant interpretation, and additional evaluation into the ideal clinical approach to assessing cause of death and the medical and psychological risks of surviving family members. This short review aims to summarize recent publications as well as provide context for future directions for the care of families faced with sudden cardiac death.

Recent Findings The molecular autopsy is a critical method in determining cause of death, particularly in autopsy-negative sudden cardiac death. Although the cost and availability of exome sequencing have improved in the past few years, its utility in the postmortem evaluation has not significantly improved yield. This is primarily due to the increase in genetic variants detected through this sequencing modality and the difficulty in interpreting these results. Medical examiners are essential in the proper identification and referral of surviving relatives to multidisciplinary clinics that include an expert cardiologist, cardiac genetic counselor, and clinical psychologist that can provide appropriate education, clinical evaluation, and psychological support.

Summary Future studies need to build on the improvement in variant interpretation and appropriate education and support of medical examiners. This will ensure family members are referred to appropriate providers such that they can receive medical and psychological care that will improve overall health outcomes and reduce the incidence of subsequent sudden cardiac death in the family.

Keywords Sudden cardiac death · Postmortem genetic testing · Variant interpretation · Molecular autopsy · Multidisciplinary care · Genetic counseling

Introduction

Sudden cardiac death (SCD) is a common and tragic event that occurs frequently in the population, often in otherwise healthy-appearing individuals [1, 2]. SCD is defined as the unexpected death of an individual within 1 h of the onset of symptoms related to an underlying cardiac disease. In the US alone, the prevalence of SCD is estimated at 300,000 to 400,000 deaths per year [2] and accounts for 15–20% of all deaths internationally [3].

In older individuals, the causes of SCD are primary coronary artery disease (CAD) and acute myocardial infarction [2]. When considering the younger population (ages 1–40 years), the incidence of SCD is estimated at 2.8 per 100,000 person-years [4], with lower frequency in children. More specifically, 19% of deaths in children ages 1 to 13 years are attributed to SCD while the incidence increases to 30% in individuals between 14 and 21 years of age [2]. In the 1–40 year age range, the highest incidence of SCD is seen in individuals over the age of 30 while the highest number of unexplained sudden death is observed in persons 16–20 years old [5•, 6].

While rare, SCD in the young is a tragic and traumatic event, particularly since these individuals are previously understood to be healthy. Surviving family members are often left with questions about the cause of this event and the potential implications for other relatives. Postmortem evaluation can be considered to

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address these questions. Traditional forensic autopsies are performed by medical examiners (ME) or forensic pathologists and can include gross and histological examination as well as toxicology studies. Along with traditional autopsy, molecular genetic testing can be integrated into the postmortem evaluation. This additional testing, referred to as a molecular autopsy, is generally considered in cases when the traditional autopsy reveals a structurally normal heart and does not uncover any other, non-cardiac related, cause of death [7]. Molecular autopsies are typically performed through sequencing of DNA extracted from whole blood or tissue (cardiac or other) postmortem. There has also been limited success with formalin-fixed samples [8, 9]. In the best-case scenario, a clinically actionable gene variant is discovered that correlates either with the decedent's clinical history or with cardiac findings in surviving relatives. This testing outcome allows for cascade screening in living family members to assess potential risk for cardiac disease and allow for hopeful prevention of SCD.

Common Genetic Causes of SCD

While coronary artery disease remains the most common cause of SCD in the population, deaths in the younger population are more commonly due to inherited cardiac disease [2, 6]. Many of these diseases are structural in nature and thus can be observed on postmortem evaluation, including hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM), arrhythmogenic right ventricular cardiomyopathy (ARVC), and left ventricular non-compaction (LVNC) [5•, 10, 11]. Other observable causes of sudden death include myocarditis, congenital heart defects, and aortic aneurysm [2, 12, 13]. However, in 25–40% of autopsies, there is no visible cause of death [5•, 13]. In addition to epilepsy [14], cardiac channelopathy syndromes such as long QT syndrome (LQTS), catecholaminergic polymorphic ventricular tachycardia (CPVT), and Brugada syndrome (BrS) are thought to make up a good portion of these unexplained sudden deaths, as they do not involve structural changes to the heart muscle. Postmortem genetic testing completed in cases of sudden unexplained death (SUD) can provide an underlying genetic etiology about 30% of the time, with the common genes associated being *KCNQ1*, *KCNH2*, *SCN5A*, and *RYR2*, as well as other genes in lower frequency [5•, 10, 15].

The Current State of the Molecular Autopsy

Genetic testing technologies have advanced rapidly over the past decades. Next-generation sequencing (NGS) technology has allowed for the expansion of gene panels and unbiased interrogation of the genome. Given the cost efficiency and workflow advantages of high throughput sequencing, it is common for laboratories to utilize NGS over traditional Sanger sequencing for many of their test offerings. Clinically, the use of targeted NGS panels and genome sequencing has resulted in overall improved diagnostic yields for many Mendelian disorders [16–18] as well as for other genetically heterogeneous phenotypes [19, 20].

Genetic testing recommendations for channelopathies and cardiomyopathies are mainly phenotype driven [7]. The observation of a structurally normal heart in the setting of SCD is more consistent with a channelopathy than a structural cardiac disease such as HCM. Given this, molecular autopsies, done following a negative traditional autopsy, have primarily focused on major channelopathy genes such as *KCNQ1*, *KCNH2*, *SCN5A* (LQTS), and *RYR2* (CPVT) [7, 21–23].

Now, in the era of NGS, gene panels have expanded to include dozens to hundreds of genes (see Table 1). Gene panels can be generated either through sequence capture technology [5•, 29] or through bioinformatics filtering [5•, 25]. Target capture ensures that only selective regions of the genome are enriched and sequenced, reducing the total amount of sequencing required. Exome sequencing uses capture methods that target all the protein-coding regions (exons) of the genome. Once sequencing is complete, computational methods, known as bioinformatics pipelines, are used to identify variants and filter, annotate, and prioritize them for human analysis [34].

Determining which genes are included in gene panels is solely up to the individual clinical laboratories. Each laboratory will have their own inclusion or exclusion criteria based on their own experiences, provider preferences, and expert options. The genes included on gene panels may be limited to genes with strong disease associations or may include additional genes that are less commonly associated with disease. Moreover, they may expand to include genes associated with a different, though related, phenotype. For cardiac disease associated with SCD, it is common to see panels include genes for both channelopathies as well as cardiomyopathies [35].

Detection Rate Variability

There have been several studies over the past few years looking at detection rates of molecular autopsies for SCD (Table 1). While the majority of likely pathogenic and pathogenic sequence variants are often still found in the major channelopathy genes (*KCNQ1*, *KCNH2*, *SCN5A*, *RYR2*) [25, 29•], improvement in detection rates are observed with expanded gene panels [5•, 25, 36]. When limiting to channelopathy genes only, pathogenic or likely pathogenic variants are generally found in 14–19% of cases [5•, 24, 31]. Detection rates increase when gene panels are expanded to include cardiomyopathy genes along with channelopathy genes [5•, 10, 27, 28, 30, 32, 33].

Table 1 Recent publications assessing postmortem genetic testing in cases of sudden death in the young (excluding sudden unexplained infant death (SUID) studies). Direct comparisons cannot be drawn as studies differ by inclusion and exclusion criteria, testing methodology, and variant classification systems. Detection rate refers to percentage yield of clinically actionable variants (likely pathogenic/pathogenic)

Author/group	Year	*N*	Geographic location	Age range in years	Sequencing technology			Detection rate
					Sanger	NGS	Genes included	
Farrugia et al. [24]	2015	16	France	1–35		X	23	19%
Anderson et al. [25]	2016	32	Minnesota, USA	2–19		X	100	44%
Hellenthal et al. [10]	2016	10	Germany	19–40		X	174	30%
Bagnall et al. [5]	2016	113	New Zealand and Australia	1–35		X	59	27%
Stattin et al. [26]	2016	15	Sweden	1–35	X		6	40%
Hata et al. [27]	2016	25	Japan	18–50		X	70	20%
Nunn et al. [28]	2016	59	Europe	1–55		X	135	29%
Lahrouchi et al. [29]	2017	302	New Zealand, UK, Netherlands	71–64		X	77	13%
Rueda et al. [30]	2017	50	San Diego County, USA	2–44		X	233	28%
Cann et al. [31]	2017	46	Scotland	1–40	X		5	15%
Shanks et al. [32]	2018	25	Illinois, USA	1–40		X	99	28%
Mak et al. [33]	2019	21	China	14-39y		X	35	29%

Detection rates will also vary depending on inclusion and exclusion criteria. For example, Anderson et al. focused on a high-risk subgroup of cases with exertion-related SCD in childhood and negative traditional autopsy results [25]. Lahrouchi et al. only included cases with a negative traditional autopsy, excluding cases with evidence of structural disease [29•], whereas Nunn et al. included cases with subtle structural cardiac abnormalities [28].

Exome sequencing is still not commonly used for molecular autopsies. The amount of data and variants generated from exome sequencing is far more than gene panel testing. When comparing variants per gene between exome and a 233-gene panel, Rueda et al. found a 50-fold reduction in the number of variants identified [30]. The majority of additional variants tend to be missense variants, many of which are located in genes not previously associated with SCD, and are ultimately classified as variants of uncertain significance (VUS) [30]. Even when limiting analysis to discrete gene panels, as much as half of identified variants end up being VUSs [10, 25]. While exome sequencing has allowed for new gene-disease associations [37, 38], the added value appears to be limited in a postmortem population given the lack of clinical information and complexities of variant interpretation.

The Complicated Process of Variant Interpretation: Looking for a Clinically Actionable Test Result

Genetic variation is common. Copy number variants (CNVs) and single nucleotide variants (SNVs) are both seen in high frequencies (> 1% allele frequency) in control populations [39, 40]. Rare variants (< 0.1% minor allele frequency), both synonymous and non-synonymous, are also found throughout the genome [41]. As more of the genome is sequenced, either by expanded gene panels or by exome sequencing, the number of gene variants identified per case will likewise increase. This adds to the complexity of result interpretation. For molecular autopsies, the classification of a variant is critical given the potential impact of family cascade testing, making responsible evaluation of putative variants essential. While variant classification guidelines are available [42, 43], the process, and final classification determination, is completely at the discretion of the laboratory issuing the test report.

In the age of NGS sequencing, when thousands of variants can be identified per case, prioritization of variants to assess for pathogenicity is key. This is usually accomplished through bioinformatics pipelines using minor allele frequencies (MAF), prior disease associations, in silico predictive algorithms, and evolutionary sequence conservation data. Manual variant curation and annotation usually follow along individual laboratory standard operating procedures.

For molecular autopsies, the greatest benefit of finding a pathogenic or likely pathogenic variant is for the family. For inherited arrhythmias and cardiomyopathies, treatment and screening guidelines rely heavily on clinical features and family history, rather than genetic testing results. Variant-specific genetic testing is recommended for family members when a causative variant in a decedent is identified [7]. A clinically actionable result would therefore be one that is considered pathogenic or likely pathogenic and would be appropriate for cascade testing in family members.

When assessing pathogenicity of novel variants, multiple factors are considered. The type of variant, when in

concordance with genetic mechanism, often provides very strong evidence for pathogenicity. Inherited channelopathy and cardiomyopathies are often due to loss of function variants in disease-associated genes. Therefore, nonsense variants, frameshift variants, exonic deletions, and other null-type variants often end up with a pathogenic or likely pathogenic classification. Missense variants may be more difficult to interpret as their impact on the gene or protein product may be unknown. Functional data, if available, can help to elucidate the effect the variant has on the gene or protein product. For novel missense variants, however, this data is often lacking. In silico prediction tools are widely used to predict pathogenicity for missense variants when biological functional studies are unavailable. While these computational methods can be useful, they should be considered cautiously as results and false positive rates can vary between models [44]. The results of in silico tools should not be considered as strong evidence for pathogenicity alone but may contribute to that conclusion especially in cases where multiple models agree on the prediction [42, 43]. Other factors in assessing for pathogenicity of a variant include its rarity (MAF), whether it is de novo, and its co-segregation with affected family members.

Postmortem genetic testing for inherited channelopathies and cardiomyopathies can be particularly challenging as many conditions associated with risk for SCD demonstrate incomplete or age-related penetrance and variable expressivity. And in many cases, sudden death can be the first clinical sign of disease in the family. Molecular autopsies can result in a diagnosis in a subset of families, but often more cases are left with findings of uncertain significance [29•]. The high prevalence of missense variants [15, 27, 30] identified in molecular autopsies adds to the difficulty in establishing causation and reinforces the need for collaborative, multidisciplinary care for these families.

Value of Collaborative, Multidisciplinary Care

Following the sudden death of a young person, surviving relatives are often faced with two main concerns: "why did this happen" and "might this happen to anyone else in our family"? These two issues are the main guiding force impacting how these families are evaluated and managed after a SCD.

Why Did This Happen?

A primary concern for relatives is to address how their loved one died through traditional autopsy, which may or may not include the addition of postmortem genetic testing. Before any of the molecular autopsy process can begin, a ME has to appropriately identify decedents that may fit into the category of young sudden death. Compared with smaller European countries as well as Australia and New Zealand [45], the USA has a remarkably de-centralized approach for evaluating cases of SCD. There are recommendations from the National Association of Medical Examiners (NAME) [46] in place on how to go about this evaluation, including proper specimen collection and storage, but this is not a regulated process.

For efficient continuity of care, it is ideal for MEs to have an established workflow with medical providers with expertise in inherited cardiac disease. Working together with a multidisciplinary clinic, the ME can provide initial referral information and facilitate communication of family members with the clinic staff. Establishing this connection between surviving relatives and a multidisciplinary clinic is critical, as the follow-up for these individuals, in order to prevent subsequent cardiac events in these families, is the primary public health concern following a SCD in a young individual [4, 47–49].

In cases of a positive autopsy (structural heart disease or other identifiable cause), assessment of cause of death can be a relatively straightforward investigation. However, this becomes exceedingly complicated in autopsy-negative SCD. In collaboration with the ME's office and their findings, the evaluation for the cause of death can be furthered by specialized centers focused on inherited cardiac diseases. In a sense, this becomes medical detective work, wherein multidisciplinary clinic staff (cardiologists, genetic counselors, nurses, etc.) work to glean clinical history from medical records as well as patient and family history from surviving relatives. Having access to prior rhythm monitoring, such as ECG, Holter data, and stress testing as well as imaging studies like echocardiogram and cardiac MRI, can help put genetic findings in context. The circumstances of an individual's death as well as their family history of concerning symptoms also impacts hypotheses made about the cause of death.

Despite efforts made on the part of the ME and the clinicians involved in the evaluation of the decedent, traditional and molecular autopsy may still provide no insight into the cause of death. As seen in the recent data reviewed, the yield for postmortem evaluation still hovers around 30%. Therefore, banking DNA for future investigations is prudent to provide future opportunities for genetic investigation [10, 46].

Might This Happen to Anyone Else in Our Family?

Whether cause of death is identified or not, young sudden death can cause significant psychosocial stressors for surviving family members. Not only may they have legitimate concerns about their own risk for cardiac symptoms, but they have also experienced an acute trauma. These issues are best addressed by a multidisciplinary clinic whose resources are introduced via the ME's office, underscoring the importance of the awareness of the ME in this process [26, 48•, 49•]. The facilitation of this referral is critical for families who are in mourning, as their decision-making abilities are hindered [48].

In addition to clinical evaluation and management, a cardiac genetics clinic provides important services for these families: genetic counseling and psychological support.

The clinical care and evaluation of these families are delicate, as the surviving relatives may be at risk for an inherited cardiac disease while still in a grieving stage. Relatives seen in a cardiac genetics clinic should be met by a care provider, ideally a genetic counselor, who is familiar and sensitive to the stages of grief. In their review, Ingles and James provide an excellent timeline of psychosocial concerns in the care of families with SCD [50], which takes into account the needs of patients in the acute stages of grief and how this progresses as relatives pursue their own clinical evaluations, postmortem testing, and long-term follow-up. As many families will require more significant psychological support, genetic counselors can then refer to a clinical psychologist who can provide bereavement services and more in-depth counseling [51–53]. Provision of interdisciplinary psychological care throughout their initial evaluation and follow-up can help ensure that families are supported and educated in a sensitive manner and may help support better health outcomes [52•, 53•, 54•, 55•, 56•, 57•].

In addition to psychological support, having clinical team members who can provide thorough and careful clinical interpretation of laboratory-reported variant data is critical in cases of sudden unexplained death [24, 32]. Postmortem genetic testing can be a double-edged sword of trying to provide genetic risk information to family members when a likely pathogenic or pathogenic variant is found through sequencing [25, 31, 58]. Surviving relatives are looking for a cause and providers want to be able to explain this sudden death, but there is also a risk of over-calling a variant or variants that may not yet be confidently clinically actionable [32]. Working together with laboratory staff, clinical cardiac genetic counselors can offer support to cardiac care teams in an effort to discern appropriate variant interpretation [59]. In addition, they can provide thorough consenting for families prior to postmortem genetic testing to ensure appropriate education about detection rate as well as the clinical, legal, and psychological implications of test results [57•, 60].

Emerging Issues Related to the Molecular Autopsy

Given the integral nature of the ME's role in providing the referral for molecular autopsy and family member screening in multidisciplinary cardiac genetics clinics, there is mounting concern given the increasing shortage of qualified MEs across the USA [61, 62]. With growing demands and staffing

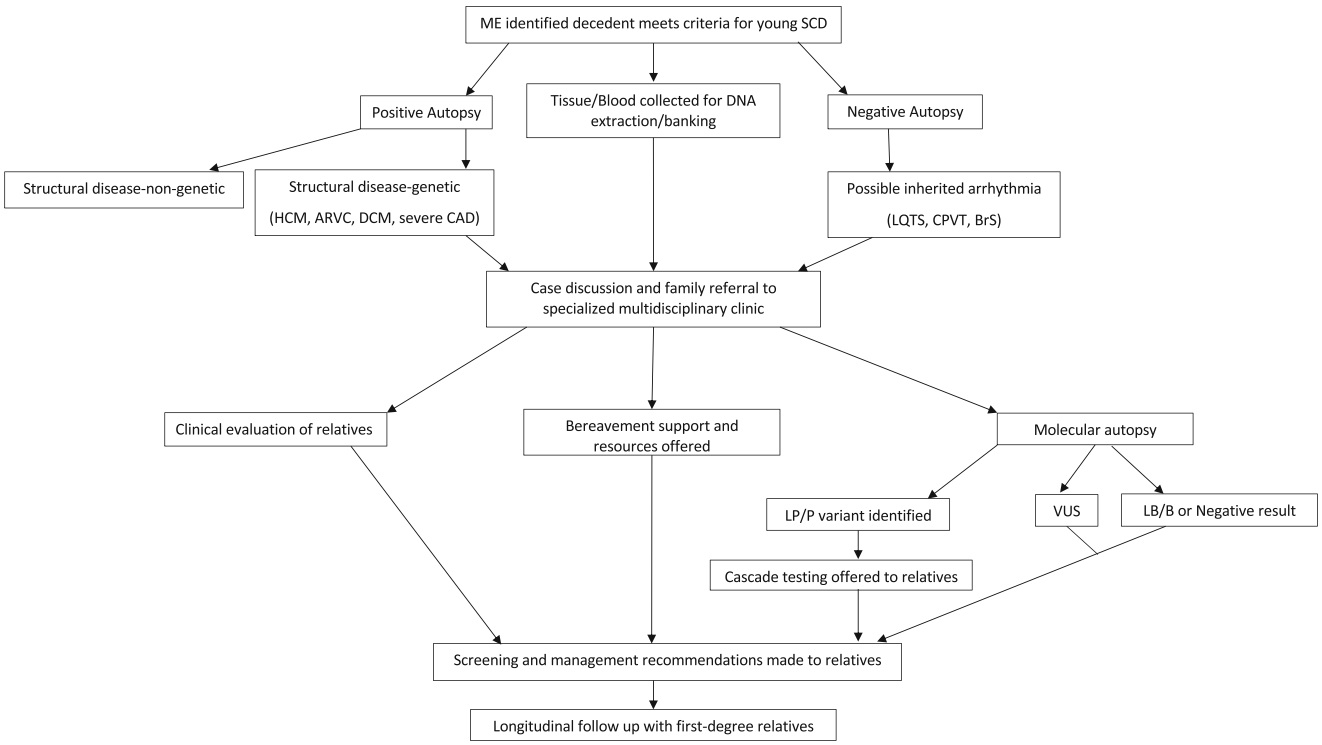

Fig. 1 Proposed workflow for the evaluation of cases of sudden death in the young and management of surviving family members. (ME = medical examiner; SCD = sudden cardiac death; LQTS = long QT syndrome; CPVT = catecholaminergic polymorphic ventricular tachycardia; BrS=Brugada syndrome; HCM = hypertrophic cardiomyopathy; ARVC = arrhythmogenic right ventricular cardiomyopathy; DCM = dilated cardiomyopathy; CAD = coronary artery disease; LP = likely pathogenic; P = pathogenic; VUS = variant of uncertain significance; LB = likely benign; B = benign)

concerns, meeting desired turn-around time for autopsy reports and death certificates (typically less than 90 days) puts at risk appropriate support for the ME to thoughtfully approach the autopsy process. This, in turn, delays the postmortem evaluation procedure for clinicians involved in the evaluation of surviving relatives. In addition, there are practical concerns for families trying to work through the logistics of settling their loved one's estate, which typically requires a death certificate. In short, without the ME, the rest of the postmortem evaluation is impossible, and steps must be taken to ensure this workforce is appropriately supported, both financially and with continuing education.

It follows that the molecular autopsy, as described in the reviewed literature, can be a key component to both identifying the cause of death as well as providing a critical risk assessment tool for surviving relatives. However, medical insurance does not typically cover the cost associated with postmortem genetic testing [63]. While the cost of next-generation sequencing continues to decrease, this financial burden is frequently passed on to surviving family members if they would like to pursue this type of evaluation. There is currently a paucity of data focusing on cost associated with molecular autopsy, thus additional dialog is needed to determine a feasible approach to help offset the family's financial responsibility.

Conclusion

The utility of postmortem genetic testing for the diagnosis of inherited channelopathies and cardiomyopathies in cases of SCD is established. This is particularly useful in cases of unexplained death, but genetic etiology can provide helpful risk assessment information for victims of inherited cardiac disease with structural involvement as well. Continued efforts and guidance in the area of variant interpretation is necessary to improve outcomes. This is not unique to postmortem genetic testing, and efforts in this area are ongoing [64–68], but postmortem testing adds difficulty to interpretation as clinical phenotype is often limited. Medical examiners will continue to play a critical role in the identification of appropriate decedents along with timely collection and storage of biological samples used for DNA extraction. They also carry the responsibility of connecting surviving relatives with a specialized cardiac genetics clinic that can provide clinical evaluations, genetic counseling and testing, and psychosocial support as the family processes their grief, searches for their loved one's cause of death, and assesses cardiac risk for family members. In Fig. 1, we propose a workflow to encompass each step of this multi-faceted process. Best outcomes will be achieved with collaborative, multidisciplinary teams that include MEs, laboratory professionals, as well as expert cardiac and genetic care providers.

Compliance with Ethical Standards

Conflict of Interest Kate Orland reports personal fees as a consultant for My Gene Counsel. Kimberly Anderson declares no potential conflicts of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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