The National Association of Medical Examiners (NAME) Position Paper: Postmortem genetic testing in forensic pathology cases

Authors:
Heather MacLeod, MS, Consultant, Genetic Counselor
Lisa Marie Castillo, MS, Consultant, Northwestern University
Joyce deJong, DO, Chair, Michigan State University
Lisa Ann Gavin, MD, MPH, Clark County Office of the Coroner/Medical Examiner
Kara L. Gawelek, MD Cuyahoga County Medical Examiner's Office
Ellen Hostetler, PhD, Consultant, John Ritter Foundation
Laura D. Knight, MD, Washoe County Regional Medical Examiner's Office and University of Nevada Reno School of Medicine
Basma Klump, DO, PhD, Consultant, Michigan State University
Elizabeth M. McNally, MD, PhD, Consultant, Northwestern University
Kathy Pinneri, MD, Montgomery County Medical Examiner's Office
Yingying Tang, MD, PhD, Consultant, Office of Chief Medical Examiner New York City
R. Gregory Webster, MD, MPH, Consultant, Northwestern University
Alison Krywanczyk, MD, Cuyahoga County Medical Examiner's Office

Introduction:

When a person dies suddenly and unexpectedly, the responsibility to determine the cause of death often falls to a forensic pathologist (FP). It is therefore not uncommon that a forensic pathologist is the first to make the diagnosis of a genetic disease. Identifying these heritable conditions at autopsy allows for accurate death certification and gives relatives the opportunity to seek diagnosis and intervention. In this sense, recognizing and diagnosing genetic disease fulfills the public health mission of most Medical Examiner Coroner (MEC) offices.

In 2013, the National Association of Medical Examiners (NAME) issued a position paper recommending the retention of a sodium ethylenediaminetetraacetic acid (EDTA) tube of blood for potential postmortem genetic testing (PMGT) ideally at each autopsy [1]. The subsequent years have shown marked advances in the diagnostic capabilities and accessibility of PMGT. As a result, NAME commissioned an expanded position paper to provide medicolegal professionals with a comprehensive framework for the evolving use of PMGT in death investigation. While resources vary between jurisdictions, these recommendations will begin to establish uniformity regarding testing protocols and procedures, including reporting and interpretation of findings, development of multidisciplinary expert teams, and recommendations for continuing education and future research.

Methods

The NAME formed a panel of experts from the fields of forensic pathology, genetic counseling, molecular pathology, and cardiology. This expert panel met, identified topics relevant to practicing forensic pathologists, and split into smaller writing groups. Literature searches were performed on PubMed and relevant articles were shared from the authors' personal collections. Search terms included postmortem genetic testing, autopsy, cardiomyopathy, channelopathy, epilepsy, aortopathy, aortic aneurysm, sudden cardiac death, sudden death, forensic pathology, inborn errors of metabolism, sudden unexpected death in epilepsy (SUDEP), thrombophilia, sudden unexpected infant death (SUID), sudden infant death syndrome (SIDS), sudden unexpected pediatric death, and sudden unexplained death in childhood (SUDC). After assembling the individual sections, the final paper was reviewed and approved by the entire panel.

Background of DNA and Genetics

Deoxyribonucleic acid (DNA) is the essential informational unit of the cell, composed of an approximately 3 billion-base pair sequence of alanine, guanine, cytosine and thymine nucleotides [2] distributed across 46 chromosomes. Human DNA is made up of 20,000-25,000 genes [3]. DNA is also known for its durability, surviving temperatures up to 190C, and has a postmortem longevity of up to 521 years [4, 5]. Given these qualities, DNA analysis has long been used in the forensic sciences for identification of decedents, parentage determinations, or identification of potential perpetrators in criminal cases. Since the advent of DNA sequencing, scientists have studied genetic variations to understand the mechanisms of human disease and identify preventative and treatment strategies [2]. Genetic testing in the postmortem setting therefore not only fulfills the well-established forensic purposes of identification and parentage but can now shed light on the cause of death.

Types of Genetic Testing

Several types of genetic testing are available. The optimal choice depends on circumstances of death, suspected disease, sample type available, and cost. Definitions of commonly used

molecular pathology and genetics terms used below are defined for reference in **Table 1.** A summary of different types of genetic testing is provided in **Table 2**.

Biochemical Genetics

Biochemical genetics uses a blood spot card to assess specific biochemical markers associated with various genetic disorders, such as phenylketonuria (PKU), hypothyroidism, sickle cell anemia, and cystic fibrosis. Most labs use techniques like tandem mass spectrometry to identify and quantify the levels of different metabolites in the blood sample. This is the methodology typically performed for universal newborn screening but can also be used at autopsy [6].

Cytogenetics

Cytogenetics uses karyotype analysis, sometimes enhanced by fluorescence in situ hybridization (FISH) or spectral karyotyping (SKY) to identify broken, missing, rearranged, or extra chromosomes. This type of testing is often used to determine a chromosomal cause of disease like trisomy 13, 18, or 21. Chromosomal microarray analysis (CMA) uses comparative genomic hybridization or single nucleotide polymorphism arrays to identify microdeletions or microduplications too small to be visualized with traditional cytogenetics [7, 8].

Next Generation Sequencing (NGS)

NGS technology sequences millions to billions of shorter DNA fragments simultaneously, providing a much higher throughput capacity than traditional Sanger or pyrosequencing [9]. The three main options for NGS in the postmortem setting are panel tests, whole exome sequencing, and whole genome sequencing.

Panel Testing

Panel tests sequence a targeted set of genes for a specific condition(s), which may involve only a handful or up to several hundred genes. Panels are typically the most affordable genetic testing option, and return a more manageable amount of data with fewer results of unknown significance or secondary findings [9].

Whole exome sequencing (WES)

WES sequences the entire protein-coding region, encompassing approximately 1-2% of the human genome. WES uses a standardized capture method to isolate the coding regions of an established set of genes - for example the current medical exome is ~4000 genes linked to human disease. Because of the amount of genetic code that is analyzed, submission of clinical information on the decedent is typically required and genes with known associations with the submitted phenotype are analyzed [10, 11]. WES may result in secondary and/or incidental findings, which are addressed in the “Interpretation of Results” section.

Whole genome sequencing (WGS)

WGS sequences both the coding and non-coding regions of the genome. However, the analysis of the results is still very focused on the reported clinical presentation of the decedent and exons are typically isolated informatically. Although WGS with short read coverage has fewer reads at each location than whole exome sequencing, the lack of

exome amplification leads to more even coverage and, in general, excellent bioinformatic quality [10]. Reported pathogenic variants are typically still within the coding regions of the genome, as this is where the most known pathogenic variants are located. WGS offers the possibility of identifying variants in new, previously unreported genes which may potentially be disease-causing. However, these variants are unlikely to be reported as "pathogenic", and it is unclear if a clinical lab would report these findings. As such, the additional value over WES may be minimal, depending on the disease of interest [11]. Like WES, WGS may result in secondary and/or incidental findings, which are addressed in the "Interpretation of Results" section.

With all forms of NGS-based testing, turnaround times are rapidly decreasing (results may take only 3 to 4 weeks). For children or young adults, trio exome or trio genome (provided the biological parents are available and willing to participate) can confirm if a variant is de novo or inherited from a parent, with de novo variants typically conferring pathogenicity. It is important to be aware whether mitochondrially-encoded genes are included in the test requested, as this may require a separate analysis.

Sample Collection

While some decedents have anatomic findings or clinical histories which immediately indicate a possible need for PMGT, many lack clear indications at the time of autopsy. Comprehensive investigation may take months, or even years, before the need for PMGT becomes evident. For example, a death initially attributed to drug intoxication might only be linked to an underlying genetic channelopathy after another relative exhibits symptoms. Thus, it is crucial to collect and store samples suitable for long-term preservation and indefinite retention. Of note, some degree of DNA degradation can occur over long periods of storage, and with any sample type (particularly if the conditions of storage are suboptimal). It is therefore optimal for the MEC to send the sample for PMGT as soon as possible once they have decided to pursue testing.

It is recommended that the autopsy protocol of an MEC office standardize collection of at least one sample suitable for genetic testing. It is beneficial to pre-emptively establish relationships with preferred laboratories for molecular genetics, biochemical genetics, and cytogenetics testing. If testing indications are identified at the time of autopsy, the laboratory can be consulted immediately to ensure proper collection, storage, and shipping of samples. The specific needs and resources of the MEC office need to be considered when determining the standard sample types to collect.

A summary of different sample types is provided in **Table 3**. The gold-standard sample for DNA testing is blood saved in a lavender/pink top K2 EDTA tube, which can be stored refrigerated (4 ºC) for 4 weeks or less or long-term in a freezer (-20º C or -80 ºC) [1]. Other common sample types include blood spot cards or tissue (either frozen or in RNALater™). Blood spot cards are easy to prepare, store at room temperature, ship via regular mail, and may be used for molecular or metabolic testing. However, these cards generally yield lower quantities of DNA compared to liquid blood or frozen tissue and not all reference laboratories can use them. Formalin fixed and/or paraffin embedded tissue (FFPE) is typically unsuitable as the formalin

and paraffin fragment the DNA, resulting in poor quality. For decomposed remains, bone marrow (stored in RNAlater™ or frozen), bone (frozen), or other frozen tissue can be collected and preserved, although commercial laboratories often have limited experience with decomposed samples and the suitability of the specimens cannot be guaranteed. All MEC offices are strongly encouraged to confirm the preferred sample type for their own reference laboratory.

Cost

The cost of genetic analysis includes sequencing instruments, labor, administration, data processing and analysis, and clinical interpretation. An indirect cost is the time commitment required to participate in multi-disciplinary teams to guide the healthcare of the family members [12]. Since its inception, the cost has significantly decreased due to method refinement, increased commercial availability, and utilization in both research laboratories and direct patient care [13]. While insurance can be billed for testing in living patients, insurance companies typically do not reimburse PMGT. The MEC office may pay for testing, in which case the office can request the same price offered to a patient for out-of-pocket cost (prices billed to insurances or institutions are typically inflated). The MEC office may request the decedent's next of kin, or a clinician, pay for testing; however, placing a financial burden on the next of kin may inadvertently disincentivize pursuit of an accurate diagnosis. Recognizing PMGT as an important forensic tool, some offices are incorporating the cost into their state/jurisdictional determined budget; the state of Kentucky has passed legislation mandating PMGT in certain cases of sudden unexplained death [14].

Pre-test investigations

A thorough forensic investigation is essential for proper contextual interpretation of PMGT. This includes examination of the death scene, interviews with family and/or clinicians, and review of medical records. Cardiomyopathies, arrhythmia syndromes, or epilepsy may be initially present as a drug-related death, drowning, or a single car motor vehicle accident. Especially in these latter two scenarios, if trauma and/or other mitigating factors like intoxication or poor environmental conditions have been excluded, it is important to consider whether a cardiac event triggered the incident through careful consideration of the circumstances and family history [1, 15]. A full autopsy, including histology, toxicology, and vitreous chemistries, is necessary to exclude specific causes of death and to generate the appropriate differential diagnosis. Ancillary studies (such as microbiology) or subspecialist neuropathology, pediatric pathology, or cardiovascular pathology consultation may be needed. Reviewing the decedent's medical records and/or interviewing the next of kin may help identify a family history of possible (or already known) genetic disease. Depending on the ultimate findings from the autopsy and death investigation (**Figure 1**), the process of PMGT can be initiated.

Diseases and circumstances in which to consider PMGT

Given the limited resources available to most MEC offices, it is impractical to create strict guidelines on when to request PMGT. The following conditions, however, should raise concern for genetic disease. A summary of the following section is in **Table 4.**

Inborn errors of metabolism

Inborn errors of metabolism (IEM) are a broad spectrum of disorders involving a biochemical deficiency which causes either deficiency of a necessary nutrient or accumulation of a toxic by-product. They may be caused by variants in either nuclear or mitochondrial DNA [16-18]. Neonatal screening programs identify many affected infants, yet some are missed because a) the specific disorder is not included in the screening, b) delayed clinical onset or c) the infant dies before the results are available [19]. Infants born at home and without traditional medical care may bypass neonatal screening. The symptoms and onset of IEMs are variable, depending on the specific deficiency. Some present within hours of birth, while others present in childhood or even adulthood. The symptoms include hypotonia, failure to thrive, seizures, cardiac arrhythmias or cardiomyopathies, lactic acidosis, and neuromuscular manifestations. Many IEMs have been associated with sudden infant death and/or Reyes syndromes. At autopsy, signs may include cardiomegaly, hepatomegaly, pallor of skeletal muscle, and/or microvesicular steatosis of the heart, liver, and kidneys [16, 18-22]. Some MEC offices screen all infants and children for IEM with blood spot card analysis; other offices reserve testing for those in whom the autopsy findings are suggestive of disease, or those without known neonatal screening results.

Chromosomal disorders/dysmorphic features

In fetuses or infants with structural abnormalities, chromosomal disorders may be suspected. In fetuses with normal chromosomal microarray and karyotype, expanded testing with WES was able to identify a genetic cause in 25% [21]. Trio WGS has also been shown to offer increased diagnostic yield (11.8%) in fetuses with structural abnormalities who have normal CMA and WES [22]. Although these studies were performed on clinical cohorts, there is no reason to suspect the yield would be different at autopsy. Of note, older children and adults may also have chromosomal disorders or dysmorphic features which were not diagnosed in life and are only identified at autopsy.

Cardiomyopathy

Heritable cardiomyopathies may be suspected when gross cardiac abnormalities are identified at autopsy. These include hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM), and arrhythmogenic cardiomyopathy/arrhythmogenic right ventricular dysplasia (ARVD) [23-25], and left ventricular non-compaction (LVNC). The gross and microscopic phenotypes of these diseases are well-described elsewhere [26-30], but it is important to highlight the widely variable expressivity and penetrance that cardiomyopathies often demonstrate. In 2023, the Society for Cardiovascular Pathology issued a consensus document regarding the recommended autopsy evaluation of sudden cardiac death in the young (SCD-Y, defined as younger than 35 years of age), including specific procedures and findings. Notably, though, the gross findings in a patient with cardiomyopathy may be subtle or inconclusive [15]. In these cases, a combined cardiomyopathy/arrhythmia panel may be considered. Also of note, lymphocytic myocarditis may be a manifestation of underlying cardiomyopathy or may reflect increased susceptibility to viral-type myocarditis in the setting of cardiomyopathy [31].

Aortic dissection/aneurysm and connective tissue diseases

Variants in multiple genes are known to confer a highly penetrant risk for aortic dissections, with or without syndromic features [32]. Syndromes (such as Marfan, Loeys-Dietz, and vascular Ehlers-Danlos) can cause aortic dissection because of a variant in a single gene which acts in an autosomal dominant fashion. The external phenotypes of these syndromes can overlap and be subtle. Furthermore, up to 20% of non-syndromic aortic dissection patients have an affected first-degree relative, indicating a significant genetic component to etiology even in the absence of syndromic features [32-35]. The yield of PMGT in the setting of thoracic aortic dissection and/or rupture has been reported as up to 23.5%, depending on the criteria used for testing [36]. PMGT should be considered when aortic dissection or rupture is identified on autopsy, particularly if the decedent is under the age of 60, has syndromic features, or has a family history of thoracic aortic aneurysms, peripheral aneurysms, or sudden unexplained death [37, 38].

Thrombophilias

Pulmonary thromboembolism (PTE) is a relatively common cause of sudden, unexpected death. Common acquired risk factors include recent trauma or immobilization, pregnancy, obesity, oral contraceptive (OC) use, and malignancy [39]. In some cases, though, no risk factors are identified, raising the possibility of hereditary thrombophilia. Functional clot-based assays are unreliable in postmortem samples, so the analysis is limited to conditions detectable by gene sequencing - including factor V Leiden and prothrombin G20210A. One study of PTE at an MEC office identified pertinent mutations in five of thirty-four decedents [40], but two other studies found low yield and concluded the cost was not justified [41, 42]. In another series, WES identified causative variants in three anticoagulant genes (*SERPINC1, PROS1, PROC*) in 13.2% of decedents with idiopathic PTE [43]. Testing for antiphospholipid antibodies postmortem is controversial, given the different pre-analytical variables compared to tests in living patients [44]. It is important to note that the presence of an identified risk factor does not necessarily exclude hereditary thrombophilia. For example, obesity is relatively common, yet a small percentage of obese people die from PTE. The American College of Medical Genetics and Genomics (ACMG) has published recommendations for testing in living patients. While these are not specific for PMGT, the recommendations do support testing in decedents younger than 50 with unprovoked PTE, recurrent PTE, thromboses of unusual sites (hepatic, portal, mesenteric, and/or cerebral veins), or PTE in the setting of pregnancy or oral contraceptive use [45, 46].

Coronary artery disease

Atherosclerosis is a common etiology of sudden cardiac death [24, 47], but premature disease may indicate familial hypercholesterolemia (FH). There is an estimated prevalence between 1/200 and 1/500 for heterozygous FH, yet many patients remain undiagnosed [48]. The European Atherosclerosis Society issued a statement in 2013 recommending screening for individuals with premature atheromatous coronary artery or cerebrovascular disease (less than 60 years of age in women, and 55 years of age in men) [49]. Up to 40% of patients with a clinical diagnosis of FH do not have an identifiable genetic cause, suggesting a possible polygenic basis or a role for modifier genes [50]. Screening of family members may be accomplished by measurement of blood lipid levels, without direct genetic testing of the decedent.

“Autopsy-negative” or unexplained sudden death

“Autopsy-negative” sudden death means the heart is grossly normal and there are no other anatomic, microscopic, toxicologic, or investigative findings to suggest another cause of death [24, 51]. While underlying etiologies of autopsy-negative death do occur outside the brain and heart, the majority of autopsy-negative deaths can be either attributed to a cardiac etiology or to sudden unexpected death in epilepsy. Scene investigation and medical history are crucial to exclude some causes of death which can leave subtle or no findings at autopsy (such as positional asphyxia or low-voltage electrocution). Genetic variants occur in sudden deaths with normal and abnormal autopsies [24, 52], but genetic testing can be exceptionally useful in autopsy negative deaths.

Highly penetrant, monogenetic causes of autopsy negative death fall into three major genetic categories – cardiac channelopathies, cardiomyopathies, and sudden unexplained death in epilepsy (SUDEP). Sudden unexpected pediatric deaths also may fall into the category of autopsy-negative death, and are therefore briefly addressed below (although the underlying genetic etiologies overlap with those of any autopsy-negative death). The reader is referred to the NAME book “Unexplained Pediatric Deaths: Investigation, Certification, and Family Needs” [53] for additional information.

Cardiac Channelopathies and Cardiomyopathies

Cardiac channelopathies and cardiomyopathies are two potential cardiovascular causes of autopsy negative death. Both of these entities can also fall under the category of “sudden cardiac death” (SCD). SCD is defined as death within one hour of collapse (or 24 hours of the time last known well, if the death is unwitnessed), and can be due to any cardiac pathology (including atherosclerosis, hypertension, and valvular heart disease, which would cause grossly apparent autopsy findings). The highest diagnostic yield for genetic causes of SCD has traditionally been in people younger than 35 years of age (SCD-Y). The annual incidence of SCD-Y has been reported between 1 per 100,000 (Veneto, Italy) and 13 per 100,000 (United States military), but most reports document a rate of approximately 2.5 deaths per 100,000 young people, with a peak in infancy, a nadir before puberty, then gradually increasing incidence during adolescence and early adulthood [54-56]. The cardiac channelopathies include long QT syndrome, catecholaminergic polymorphic ventricular tachycardia (CPVT), Brugada syndrome, and short QT syndrome [1, 15, 57]. The reported yield of PMGT in these circumstances is highly variable, between 13% and 27% [47, 51, 58] depending on the population and the scope of testing. As stated earlier, pathogenic and likely pathogenic variants in cardiomyopathy genes are well-established findings in autopsy-negative sudden death series [52], and arrhythmogenic death can precede structural changes. The rate of “normal” hearts has ranged from 6% to 54% in autopsy series [52, 56, 59-63], with one study showing that expert analysis of post-mortem tissue can reduce overinterpretation of cardiomyopathic findings [64].

Sudden Unexpected Death in Epilepsy (SUDEP)

In individuals diagnosed with epilepsy or a seizure disorder, sudden unexplained death in epilepsy (SUDEP) is a possible cause of death when autopsy does not reveal any competing cause [65]. The mechanisms of death in SUDEP continue to be an area of

investigation. Progressive apnea, bradycardia, and asystole during generalized tonic-clonic seizures are likely the common pathophysiologic endpoint [66]. However, in population-based data, patients with epilepsy have an increased risk of all cardiac arrhythmias, and in a systematic review, rare cardiac ion-channel variants were present in 11% of SUDEP cases [67-69]. Thus, there is likely an overlap between neurological and cardiac causes of SUDEP. In addition, some medications used to treat epilepsy have clinically relevant effects on cardiac repolarization [70]. The full expression of SUDEP is likely multifactorial.

Sudden Unexplained Pediatric Deaths

Sudden Unexplained Infant Death

Sudden unexplained infant death (SUID) is the sudden and unexpected death of an infant less than 1 year of age which remains unexplained following a thorough autopsy and investigation, including scene re-enactment and ancillary testing. Scene investigation with re-enactment is critical, as it may reveal a specific cause of death like accidental suffocation or strangulation in an unsafe sleeping environment. The vast majority of SUID occur during sleep (95%); most have unsafe sleep factors present. [53, 71, 72]. Even if known risk factors for SUID are identified during the investigation, these are often insufficient to fully explain the death and thus the death remains classified as “unexplained” (SUID). These deaths were formerly called Sudden Infant Death Syndrome or SIDS. As access to PMGT has increased over the past decades, there have been several potential genetic causes of SUID identified. These include variants associated with both cardiac channelopathies and/or epilepsy, although some studies report a higher rate of potentially pathogenic variants in genes associated with epilepsy, (especially Dravet syndrome), and a lower rate of pathologic variants in cardiac genes [53, 73, 74].

Sudden Unexplained Death in Childhood

Sudden unexplained death in childhood (SUDC) occurs in children between 1 and 18 years of age and is a sudden and unexpected death which remains unexplained following a thorough autopsy and investigation. While rare, it is more common among males and Black children. Of note, sudden deaths in children (ages of 1 and 18) are more likely to have an identifiable underlying cause than sudden deaths in infants (age less than one year), with one study finding 57% had an underlying cause of death identified and only 43% remained unexplained [75]. The most common age groups for sudden death in children are 1 year of age and 14-17 years. Among cases that remain unexplained, the rate of pathogenic variants in epilepsy and cardiac arrhythmia-associated genes has ranged from 6% to 25%, depending on the scope of testing and population tested [47, 52, 76, 77].

Other Known or Suspected Genetic Diagnosis

The genetically-mediated diseases included thus far are those most likely to cause sudden and unexpected death and therefore are more likely to present at forensic autopsy. There are many other genetic diagnoses and syndromes which could potentially be identified at forensic autopsy

- however, the clinical course is often protracted, and these patients are more commonly diagnosed during life. These include hereditary cancer syndromes (e.g. Lynch Syndrome, Hereditary Breast and Ovarian Cancer), chronic kidney diseases (e.g. autosomal dominant polycystic kidney disease), and many others. If suspected at autopsy, PMGT may help confirm the diagnosis. At times, the decedent may already have a clinically known or suspected genetic diagnosis but may pass away before definitive genetic testing is pursued. PMGT may therefore be the last chance to confirm the diagnosis, and to identify a specific causative genetic variant [3].

Interpretation of Results

The results of PMGT will report findings according to the five-tier classification system created by the American College of Medical Genetics and Genomics (ACMG) and Association for Molecular Pathology (AMP) [78]. The classifications are pathogenic, likely pathogenic, variant of uncertain significance (VUS), likely benign, and benign (**Table 5**). Consultation with a clinical genetics provider (including either or both clinical geneticists and genetic counselors) may help clarify the significance of the results, particularly in the context of the autopsy findings. Of note, with WES and WGS there is a possibility of secondary and incidental findings; and as more genes are tested (expected as one escalates from panel testing to WES and then WGS), the likelihood increases for obtaining a VUS.

Pathogenic Variants or Likely Pathogenic Variants (P/LP): If identified in a gene linked to the observed phenotype, P/LP variants provide direct supporting evidence of a genetic contribution to the cause of death. Referrals for cascade family genetic testing can be made by the MEC, testing laboratory, or by another clinician involved in the family's care.
It is important to consider that variants with late-onset or incomplete penetrance may not have contributed to death. For example, a *TTR* variant causing late-onset cardiac amyloidosis (typically after age 65) [79], is unlikely to be the cause of death in a younger decedent without histologic amyloid deposition. Similarly, adult-onset cardiomyopathy variants found in deceased infants are likely incidental though the results may inform family care. Pseudogene sequences can also be misidentified as disease-causing variants by default analysis programs. For example, one *FLNC* pathogenic variant is indistinguishable from the pseudogene sequence in LOC392787 by next-generation sequencing testing [80]. Confirmatory tests are necessary to ensure that the reported variant is not a pseudogene sequence. Laboratories can be consulted to understand the methodologies used in these cases.

Variants of Uncertain Significance (VUS): These variants have unclear clinical implications, either due to insufficient information or due to conflicting evidence. They are a common, often frustrating, outcome of PMGT, but VUS are not devoid of value. Further studies (such as family or functional analysis) may clarify their relevance, particularly as clinical research advances. VUS reporting practices vary by laboratory. Some labs report all, while others report only those considered "clinically suspicious". Targeted panel testing often reports VUS in known disease-causing genes, while WES/WGS testing may detect VUS in genes of unknown relevance to the cause of death. Cascade testing of family members for a suspicious VUS may be warranted, as the identification of other affected family members could prompt reclassification; this assessment is best accomplished with the guidance of a clinical genetics provider. VUS data can be added to genetic databases used by clinical researchers, and as new evidence becomes available, a VUS~~s~~ may be reclassified as either benign or pathogenic. This benefits not only the affected family but also other unrelated patients who undergo testing in the future [81].

Negative (Benign/Likely Benign Variants (B/LB)): A negative result means only benign or likely benign variants are identified. While the likelihood of the death being related to a variant in the genes tested is reduced, it is important to note that B/LB results do not completely rule out a genetic etiology of cause of death. First degree family members may still need clinical assessment.

Secondary findings: These are pathogenic or likely pathogenic variants identified in genes which are sequenced as a part of the testing process, but which are ultimately unrelated to the primary indication for testing [82]. For example, pursuing WES or WGS in a teenager with cardiomyopathy may identify a P/LP *BRCA* gene variant. As these findings still have implications for biologic relatives, they are also important to communicate to the family. A list of reportable pathogenic secondary findings has been established by the American Board of Medical Genetics, determined as those variants which have implications for medical management [83]. The specific genes recommended to be reported are re-assessed at regular intervals, though most listed at this time are responsible for inherited cancers and inherited cardiovascular diseases [84]. Clinical laboratories typically offer an "opt-out" option from receiving secondary findings for individuals undergoing clinical genomic sequencing. In PMGT, laboratories may also provide the "opt-out" option to avoid a scenario in which secondary findings, unrelated to the cause of death, are discovered, and the responsibility then falls to the MEC to inform the next-of-kin.

Incidental findings: These are findings which are discovered unexpectedly as part of the genetic testing process (i.e. are not a finding in an intentionally sequenced gene) and are unrelated to the primary reason for testing. For example, some forms of genetic testing (i.e. trio sequencing) may reveal non-paternity or consanguinity [82].

Practical Guidelines for Implementation

This section focuses on the collection and management of postmortem specimens for PMGT, with particular attention to the operations of MEC offices in the United States.

Because of the variable financial resources of MEC offices, it is impractical to require universal PMGT for any condition. Under optimal conditions, MEC offices would request PMGT when a cause of death cannot be identified (including SUID, SUDC, SUDEP, potential channelopathies, and other sudden unexplained deaths, particularly under age 40), suspected cardiomyopathies, and thoracic aortic dissections or ruptures under the age of 60. At a minimum if a genetic diagnosis is suspected, the MEC should, wherever possible, save a specimen for testing and notify the family. Of note, the legal next of kin may not be biologically related to the decedent (such as a spouse), and if permissible by law the MEC may try to contact first-degree relatives (parents, siblings, and adult children). This notification may be by telephone call or letter (although the latter has the benefit of written documentation), and should describe the suspected genetic condition, and provide contact information for local hospital departments with clinicians or clinical genetics providers to help coordinate genetic testing and/or screening. It is reasonable to consider saving DNA samples for up to 1 year after the family has been informed of the findings, if not longer, depending on the capacity and resources of the offices. If testing ~~or~~

is not performed, it is best to offer families the option to request the sample for the purposes of DNA banking (see sample notification letters in **Appendix A**) before it is discarded.

WES and WGS can improve the likelihood of diagnosis but may require testing of biological family to correctly interpret results. Given these limitations and the increased complexity of test interpretation, some MEC offices refer family members to a clinical genetics provider who then requests the decedent's samples for testing. This ensures the appropriate test is ordered, facilitates coordination of the results with ongoing medical care and testing of family members, and allows any re-interpretation or re-classification of the results to be reported to the clinician engaged in the ongoing care of the family. Some MEC offices have formed regional referral systems, connecting families to clinical genetics providers and clinicians who subsequently request the decedent's sample for PMGT [85]. Such multidisciplinary systems can be challenging to establish throughout the United States, given the variability in death investigations systems and access to specialty medical care. The drawbacks of this approach include the need to coordinate transfer and send-out of samples when clinically requested and ensuring that the MEC office receives the results of any testing to update the death certificate and autopsy report [85]. In addition, if PMGT is not requested by the MEC office, many families do not follow up for specialized care.

There is a wide spectrum of possible approaches, and the type of testing requested and the indications for testing will need to vary with the resources of the office. However, it is important not to allow financial considerations to be the main influence on an office's approach to PMGT.

Ethical Considerations, Benefits, and Risks

The most direct benefit of PMGT is determining the cause of sudden death, thereby providing answers to the family and improving death certificate accuracy for national statistics. Also important, though, is the ability to inform relatives of their own risk for a disease which may or may not have yet manifested in a distinct phenotype. This can allow access to individualized care targeted toward prevention of the disease, which may require establishing care with a cardiologist and/or geneticist, regular physical examination or tests (including electrocardiograms (ECGs) and/or imaging), initiating treatment or altering monitoring frequency, patient education, and follow up for continuity of care [12, 86]. As noted, the more genes that are tested, the greater likelihood of obtaining a "VUS" result, and with WES and WGS there is a possibility of receiving secondary and/or incidental findings. However, these can be managed with medical evaluation by a clinician or geneticist to help establish clinical suspicion for disease. If WES or WGS is pursued, a clinical genetics provider can be enlisted to aid with the reporting of secondary findings.

In most jurisdictions, the MEC has legal authority to investigate sudden and unexpected death of unknown cause. This includes performing PMGT without informed consent from next of kin and including a genetic diagnosis on a document which may be open to public access (depending on state/regional law). While these two factors have raised some ethical questions surrounding PMGT, regardless of whether genetic testing is performed a potentially genetic cause of death will still be reported (i.e. hypertrophic cardiomyopathy) on the death certificate. Therefore, the benefits of PMGT are not outweighed by the risk of potential disclosure [87, 88]. The family of the deceased should be informed of the genetic analysis results including pathogenic, likely pathogenic and variants of uncertain significance and given a copy of the

genetic testing report. Individual family members may choose to discuss the results with the medical examiner but additional evaluation by a clinical genetics provider and/or clinician is prudent [87].

Results Reporting

Autopsy report

If a heritable or genetic disorder is suspected but PMGT cannot be performed, it is still important to document this concern. The autopsy report can reflect this information and specifically describe which disorder(s) is suspected, recommendations to the biological family, and if a sample was saved for potential genetic testing. Some pathologists include this in an “Opinion” or “Comment” as part of the report. It is recommended that the specimen collected at autopsy is made available to the next of kin for independent pursuit of testing, once the MEC investigation is complete. State laws and regional/local policies may differ in their requirements for release of specimens, but it is best practice for MEC Offices to facilitate the process whenever possible. This will allow for quicker testing and earlier diagnosis for an affected family member.

Example: The anatomic and microscopic findings at autopsy are suggestive of hypertrophic cardiomyopathy (HCM). Immediate biologic family members are encouraged to seek evaluation from a clinical genetics provider and/or cardiologist. A sample of blood collected at autopsy has been stored in a frozen EDTA tube and can be released for genetic testing upon clinician request.

If PMGT has been performed, it is recommended to state this clearly in the autopsy report. In the event the genetic report becomes separated from the autopsy report, it is important to describe specifically which test was performed and by which company, as this communicates the scope of testing to future clinicians. Similarly, the specific variant detected should be listed (not just the interpretation) to allow for independent interpretation and/or future reclassification. Because additional information pertinent for clinicians and clinical genetics providers is often included in the original report of the genetic results, it is recommended that copies of the autopsy report also contain the original genetics report. Depending on local resources, offices may consider providing a copy of the original report to biological family members and/or their clinicians on request for no charge.

Death certification

In trying to arrive at the cause of death and/or contributing conditions, the certifier integrates all data from the investigation (circumstances surrounding the death), the family history, the medical records, the autopsy findings, the microscopic/histologic observations, and any additional relevant ancillary studies (toxicology/laboratory results) to include postmortem genetic testing (PMGT); each of these factors are weighed for their relative contributions to the death. Hence, it is recommended that PMGT results are interpreted within the context of each case.

If PMGT identifies a P/LP variant in a gene related to the cause of death, and the clinical history, autopsy, and histologic findings are corroborative, then it is advisable for the variant to be documented on the death certificate. Either part I or part II are appropriate, depending on whether the gene variant was the main cause of death or a secondary, contributing factor.

If PMGT identifies a VUS, inclusion on the death certificate can only be done with careful clinical assessment of the context and findings of the individual case. Guidance from expert clinicians and clinical genetics providers may be helpful in correlating the decedent’s phenotype with the gene variant, and studies on surviving relatives may help to clarify the pathogenicity of the

variant. VUS results are not uncommon in PMGT, particularly in SUID, SUDEP, and SCD-Y. As with all deaths, the results of PMGT are interpreted relative to the case information and findings. Thus, if within the context of the case and in the opinion of the certifier the VUS is considered likely to be the cause of death, it may be appropriate to include the VUS on the death certificate [15].

Overall, documentation of PMGT results within certification may improve death surveillance, ensuring the acquisition of important epidemiologic data and allowing for better understanding of mortality patterns.

See **Appendix B** for examples of how to incorporate the results of PMGT on death certificates.

Reporting to the family

PMGT results can be reported to the family with the full autopsy results, preferably with recommendations for clinical follow-up. While this may be done verbally, providing a written letter (mailed or emailed) helps document this communication. Ideally, recommendations for follow up medical screening can be shared with the biological family as well as the primary care provider for the family (pediatrician for child or primary care provider for older individuals) (see **Appendix A**). Because of the risk to other family members, it is best for results to be shared in a timely manner.

As this can be a time and energy-intensive process, multidisciplinary collaboration, whenever feasible, is beneficial. PMGT may bring up psychological, emotional, social and interfamilial challenges. Clinical genetics providers have expertise in medical genetics and psychosocial counseling and can aid in interpretation of PMGT results and direct screening of relatives. In addition, they are skilled in providing emotional support, resources, and further obtaining and assessing medical and family history. Unfortunately, despite existing guidelines, families are not being referred for follow-up medical screening on a routine basis [89].

A clinical genetics provider in the appropriate subspecialty can help facilitate phenotypic and genotypic family screening as detailed in the chart below (**Figure 1**), particularly in the setting of a VUS result [90]. Thorough evaluation of first-degree relatives requires expert understanding of the various diseases and their phenotypes and may help to confirm or establish the diagnosis in the decedent [24, 91, 92].

Additional resources for families may include disease-specific support groups and open research studies. The next of kin should be given the opportunity to bank any remaining DNA, or to save and share their family's member's sequence data if WES or WGS was done.

Reporting secondary and/or incidental findings

If WES or WGS is performed, secondary and/or incidental findings may be identified. Secondary findings have implications for the decedent's relatives, who may be at risk for the condition in the future. Therefore, additional clinical referrals are indicated for the secondary finding variant (in addition to any evaluations warranted by the decedent's phenotype or cause of death). This is especially important when considering PMGT in children. The majority (60% to 70%) of sudden deaths under the age of 18 occur in infants [75]. The parents of these children are still in their reproductive years; if WES or WGS is performed, secondary findings and carrier status may be identified which can have implications for future pregnancies.

Conclusions and Future Directions

The accessibility of genetic testing is rapidly improving, due to more efficient technology and decreased costs. To implement PMGT as a routine part of the forensic autopsy will require increased MEC and consumer education. Creating connections with local hospitals will help MEC refer families to clinical genetics providers and other medical specialists for follow-up, and to stay updated on developments in the field. Broad population-based approaches to genetic testing have already helped to identify pre-clinical or undiagnosed heritable conditions [93]. Additional research is needed to identify more candidate genes for many hereditary diseases, which will in turn improve the yield of PMGT. Several heritable diseases are now recognized as being polygenic (influenced by multiple genes, rather than a single-gene trait) [94]. Newer testing methods and computational tools are also in development, which may further the identification of new variants and better predict their pathologic status [11, 95].

As the understanding of these diseases expands, the need for PMGT will also expand. Eventually, the authors of this paper hope that PMGT becomes universally accessible for all deaths, both natural and unnatural.

Final Recommendations

1. PMGT is becoming increasingly affordable. Identifying heritable disease at autopsy is important to enable families to access preventative care. All MEC offices are encouraged to establish relationships with local or regional hospitals in the event families need referrals or other expert consultation.
2. MEC offices are recommended to pre-emptively establish relationships with a company or institution capable of providing PMGT, to include acceptable sample types and establishing expected costs. MEC should be offered the same price point as “self-pay” or “out of pocket” patients (or lower).
3. Governing bodies which control budgets for MEC offices should prioritize the inclusion of adequate funding for PMGT.
4. It is recommended that a sample for genetic testing is proactively saved during each autopsy, regardless of whether indications for testing are known at the time. The sample type and duration of storage will be determined by the needs and resources of the office. If the MEC investigation is completed without PMGT, it is recommended that the next of kin be given access to the sample for independent pursuit of PMGT, if requested.
5. Establishing multidisciplinary teams involving forensic pathologists, clinical genetics providers, and other clinical experts can help to improve the diagnostic potential of PMGT and improve the screening and care provided to the surviving family.
6. Ideally, the MEC office will attempt to notify families of the potential for hereditary disease in the setting of premature atherosclerosis, possible inborn errors of metabolism, sudden and unexplained deaths, cardiomyopathy, suspected channelopathy, thoracic aortic dissections or ruptures, pulmonary thromboemboli without associated risk factors, sudden unexpected death in epilepsy, or any other situation where genetic disease is strongly suspected. Additional resources, such as improved access to clinical genetics providers and increased administrative support, may be necessary to accomplish or optimize this.
7. Developers of continuing education for forensic pathologists, medical examiners, and coroners may consider topics regarding molecular pathology, genetic counseling, and

hereditary diseases, to ensure optimum utilization of resources as the cost and ability of testing changes over time.

References:

1. Middleton O, B.S., Demo E, Honeywell C, Jentzen J, Miller F, Pinckard JK, Reichard RR, Rutberg J, Stacy C, MacLeod H, *National Association of Medical Examiners Position Paper: Retaining postmortem samples for genetic testing.* Academic Forensic Pathology, 2013. **3**(2): p. 191-194.
2. Nurk, S., et al., *The complete sequence of a human genome.* Science, 2022. **376**(6588): p. 44-53.
3. Services, G.A.T.N.Y.-M.-A.C.f.G.a.N.S., *Genetics 101*, in *Understanding Genetics: A New York, Mid-Atlantic Guide for Parents and Health Professionals*. 2009, Genetics Alliance: Washington DC.
4. Allentoft, M.E., et al., *The half-life of DNA in bone: measuring decay kinetics in 158 dated fossils.* Proc Biol Sci, 2012. **279**(1748): p. 4724-33.
5. Karni, M., et al., *Thermal degradation of DNA.* DNA Cell Biol, 2013. **32**(6): p. 298-301.
6. Children, H.R.a.S.A.A.C.o.H.D.i.N.a., *Recommended Uniform Screening Panel.* 2024.
7. Trask, B.J., *Human cytogenetics: 46 chromosomes, 46 years and counting.* Nat Rev Genet, 2002. **3**(10): p. 769-78.
8. Levy, B. and R. Wapner, *Prenatal diagnosis by chromosomal microarray analysis.* Fertil Steril, 2018. **109**(2): p. 201-212.
9. Satam, H., et al., *Next-Generation Sequencing Technology: Current Trends and Advancements.* Biology (Basel), 2023. **12**(7).
10. Wojcik, M.H., et al., *Genome Sequencing for Diagnosing Rare Diseases.* N Engl J Med, 2024. **390**(21): p. 1985-1997.
11. van Karnebeek, C.D.M., et al., *Leaving no patient behind! Expert recommendation in the use of innovative technologies for diagnosing rare diseases.* Orphanet J Rare Dis, 2024. **19**(1): p. 357.
12. Martinez-Barrios, E., et al., *Molecular autopsy: Twenty years of post-mortem diagnosis in sudden cardiac death.* Front Med (Lausanne), 2023. **10**: p. 1118585.
13. Institute, N.H.G.R. *The cost of sequencing a human genome*. 2021.
14. Kentucky, S.o., *Kentucky Revised Statutes Chapter 72: Coroners, Inquests, and Medical Examinations.* 2023.
15. Kelly, K.L., et al., *Sudden cardiac death in the young: A consensus statement on recommended practices for cardiac examination by pathologists from the Society for Cardiovascular Pathology.* Cardiovasc Pathol, 2023. **63**: p. 107497.
16. Barbosa-Gouveia, S., et al., *Utility of Gene Panels for the Diagnosis of Inborn Errors of Metabolism in a Metabolic Reference Center.* Genes (Basel), 2021. **12**(8).
17. Ferreira, C.R., et al., *A proposed nosology of inborn errors of metabolism.* Genet Med, 2019. **21**(1): p. 102-106.
18. Sue, C.M., et al., *Neonatal presentations of mitochondrial metabolic disorders.* Semin Perinatol, 1999. **23**(2): p. 113-24.
19. van Rijt, W.J., et al., *Inborn Errors of Metabolism That Cause Sudden Infant Death: A Systematic Review with Implications for Population Neonatal Screening Programmes.* Neonatology, 2016. **109**(4): p. 297-302.
20. Emery, J.L., et al., *Investigation of inborn errors of metabolism in unexpected infant deaths.* Lancet, 1988. **2**(8601): p. 29-31.

21. Fu, F., et al., *Whole exome sequencing as a diagnostic adjunct to clinical testing in fetuses with structural abnormalities.* Ultrasound Obstet Gynecol, 2018. **51**(4): p. 493-502.
22. Qi, Q., et al., *Whole-genome sequencing analysis in fetal structural anomalies: novel phenotype-genotype discoveries.* Ultrasound Obstet Gynecol, 2024. **63**(5): p. 664-671.
23. Ezekian, J.E., et al., *Interpretation of Incidental Genetic Findings Localizing to Genes Associated With Cardiac Channelopathies and Cardiomyopathies.* Circ Genom Precis Med, 2021. **14**(4): p. e003200.
24. Stiles, M.K., et al., *2020 APHRS/HRS expert consensus statement on the investigation of decedents with sudden unexplained death and patients with sudden cardiac arrest, and of their families.* J Arrhythm, 2021. **37**(3): p. 481-534.
25. Lahrouchi, N., et al., *The yield of postmortem genetic testing in sudden death cases with structural findings at autopsy.* Eur J Hum Genet, 2020. **28**(1): p. 17-22.
26. Basso, C., et al., *Cardiac hypertrophy at autopsy.* Virchows Arch, 2021. **479**(1): p. 79-94.
27. Japp, A.G., et al., *The Diagnosis and Evaluation of Dilated Cardiomyopathy.* J Am Coll Cardiol, 2016. **67**(25): p. 2996-3010.
28. Aoki, J., et al., *Clinical and pathologic characteristics of dilated cardiomyopathy in hemodialysis patients.* Kidney Int, 2005. **67**(1): p. 333-40.
29. Bueno-Beti, C. and A. Asimaki, *Histopathological Features and Protein Markers of Arrhythmogenic Cardiomyopathy.* Front Cardiovasc Med, 2021. **8**: p. 746321.
30. Thiene, G., et al., *Right ventricular cardiomyopathy and sudden death in young people.* N Engl J Med, 1988. **318**(3): p. 129-33.
31. Kontorovich, A.R., et al., *Myopathic Cardiac Genotypes Increase Risk for Myocarditis.* JACC Basic Transl Sci, 2021. **6**(7): p. 584-592.
32. Albornoz, G., et al., *Familial thoracic aortic aneurysms and dissections--incidence, modes of inheritance, and phenotypic patterns.* Ann Thorac Surg, 2006. **82**(4): p. 1400-5.
33. Biddinger, A., et al., *Familial thoracic aortic dilatations and dissections: a case control study.* J Vasc Surg, 1997. **25**(3): p. 506-11.
34. Chou, A.S., et al., *Do Familial Aortic Dissections Tend to Occur at the Same Age?* Ann Thorac Surg, 2017. **103**(2): p. 546-550.
35. Coady, M.A., et al., *Familial patterns of thoracic aortic aneurysms.* Arch Surg, 1999. **134**(4): p. 361-7.
36. Saxton, S., et al., *Molecular genetic characterization of sudden deaths due to thoracic aortic dissection or rupture.* Cardiovasc Pathol, 2023. **65**: p. 107540.
37. Isselbacher, E.M., et al., *2022 ACC/AHA Guideline for the Diagnosis and Management of Aortic Disease: A Report of the American Heart Association/American College of Cardiology Joint Committee on Clinical Practice Guidelines.* Circulation, 2022. **146**(24): p. e334-e482.
38. Krywanczyk, A., et al., *Thoracic Aortic Aneurysm and Dissection: Review and Recommendations for Evaluation.* Am J Forensic Med Pathol, 2023. **44**(2): p. 69-76.
39. Miller, E.J., M.B. Marques, and G.T. Simmons, *Etiology of pulmonary thromboembolism in the absence of commonly recognized risk factors.* Am J Forensic Med Pathol, 2003. **24**(4): p. 329-33.
40. Ely, S.F. and J.R. Gill, *Fatal pulmonary thromboembolism and hereditary thrombophilias.* J Forensic Sci, 2005. **50**(2): p. 411-8.
41. Kohlmeier, R.E., et al., *Prothrombin gene mutation uncommon in pulmonary embolism.* South Med J, 2000. **93**(11): p. 1073-7.
42. Rulon, J.J., et al., *Activated protein C resistance is uncommon in sudden death due to pulmonary embolism.* J Forensic Sci, 1999. **44**(6): p. 1111-3.

43. Halvorsen, M., et al., *Whole Exome Sequencing Reveals Severe Thrombophilia in Acute Unprovoked Idiopathic Fatal Pulmonary Embolism.* EBioMedicine, 2017. **17**: p. 95-100.
44. Hudzik, B., J. Szkodzinski, and L. Polonski, *Testing for antiphospholipid antibodies at autopsy.* Forensic Sci Med Pathol, 2014. **10**(2): p. 288-9.
45. Kang, B.E., et al., *Venous thromboembolism laboratory testing (factor V Leiden and factor II c. *97G>A), 2025 revision: A technical standard of the American College of Medical Genetics and Genomics (ACMG).* Genet Med, 2025. **27**(8): p. 101466.
46. Croles, F.N., et al., *Pregnancy, thrombophilia, and the risk of a first venous thrombosis: systematic review and bayesian meta-analysis.* BMJ, 2017. **359**: p. j4452.
47. Bagnall, R.D., et al., *A Prospective Study of Sudden Cardiac Death among Children and Young Adults.* N Engl J Med, 2016. **374**(25): p. 2441-52.
48. Buchanan, A.H., et al., *Clinical outcomes of a genomic screening program for actionable genetic conditions.* Genet Med, 2020. **22**(11): p. 1874-1882.
49. Nordestgaard, B.G., et al., *Familial hypercholesterolaemia is underdiagnosed and undertreated in the general population: guidance for clinicians to prevent coronary heart disease: consensus statement of the European Atherosclerosis Society.* Eur Heart J, 2013. **34**(45): p. 3478-90a.
50. Abifadel, M. and C. Boileau, *Genetic and molecular architecture of familial hypercholesterolemia.* J Intern Med, 2023. **293**(2): p. 144-165.
51. Lahrouchi, N., et al., *Utility of Post-Mortem Genetic Testing in Cases of Sudden Arrhythmic Death Syndrome.* J Am Coll Cardiol, 2017. **69**(17): p. 2134-2145.
52. Webster, G., et al., *Genomic Autopsy of Sudden Deaths in Young Individuals.* JAMA Cardiol, 2021. **6**(11): p. 1247-1256.
53. Bundock EA, C.T., *Unexplained Pediatric Deaths: Investigation, Certification, and Family Needs.* . 2019, Academic Forensic Pathology International: San Diego, CA.
54. Corrado, D., et al., *Does sports activity enhance the risk of sudden death in adolescents and young adults?* J Am Coll Cardiol, 2003. **42**(11): p. 1959-63.
55. Eckart, R.E., et al., *Sudden death in young adults: a 25-year review of autopsies in military recruits.* Ann Intern Med, 2004. **141**(11): p. 829-34.
56. Winkel, B.G., et al., *Nationwide study of sudden cardiac death in persons aged 1-35 years.* Eur Heart J, 2011. **32**(8): p. 983-90.
57. Basso, C., et al., *Sudden cardiac death with normal heart: molecular autopsy.* Cardiovasc Pathol, 2010. **19**(6): p. 321-5.
58. Skinner, J.R., et al., *Prospective, population-based long QT molecular autopsy study of postmortem negative sudden death in 1 to 40 year olds.* Heart Rhythm, 2011. **8**(3): p. 412-9.
59. Corrado, D., C. Basso, and G. Thiene, *Sudden cardiac death in young people with apparently normal heart.* Cardiovasc Res, 2001. **50**(2): p. 399-408.
60. Morentin, B., M.P. Suarez-Mier, and B. Aguilera, *Sudden unexplained death among persons 1-35 years old.* Forensic Sci Int, 2003. **135**(3): p. 213-7.
61. Margey, R., et al., *Sudden cardiac death in 14- to 35-year olds in Ireland from 2005 to 2007: a retrospective registry.* Europace, 2011. **13**(10): p. 1411-8.
62. Puranik, R., et al., *Sudden death in the young.* Heart Rhythm, 2005. **2**(12): p. 1277-82.
63. Doolan, A., N. Langlois, and C. Semsarian, *Causes of sudden cardiac death in young Australians.* Med J Aust, 2004. **180**(3): p. 110-2.
64. de Noronha, S.V., et al., *The importance of specialist cardiac histopathological examination in the investigation of young sudden cardiac deaths.* Europace, 2014. **16**(6): p. 899-907.
65. Middleton, O.L., et al., *National Association of Medical Examiners Position Paper: Recommendations for the Investigation and Certification of Deaths in People with Epilepsy.* Acad Forensic Pathol, 2018. **8**(1): p. 119-135.

66. Ha, F.J., et al., *Epilepsy and Cardiac Arrhythmias: A State-of-the-Art Review.* JACC Clin Electrophysiol, 2025. **11**(1): p. 217-229.
67. Wang, J., et al., *Epilepsy and long-term risk of arrhythmias.* Eur Heart J, 2023. **44**(35): p. 3374-3382.
68. Chahal, C.A.A., et al., *Systematic Review of the Genetics of Sudden Unexpected Death in Epilepsy: Potential Overlap With Sudden Cardiac Death and Arrhythmia-Related Genes.* J Am Heart Assoc, 2020. **9**(1): p. e012264.
69. Mellor, G., et al., *Clinical characteristics and circumstances of death in the sudden arrhythmic death syndrome.* Circ Arrhythm Electrophysiol, 2014. **7**(6): p. 1078-83.
70. Feldman, A.E. and B.E. Gidal, *QTc prolongation by antiepileptic drugs and the risk of torsade de pointes in patients with epilepsy.* Epilepsy Behav, 2013. **26**(3): p. 421-6.
71. Parks, S.E., et al., *Explaining Sudden Unexpected Infant Deaths, 2011-2017.* Pediatrics, 2021. **147**(5).
72. Erck Lambert, A.B., et al., *Characteristics of Sudden Unexpected Infant Deaths on Shared and Nonshared Sleep Surfaces.* Pediatrics, 2024. **153**(3).
73. Tester, D.J., et al., *Exome-Wide Rare Variant Analyses in Sudden Infant Death Syndrome.* J Pediatr, 2018. **203**: p. 423-428 e11.
74. Puckelwartz, M.J., et al., *The impact of damaging epilepsy and cardiac genetic variant burden in sudden death in the young.* Genome Med, 2024. **16**(1): p. 13.
75. Burns, K.M., et al., *Epidemiology of Sudden Death in a Population-Based Study of Infants and Children.* J Pediatr X, 2020. **2**.
76. Hofman, N., et al., *Yield of molecular and clinical testing for arrhythmia syndromes: report of 15 years' experience.* Circulation, 2013. **128**(14): p. 1513-21.
77. Halvorsen, M., et al., *De novo mutations in childhood cases of sudden unexplained death that disrupt intracellular Ca(2+) regulation.* Proc Natl Acad Sci U S A, 2021. **118**(52).
78. Richards, S., et al., *Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology.* Genet Med, 2015. **17**(5): p. 405-24.
79. Chandrashekar, P., et al., *Prevalence and Outcomes of p.Val142Ile TTR Amyloidosis Cardiomyopathy: A Systematic Review.* Circ Genom Precis Med, 2021. **14**(5): p. e003356.
80. Odgerel, Z., et al., *DNA sequencing errors in molecular diagnostics of filamin myopathy.* Clin Chem Lab Med, 2010. **48**(10): p. 1409-14.
81. Chen, E., et al., *Rates and Classification of Variants of Uncertain Significance in Hereditary Disease Genetic Testing.* JAMA Netw Open, 2023. **6**(10): p. e2339571.
82. Counselors, N.S.o.G. *Secondary and Incidental Findings in Genetic Testing*. 2023.
83. Green, R.C., et al., *ACMG recommendations for reporting of incidental findings in clinical exome and genome sequencing.* Genet Med, 2013. **15**(7): p. 565-74.
84. Miller, D.T., et al., *ACMG SF v3.2 list for reporting of secondary findings in clinical exome and genome sequencing: A policy statement of the American College of Medical Genetics and Genomics (ACMG).* Genet Med, 2023. **25**(8): p. 100866.
85. Duncanson ER, L.K., Baker A, Abdelhadi RH, Mackey-Bojack SM, *Establishing a Multidisciplinary Network for the Workup of Sudden Cardiac Death.* Acad Forensic Pathol, 2015. **5**(2).
86. Hershberger, R.E., et al., *Genetic Evaluation of Cardiomyopathy-A Heart Failure Society of America Practice Guideline.* J Card Fail, 2018. **24**(5): p. 281-302.
87. McGuire, A.L., et al., *The ethics of conducting molecular autopsies in cases of sudden death in the young.* Genome Res, 2016. **26**(9): p. 1165-9.

88. Elger, B.S., et al., *Sudden death: ethical and legal problems of post-mortem forensic genetic testing for hereditary cardiac diseases.* Clin Genet, 2010. **77**(3): p. 287-92.
89. Kannankeril, P.J., et al., *Family Screening After Sudden Death in a Population-Based Study of Children.* Pediatrics, 2022. **149**(4).
90. MacLeod H, D.E., Honeywell C, Rutberg J, *Genetic counselors: An important resource for families following a young sudden cardiac death.* Acad Forensic Pathol, 2013. **3**(2).
91. Priori, S.G., et al., *Executive summary: HRS/EHRA/APHRS expert consensus statement on the diagnosis and management of patients with inherited primary arrhythmia syndromes.* Heart Rhythm, 2013. **10**(12): p. e85-108.
92. Gray, B., et al., *Influence of age and sex on the diagnostic yield of inherited cardiac conditions in sudden arrhythmic death syndrome decedents.* Eur J Prev Cardiol, 2024.
93. Forrest, I.S., et al., *Genome-first evaluation with exome sequence and clinical data uncovers underdiagnosed genetic disorders in a large healthcare system.* Cell Rep Med, 2024. **5**(5): p. 101518.
94. Coll, M., et al., *Incomplete Penetrance and Variable Expressivity: Hallmarks in Channelopathies Associated with Sudden Cardiac Death.* Biology (Basel), 2017. **7**(1).
95. Karchin, R., et al., *Improving transparency of computational tools for variant effect prediction.* Nat Genet, 2024. **56**(7): p. 1324-1326.

Table 1: Definitions of Common Genetics Terminology

Term	Definition
Read depth	The number of times a single base pair is sequenced by a specific test
Coverage	The percentage of the overall genome sequenced by a specific test
Secondary Finding	A pathogenic/likely pathogenic variant identified in a gene which is typically sequenced during the requested testing, yet is ultimately unrelated to the primary reason for testing
Incidental Finding	An finding of genetic testing which is both unexpected and unrelated to the primary reason for testing
De novo variant	A genetic change occurring for the first time in the proband/decedent. Does not appear in previous generations, and is not inherited from the parents.
Trio sequencing	This is an option with exome and genome sequencing. It involves genetic testing of the proband (decedent) and both biological parents to identify pathogenic and likely pathogenic variants, which makes interpretation of variants easier. For example it can help to determine if a variant is de novo in the proband, and can aid interpretation of the variant's pathogenicity.
SCD	Sudden cardiac death: death within one hour of the onset of symptoms, or within 24 hours of the time last known alive (if the death was unwitnessed)
SCD-Y	Sudden cardiac death in the young (less than 35 years of age)

SUID	Sudden unexplained infant death: Death younger than 12 months of age. When unexplained despite thorough investigation, including autopsy and scene re-enactment, these may be referred to as “SIDS” (sudden infant death syndrome).
SUDC	Sudden unexplained death in childhood: Death between 12 months and 18 years of age which remains unexplained despite complete autopsy, toxicology, and review of the scene and medical history
SUDEP	Sudden unexpected death in epilepsy: death in a patient with epilepsy which is not due to trauma, drowning, status epilepticus, or other known causes, but for which there is often evidence of an associated seizure

Table 2: Comparison of the Types of Genetic Testing

	Read depth	Coverage	Cost	Turn-around-time	Advantages/Disadvantages
Biochemical Genetics	N/A				
Cytogenetics	N/A				
Targeted (single gene or multiple gene) panel	>500x	10-500 genes	$250-$300	Days	Smallest number of VUS* Does not include genes which are not yet known to be clinically significant
Whole Exome Sequencing (WES)	>50-100x	Entire exome (20-25k genes)	$1500-$3000	Months	Increased secondary findings and VUSs
Whole Genome Sequencing (WGS)	>30x	All genes and non-coding DNA	Most expensive option	Variable	Most VUSs and secondary findings

**variant of uncertain significance*

Table 3: Options of sample types for postmortem genetic testing

Genetic Testing Type	Testing Methods	Postmortem Samples	Collection Materials	Storage	Shipping	DNA yield
Molecular Testing	Panels, whole exome sequencing, whole genome sequencing	Liquid blood	Whole blood 10-15 mL, purple tube (EDTA)	< 4° C short term (< 6months); < -20° C long term	Same as storage	High
		Tissues in RNAlater	Coin size tissue in RNAlater	Room temperature 1 week; 4° C one month; < -20° C long term	Same as storage	High
		Tissues (without media)	Coin size tissue in collection tube	< -20° C long term	Same as storage	High
		Buccal swabs	Rinse mouth with water; ORAcollect•Dx kit	Room temperature 2 years; frozen long term	Same as storage	Medium to high
		Bone marrow	In RNAlater, or frozen	In RNAlater: Room temperature 1 week; 4° C one month; < -20° C long term	Same as storage	Medium to high
		Bone	Collection tube	Frozen	Same as storage	Medium to high
		Bloodstain cards	Thoroughly dry on Filter paper (e.g. Watman)	Room temperature indefinite	Room temperature	Low to medium
		DNA banking	Collection tube	4°C for up to 12 months; long term <-20°C	Same as storage	Medium to high
		FF/FFPE samples	The last source. Artifacts in results not amenable for confirmation by Sanger sequencing.			
Metabolic Testing	Metabolic Screening	Bloodstain cards	Thoroughly dry on Filter paper (e.g. Watman)	Room temperature indefinite		N/A
		Body fluids (bile, plasma, urine)	Collection tubes	< 4° C short term (< 6months); < -20° C long term	Same as storage	
	Enzymatic Activities	Tissues (liver or muscle)	Collection tubes	Frozen	Same as storage	

Cytogenetics	Karotyping, Fluorescence in-situ hybridization (FISH), microarray	Liquid blood	Whole blood 10-15 mL, purple tube (EDTA)	4° C short term (< 7 days)	Same as storage	N/A
		Cultured tissue cells	Contact lab for specific requirements			
Indications unknown		Bloodstain cards	Thoroughly dry on filter paper (e.g. Watman)	Room temperature, indefinite		Low to medium
		Bone marrow, or bone	In RNAlater or frozen	In RNAlater : room temperature 1 week; 4° C one months; < -20° C long term	Same as storage	Medium to high

Table 4: When to consider postmortem genetic testing (PMGT)

Diagnosis at Autopsy of:

- Cardiomyopathy
- Premature coronary artery disease*
    - Under the age of 55 for men, 60 for women.

    **May be tested by evaluating lipid levels in biologic relatives.*
- Aortic dissection/aneurysm
    - Under the age of 60.
    - Findings concerning for genetic aortic disease.
    - Family history of aortic dissection/aneurysm.
- Pulmonary thromboembolism (PTE)
    - Unprovoked PTE under the age of 50, recurrent PTE, PTE in the setting of pregnancy or hormonal contraceptive use, and/or thrombosis of an unusual site.

Sudden or Unexpected Death including:

- SUID
- SUDC
- SUDEP
- Autopsy-negative sudden cardiac death (SCD)

Suspected inborn error of metabolism with hypotonia, failure to thrive, seizures, cardiac arrhythmias or cardiomyopathies, lactic acidosis, and/or neuromuscular manifestations.

Congenital anomalies/dysmorphic features

Family History of:

- Sudden death less than age 50
- Heart disease less than age 50

Fetal Demise or Stillbirth, especially in the presence of congenital anomalies

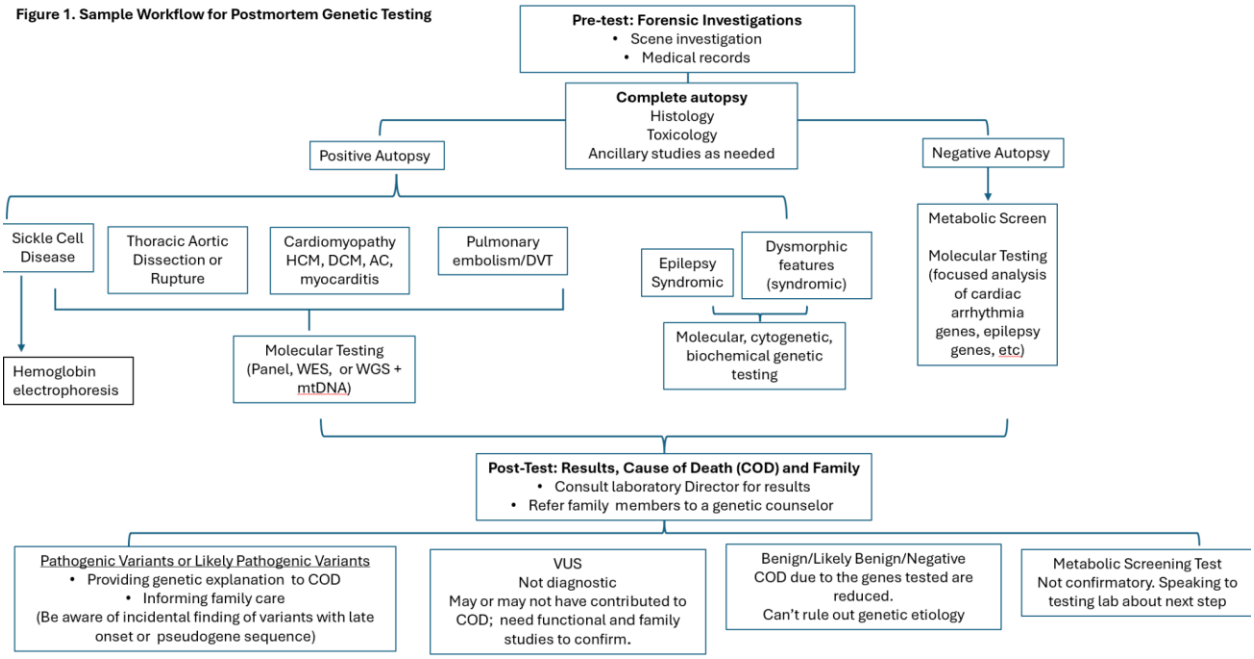
Other Known or Suspected Genetic Diagnosis:

- If there is a known genetic diagnosis in the family, postmortem testing can help confirm or rule out the same condition in the deceased.
- Genetic diagnoses and syndromes which are less likely to cause sudden and unexpected death, but may still present at forensic autopsy (e.g. hereditary cancer syndromes)

Table 5: ACMG and AMP Classification Guidelines [78]

Classification	**Interpretation for the forensic pathologist**
Pathogenic **Likely pathogenic**	*If associated with the phenotype and/or cause of death identified at autopsy, this variant is likely to have played a role in the death.*
Variant of uncertain significance (VUS)	*Significance is uncertain either due to a lack of evidence or the presence of conflicting evidence. May or may not be related to the cause of death; further investigation is needed for appropriate interpretation. May be reclassified as pathogenic or benign in the future with additional data.*
Likely benign **Benign**	*No known pathogenic genetic variants identified. Note that this does not entirely exclude a genetic basis for the phenotype/cause of death identified at autopsy, as the full spectrum of genes associated with disease is not fully elucidated yet.*

Figure 1. Sample Workflow for Postmortem Genetic Testing



Appendix A: Sample notification letters

1. Addressed to family/next of kin:

We are so sorry for the recent sudden loss of [DECEDENT'S NAME].

We are concerned that a genetic disorder may be present in your family.

We recommend you share this information with your primary care physician. We also recommend biologic relatives (siblings or children) be seen by a cardiologist for clinical evaluation for heart disease.

We have identified that [BLANK] is a local cardiologist who specializes in irregular heart rhythms and sudden death who can evaluate adults in the family:

BLANK, MD
ADDRESS
PHONE NUMBER

We are also sending a letter to [decedent's primary care provider], in case they know of a closer pediatric cardiology clinic.

Genetic testing has been sent for [DECEDENT'S NAME], and we will contact you when results are available.

We will mail you this information as well so you can share it with your doctors.

2. Addressed to Primary Care Provider:

Dear [PROVIDER]

On behalf of the [ME/C OFFICE/HOSPITAL], we would like to notify you that your patient, [NAME AND DOB], passed away suddenly and unexpectedly on [DATE]. At this time the autopsy is still pending. The final death certificate and autopsy report may not be available for several months due to the time required to complete all necessary diagnostic tests. [If known, add additional family history, autopsy findings, circumstances]. We are concerned about this family.

Based on current Heart Rhythm Society guidelines (attached) family members should be referred for cardiac evaluation including siblings and parents. If you are in touch with this family or the sibling are under your care, it is important they be seen as soon as possible for an echocardiogram and EKG by a cardiologist who is knowledgeable about inherited heart disease. We are sending this information directly to the parents as well.

We have identified that [BLANK, ADDRESS, PHONE NUMBER] can see the siblings for cardiac screening and refer the parents for cardiac screening.

[IF A PEDIATRIC DEATH]:

In, addition, this case will be reviewed by child death review and we invite you to attend this review with the goal of decreasing the number of deaths in children. It would benefit this committee, if you as the provider, would bring any significant information relevant to the care of the child to your patient's review.

Once the autopsy of your patient is finalized, the program would like to invite you to attend the case review. A member of the SUID and SDY committee will be contacting you to discuss the importance of your attendance in the upcoming weeks.

Please save the date, as your patient will be reviewed at one of the following dates:

DATE
DATE

*Each case review is approximately 30 minutes, your attendance at the entire case review is not necessary *

Sincerely,

BLANK

Appendix B: Suggested Wording for Cause of Death Statements with Genetic Test Results

Case #1:
A 35-year-old man was found deceased in bed after a night light alcohol consumption with roommates. Per roommates and family, he engaged in social alcohol use, occasional vaping, and no illicit drug use. Full investigation, autopsy, microscopy, and toxicology (BAC 0.032%; Vit EtOH 59 mg/dL; caffeine positive) was performed. Autopsy identified transmural fibrofatty replacement of right ventricular wall with thinning of the compact zone. No injuries or other potentially lethal natural diseases were noted. A panel of genes associated with arrhythmia and cardiomyopathy identified a pathogenic variant of *PKP2*. Death was certified as:

Part I. Cause of Death
A. Arrhythmogenic cardiomyopathy.
B. Pathogenic *PKP2* gene variant.

Manner of Death: Natural.

Case #2:

A 19-year-old man was found unresponsive supine in bed. Medical history significant for epilepsy, first diagnosed at age 7, with his last grand mal seizure two days prior. History of poor medication adherence, and old filled bottles of prescription antiepileptic medications were on scene. Full autopsy, including specialist neuropathology evaluation of the brain, and toxicology was performed. Autopsy showed moderate pulmonary edema and a tongue contusion. Neuropathology revealed subcortical band heterotopia; toxicology was remarkable for the absence of anti-epileptic medications. Genetic analysis for genes associated with epilepsy and brain malformations identified a likely pathogenic variant in DCX. Death was certified as:

Part I. Cause of Death
A. Epilepsy
B. Subcortical band heterotopia.
C. Likely pathogenic *DCX* gene variant.

Manner of Death: Natural

Case #3:

A 23-year-old man collapsed while playing basketball and was unable to be resuscitated. He had sustained no physical injuries and was not struck at any time during the game. In the preceding months he had complained of occasional lightheadedness and “palpitations”, but this was attributed to poor sleep quality. A full investigation including autopsy, toxicology (caffeine positive) and microscopy was performed. At autopsy the heart was markedly enlarged with asymmetric hypertrophy of the interventricular septum (left ventricle: 1.2 cm, interventricular septum: 1.5 cm). White fibrous endocardial thickening was noted in the left ventricle outflow tract. The coronary arteries were unremarkable in course and were without atherosclerosis. Microscopic analysis showed myocyte hypertrophy with interstitial and perivascular fibrosis, conspicuous dysplastic small intramyocardial arteries, and prominent myocyte disarray in the interventricular septum. Genetic analysis for genes associated with arrhythmia and cardiomyopathy identified a variant of

uncertain significance in *MYH7*. The biologic relatives were contacted and encouraged to see a cardiologist and genetic counselor. Copies of the test results were sent to the relatives, and to the clinicians they opted to see. Because of the uncertain nature of the *MYH7* variant, death was certified as:

Part I. Cause of Death
A. Hypertrophic cardiomyopathy.

Manner of Death: Natural

Case #3, continued:
Months later, the clinical team caring for the family of the decedent contacts you. Based on the results of cascade testing of family members, in association with cardiac imaging studies, they will be reclassifying the *MYH7* variant as "likely pathogenic". The death certificate is amended to read:

Part I. Cause of Death
A. Hypertrophic cardiomyopathy.
B. Likely pathogenic *MYH7* gene variant.

Manner of death: Natural

Case #4
A 17-month-old white male with a medical history of mild developmental delay and recent febrile seizure (anamnestic) was found unresponsive in a playpen and was pronounced dead in the home by EMS. Autopsy showed mild pulmonary edema; specialist neuropathologic examination showed features suggestive of cerebral and hippocampal dysgenesis. Postmortem blood and cerebrospinal fluid cultures were non-informative, and respiratory viral panel on a nasopharyngeal swab was negative. Toxicology was unremarkable.
Postmortem genetic testing was performed, using two separate panels to include epilepsy-focused and cardiac-focused causes of sudden death. The cardiac-focused sudden death molecular analysis showed a heterozygous, likely pathogenic variant in the *DSP* gene. This variant was not considered to be contributory to the cause of death, because affected carriers live into adulthood before the onset of symptoms.

Cause Of Death: Sudden unexplained death in childhood
Manner Of Death: Natural