The Promise of Molecular Autopsy in Forensic Pathology Practice

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ABSTRACT

Molecular autopsy is changing the practice of forensic pathology. Under some circumstances, one must contemplate the involvement of genetic factors to help explain why someone has died unexpectedly. Such considerations most commonly occur when a young person dies by natural means. However, there are deaths that occur by nonnatural means that the forensic pathologist will be asked to investigate, which could involve natural disease that has a significant genetic underpinning. Elucidation of genetic mutations may not only further an understanding of the pathophysiology at hand, but also speak to underlying susceptibilities in an individual who dies that may not have been recognized. In addition, one may occasionally identify pathological findings that are confused for trauma that may actually be better explained by an underlying disease process. Using molecular medicine as a tool to explore such possibilities can improve the quality of death investigations and provide a new lens to probe challenging and contentious forensic cases that have proved resistant to traditional methods. *Acad Forensic Pathol. 2017 7(4): 551-566*

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INTRODUCTION

What is the Molecular Autopsy and How is it Currently Situated in the Greater Paradigm of Precision Medicine?

Molecular medicine continues to evolve and transform health care. The precision medicine initiative, launched by the National Institute of Health in 2015, provides a platform to develop protocols that better integrate clinical medicine with medical genetics and genomics. To this end, most subspecialties of medicine are actively developing and aligning their research initiatives as well as health care delivery efforts to harness the diagnostic clout that genetic analysis can provide. Precision medicine is an approach to disease management that considers lifestyle, environment, and genetic variability (through mutational analysis) to enhance patient-centered care and treatments targeted to individual needs. This approach is greatly enhanced by the development of large-scale databases and bioinformatics tools to evaluate individual and population genetic variability (1). Since release of the first complete human genome sequence in 2001 (2), we have rapidly come to appreciate the great complexity of our genome and its contribution to many forms of disease. Critical to incorporating such information into health care delivery is the availability of reliable historical information (including both personal and family histories), clinical and pathological data, ancillary studies, and under some circumstances, a postmortem examination of a deceased family member. Without these varied sources of data, it may not be possible to interpret the relevance of individual genetic findings.

This scientific and medical revolution does not exclude forensic medicine (3-9). The premise being proffered is that the investigation of criminally suspicious, accidental, or natural deaths may require elucidation of genetic conditions through the autopsy, which is further characterized through genetic testing. The enhanced information that may flow from consideration of clinical, pathological, and genetic information can identify individual susceptibilities and perhaps explain why an individual died under a given set of circumstances. In addition, such information may potentially provide explanatory value for other opinions proffered within a medicolegal context. Genetic information is not only potentially important for the death investigation at hand, but the circumstances under which a person dies suddenly and unexpectedly may be the first manifestation of disease in a particular family. Thus, there is an ethical duty as physicians to recommend referral of relevant surviving family members to help prevent further deaths.

Numerous commercially available genetic tests are now available for a rapidly growing range of conditions, the results of which, when considered carefully and under the appropriate circumstances, may aid in the diagnosis of a given condition that is clinically (or pathologically) suspected. Interpretation requires consideration of the role of the gene product impacted by a particular mutation and evaluation within the context of the known pathophysiology of a disease entity. Mutations may take multiple forms that could include missense and nonsense mutations, alteration of splice sites, frameshift mutations, nucleotide repeat expansions, small insertions and deletions, or copy number variations. Genetic information may influence treatment decisions, prognosis, or allow cascade screening of relevant family members. An understanding of pathophysiology is also advanced when systematically evaluating these deaths in the context of relevant genetic mutations, whether by natural or nonnatural means. This approach to death investigation can enhance our understanding of how and why our citizens die, which is not only necessary to discharge our responsibilities to our community, but also helps to protect surviving family members, support our clinical colleagues, and advance science.

Nevertheless, great caution should be exercised in integrating the principals of molecular medicine within forensic pathology practice through a molecular autopsy. Genetic testing should only be performed when carefully considered. One should be sensitive to the concept of "genetic purgatory," whereby a genetic variant that is identified is neither necessarily pathogenic nor clinically actionable, yet its significance for the family is unclear. This may lead to anxiety for the

surviving family and frustration for genetic clinics tasked with managing the care of the family going forward (10). In addition, inappropriate use of genetic testing, inappropriate test selection, and misinterpretation of results are factors that can cause confusion for families, other investigators, and impede the work of our clinical colleagues in providing the best care for surviving family members. Thus, in general, the best outcomes arise when genetic testing is considered not only as an aide to answering questions arising from the death investigation, but also as part of a continuum of care with other clinical and genetic specialists who will manage the health care of the surviving families. There are enormous benefits of ongoing collaboration between death investigation systems and genetic clinics, including enhanced information sharing, cooperative trouble-shooting in difficult cases (e.g., detailed reviews of the pathology, exploring limitations of pathological interpretation, or contextualizing the pathology within the clinical and family history), potential for research partnerships, and ongoing education for the pathologist in the practical issues managing genetic disease.

The concepts of variable expressivity, incomplete penetrance, and the typical degree of genetic variation that are seen in many monogenetic conditions brings great complexity to establishing the relevance of specific genetic variants. This implies that not all potentially relevant variants are necessarily disease causing; one must carefully correlate with phenotype and history to ascertain if they are clinically relevant. The flip-side of this issue is that because of the ever-progressing nature of medical genetics and our improving understanding of pathophysiology, a negative result does not necessarily exclude a genetic condition from being present if the clinical (or pathological) suspicion is high. Relevant genetic variants in other genes that could be disease causing may be found at a later date. Some genetic conditions may be caused by mutations in two different genes that work synergistically to cause a particular phenotype (compound hemizygosity).

Making pathological (i.e., phenotypic) diagnoses in complex natural or nonnatural cases may be difficult. Part of this is due to the incomplete datasets available to the forensic pathologist regarding the quality of the medical history or the circumstances of a particular death. Thus, precisely defining the pathological phenotype may be problematic and genetic information should be evaluated by the pathologist and geneticist with great care. Like the case for clinical medicine, the greater the quality of information available, the greater the confidence one may have for pathological diagnoses proffered. For their part, clinicians and geneticists use established criteria to help define the relevance of specific genetic variants, such as those set out by the American College of Medical Genetics and Genomics (ACMG) (11, 12). Such guidelines enable geneticists and genetic counselors to use an evidence-based approach to interpreting genetic variants and guide further treatment.

DISCUSSION

How Might Molecular Autopsy Impact Forensic Pathology Practice?

First and foremost, consideration of genetic alterations will provide a new lens with which to evaluate disease, injury interpretation, and unexplained or controversial forensic "entities." At the case specific level, examples of challenging forensic investigations could include establishing which cases of apparent sudden cardiac death may have a genetic anomaly requiring collection and retention of a DNA source, establishing whether an unexplained subdural hemorrhage was the result of an unrecognized bleeding disorder or evidence of a criminal act (when it presents under suspicious circumstances), or ascertaining if a highly agitated individual who died suddenly and unexpectedly in the setting of police restraint had an underlying susceptibility for arrhythmia that was not otherwise explained.

At the phenomenon level, a molecular lens is expected to improve our understanding of some of the most challenging and controversial deaths that have defied further elucidation using traditional methods. In addition to those mentioned above, other examples might include some cases of sudden and unexplained deaths

in infants and children (SIDS/SUDI/SUDC) (13-16), sudden and unexplained deaths in epilepsy (SUDEP) (7, 17-19), triad cases in infants (20-23), as well as sudden death in individuals exhibiting features of excited delirium. This is not to indicate that a genetic anomaly is responsible for the signs and symptoms associated with excited delirium *per se*, but only that if an individual were to have an underlying genetic predisposition for an arrhythmia while in a state of great agitation, then this could help answer why some individuals die suddenly and unexpectedly when interacting with police. In these and other instances, the circumstantial and pathological findings are often not entirely determinative, yet forensic pathologists are asked to provide a cogent explanation for these deaths that are, at times, highly contentious and may be vetted at public inquest or in criminal justice proceedings. The molecular autopsy may assist in understanding these deaths if considered carefully and elucidated in a transparent manner.

Most deaths that the forensic pathologist is asked to evaluate will not be criminally suspicious or highly contentious. These cases include sudden and unexpected deaths of young people who are typically less than 40-50 years of age. In most instances, a clear structural cause of death is identified; however, in approximately 5% of cases (or more depending on the study) (7, 24) no anatomical or toxicological cause for death is found. In many of these instances, whether there is structural disease or not, there may be a genetic abnormality underlying the disease that needs to be recognized. Or, at the very least, the possibility that a genetic anomaly needs to be raised if the circumstantial and pathological findings cohere with a recognized genetic condition. Examples could include a thrombophilia in a young person with unexplained pulmonary thromboembolism, autosomal dominant polycystic kidney disease in a person with a ruptured berry aneurysm, metastatic disease in someone with an unsuspected cancer syndrome, or unexplained cardiac amyloidosis in a young person. The most common cases in young people that forensic pathologists need to consider as potentially harboring monogenic abnormalities are 1) unexplained aneurysms and dissections due to aortopathies/arteriopathies, 2) apparent sudden cardiac deaths in the absence of sufficient structural or toxicological disease to explain death (primary arrhythmia syndromes/conduction system disease), and 3) cardiomyopathies.

The pathologist also needs to keep in mind that genetic disease may yet be present even if the pathological condition is only potentially explained by an acquired disease process. While acquired diseases are generally more common than most genetic conditions, the pathologist must carefully weigh the facts to decide if a genetic condition needs to be excluded or investigated further by other specialists if the evidence is not clear. For instance, consider the sudden death of a healthy, young, obese individual with pulmonary thromboembolism that is otherwise unexplained. Is the degree of obesity and possible inactivity sufficient to likely explain the risk of deep vein thrombosis in this individual? Are there other findings identified at autopsy that may suggest a thrombophilia is present (e.g., evidence of multiple thromboses over time, thrombi in other vascular beds, or thrombosis of both arteries and veins)? Thus, many factors may need to be considered by the forensic pathologist when assessing the likelihood that a genetic condition may be present. This includes familiarity with the spectrum of risk factors to evaluate, the spectrum of pathological presentations that the pathologist may encounter, the epidemiology of the condition under consideration, as well as awareness of the range of common deleterious monogenic entities present in the population (such as Factor V Leiden or Prothrombin gene mutations) that may cause a given disease condition.

It is not rare that forensic pathologists will be confronted by the situation in which they are asked to perform an autopsy on a person where another family member also died by an unexplained etiology in the past. If the second death in the family is also unexplained, it may suggest an underlying genetic anomaly is present and needs to be evaluated. An example could include independent siblings who over a period of years die in the setting of bed sharing or as crib deaths or perhaps as adolescents who die suddenly and unexpectedly while asleep or while physically active. Under the best of circumstances, a DNA source from the first child

will still be available for genetic testing to potentially bolster the importance of any mutations identified in the second child. Additionally, even if the first person's death was not initially recognized as potentially having a heritable contribution, reassessment of the circumstances and pathological findings of the first death in light of the second may assist in characterizing what may be happening within this family.

What are the Issues to Consider When Setting Up a Molecular Autopsy Program in a Forensic Pathology Setting?

Characterize Clinical Phenotype as Much as Possible

This may be the most important contribution pathologists can make in supporting the successful interpretation of an actionable genetic variant. The issue of whether a genetic condition is potentially present must be engaged either before the autopsy, and thus considered by a death investigator or coroner who must decide whether to request an autopsy, or at time of autopsy itself by the pathologist. Engagement will inform the extent of the postmortem examination performed, what ancillary tests may be requested, and what consultants may be asked to assist in the diagnosis (**Table 1**). Directed activities may include special dissections, urine drug screens performed at the time of autopsy to triage a case, collection of a DNA source, requesting additional ancillary studies such as toxicology (as well as biochemistry and microbiology as circumstances dictate), retention of an organ for specialty cardiac or neuropathological consultation (25), ascertaining the quality of documentation needed for reviewability, and engaging other individuals early in the death investigation to elucidate additional information (e.g., family physicians, family members, pharmacists). Such inquiries could include targeted questions about personal and family medical histories, medications prescribed, the use of illicit drugs, and social history. Clinicians and genetic counselors often read pathology reports with great care when assessing surviving family members in order to extract as much phenotypic information available and, thus, such cases warrant a greater attention to detail.

A Detailed Autopsy is Necessary to Generate Exclusionary Data if a Clear Anatomical or Toxicological Cause for Death is Not Evident

This generally means that a full autopsy is warranted with a clear understanding of the scene and circumstances of death. Every forensic pathologist recognizes that there are a number of different causes for a "negative" autopsy. Understanding and evaluating

Table 1: Important Systemwide Activities That Can Support a Molecular Autopsy Program

Cardiovascular pathology and neuropathology service	Detailed consultation services to assist in characterizing structural disease, identify subtle disease phenotypes and pathological artifacts that may be over-interpreted as disease.
Dedicated DNA bank	Provides for a systematic process to obtain and store DNA under the appropriate conditions. Provides for long-term retention (many years) and requires a formal database to track samples. Continuity, sample integrity and quality assurance are important considerations.
Single point of contact for molecular autopsy program	The benefit of a single contact, such as a cardiovascular pathologist, is that all cases can be vetted prior to consideration for genetic testing, that there is a single contact for all external clinics to interface with the death investigation system to facilitate document and information retrieval (e.g. obtain documents, pathological reviews, or determine if a DNA source is available).
Close working relationship with regional genetics clinics / heritable heart disease clinics	Inter-professional collaboration is key to success of program and provides best outcomes for families and death investigation system. May include ongoing telephone and e-mail correspondence between relevant specialists on a case-by-case basis as well as periodic educational and work rounds. Also allows for ready subspecialty consultation for pathologists when needed in contentious and criminally suspicious cases.
Quality assurance program to review autopsies	If a quality assurance program exists within the death investigation system to review autopsies, then one is able to screen for cases that may represent missed opportunities to identify a genetic condition as well as to correct over-interpretation of findings that are interpreted as potentially genetic when they are unlikely to be.
Ongoing education for pathologists as well as death investigators / coroners	This is a key component in improving the quality of a molecular autopsy program, improving the identification of relevant cases for assessment and improving the management of relevant cases. This could include didactic lectures, practice guidelines, and workshops.

these are important to generate an exclusionary dataset and increases the pretest probability that one may be dealing with a primary arrhythmia syndrome in a sudden, unexplained death. As a practical point, we utilize bedside toxicological screens at the time of the postmortem examination to triage cases in order to ascertain if the heart may need a more detailed examination by a cardiovascular pathologist. In all cases, a formal toxicological assessment is still performed.

Develop a Process to Obtain and Bank DNA

If a pathologist suspects that a death may have an underlying genetic contribution, then collecting a sample of tissue or blood as a DNA source for the possibility of future testing is ethically sound. The best opportunity to collect DNA is at the time of autopsy. If feasible, a forensic pathology service could also extract and safely store the material in a dedicated DNA bank (whether it is submitted for genetic testing or not). Retention of DNA should be disclosed in the autopsy report. Formalin fixed and paraffin embedded tissues are generally not good substrates for genetic testing when genetic panels are being considered, although some laboratories have success with variant specific testing from paraffin blocks.

Select the Correct Genetic Testing Panels

In many jurisdictions, the forensic pathology service will not initiate genetic testing unless it clearly correlates with phenotype and is needed for the death investigation itself. This is usually due to fiscal considerations or lack of the appropriate expertise within the system to coordinate such testing. In Ontario, we perform approximately 7000 medicolegal postmortem examinations each year for a population of approximately 13 million residents. Forensic pathology delivery is a highly coordinated service across the province with a head office and multiple regional forensic pathology units. This administrative structure assists in coordinating our molecular autopsy program as a provincial initiative and facilitates collaboration with many heritable heart disease clinics and medical genetics clinics across the province. For the more common cases, molecular autopsy, when deemed appropriate, is generally a collaborative effort between the forensic pathology service and these clinics with ongoing adjustments made to meet the needs of the death investigation system and health care delivery for families (e.g., which commercial labs to use, what panels to use, special requests for genetic testing). One needs to be very cautious in striking a balance between limiting testing to relevant phenotypic considerations and keeping panels broad enough to capture differential diagnostic considerations. Most commercial laboratories provide genetic panels that include genes for the most common entities (as well as a number of rare conditions), which simplifies the approach to testing and are based on the clinical or pathological phenotype present.

Clearly Define the Issues in the Report of Postmortem Examination

In other words, clearly state that the pathological process identified may have a genetic and possibly heritable etiology, provide a detailed description of the pathology present, define the differential diagnostic considerations based on the nature of the case, and encourage first degree family members to consider assessment in the appropriate clinic. Also, indicate that DNA has been banked if needed in the future. It is also important that information from all relevant ancillary studies (such as toxicology) is described. It is recognized that some offices do not generally provide opinion statements in their reports; however, under circumstances where one believes that there may be a genetic condition present, it is recommended that such information be explicitly stated if possible, or provided as part of a supporting document that accompanies the autopsy report. As the autopsy report may be the only document provided to family or to a clinic, one does not want to lose the opportunity to effectively communicate the issues at hand, including the limitations on your opinions.

Recognize Limitations of the Pathologist and the Autopsy

We are pathologists, not geneticists. While pathologists have a prominent role to play in helping to iden-

tify genetic disease, full characterization of that disease requires many other specialists, which includes clinicians, geneticists, and genetic counselors. Even if genetic testing is undertaken by a forensic pathology service, first-degree family members require assessment in an appropriate clinic and genetic counseling, regardless of whether a genetic variant is identified or not. This is because not all genetic anomalies for each heritable condition are known.

If there is a clear pathological phenotype identified at autopsy that presents in the appropriate clinical context (e.g., cardiomyopathy, aortopathy, thrombophilia, spherocytosis), then providing an etiologically specific diagnosis is appropriate in the autopsy report. If, however, no etiologically specific cause for death is identified, then one must be careful in assuming that death was due to a sudden cardiac death. These autopsies should be complete and fairly comprehensive, the extent of which should be guided by the quality of the information available and considered in the context of all pathological findings identified at autopsy. When the circumstances of the death are well understood and it is highly likely after all classical investigations that an apparent death is likely due to a cardiac etiology, then our approach is to provide the cause of death as "unascertained," discuss the issues outlined above, and to refer the family for further assessment in a heritable heart disease clinic. The benefit to this approach is that there is only one diagnosis provided to the family (by the clinic) and it aligns the ultimate responsibility for managing the diagnosis with the people responsible for managing the care of families. Under circumstances where the cause of death is best explained as being cardiac in etiology, the advantage of a heritable heart disease clinic, if available, over a medical genetics clinic is that both a cardiologist and a geneticist/genetic counselor assess the family as a team. When genetic testing is important for a contentious death or criminally suspicious death and a variant of uncertain significance (VUS) is identified, then after considering all factors (including the variant-specific interpretation provided by the commercial laboratory, which should follow ACMG criteria), suggesting that the genetic anomaly identified might have played a role in that individual's death is not unreasonable in carefully constructed opinions. A forensic pathologist may also seek consultation with an appropriate clinical or genetic specialist to discuss the case. In sudden, natural deaths where a primary arrhythmia syndrome is thought likely, forensic pathologists are not encouraged to provide etiologically specific diagnoses given the complexity of interpreting genetic findings and are best left to the appropriate specialists. One must also allow for the possibility that the genetic variant identified is not clinically significant.

A Robust Mechanism to Communicate Results to Families (In Writing) is Necessary

This may also be followed up at a somewhat later date with a phone call to discuss the issues at hand. Families are encouraged to bring copies of the autopsy report and all relevant documents to their family physician to discuss the value of a referral to a subspecialty clinic. In our jurisdiction, in addition to written notice, we have also initiated a next of kin clinic, where we provide the opportunity for family members to visit our facility (or teleconference if preferred) and ask questions about the death investigation and the autopsy report. If genetic testing was performed, we indicate why such testing was done, but we do not interpret the results – this is best left to the appropriate specialists, as the genotype-phenotype correlation and interpreting genetic variants of uncertain significance can be quite challenging. As physicians, pathologists have an important part to play in conveying the significance of the pathological and genetic findings to family members, yet this should be seen as part of a continuum of care that will be taken over by the appropriate specialist clinic. Visiting with the family provides another opportunity to provide some clarity about what the autopsy can address, discuss the possibility of a genetic condition, the importance for families to be formally assessed, and provide contact information for subspecialty clinics in their region. The next of kin clinics are attended by a forensic pathologist as well as a family liaison coordinator and a dedicated member of the coronial service, who are able to provide support and follow-up on any relevant issues that may arise for the family following the death investigation.

Evaluating Sudden Cardiac Death in the Forensic Setting: Brief Synopsis of How Cardiovascular Genetics is Changing How We Think About Cardiovascular Pathology

Is it Actually a Sudden Cardiac Death and How Confident Are You in This Conclusion?

When someone is witnessed to die suddenly and unexpectedly, who had appeared well just a short time earlier, and for whom no anatomical or toxicological cause for death was identified outside of the heart after a detailed postmortem examination, then inferring that the death is due to a cardiac etiology is not unreasonable. In most cases that present to the forensic pathologist, these deaths exhibit underlying structural pathology that was due to a chronic disease process and the mechanism of death was likely arrhythmic. In people under the age of 50 years who die suddenly and unexpectedly, most will still exhibit severe, premature atherosclerotic coronary artery disease or hypertensive heart disease. Yet, in this age group, there is an enriched proportion of people with genetic cardiovascular disease that need special attention, particularly those under 35 years. The strength of this inference is increased the younger the individual was at the time of death, if there was a family history of premature cardiac death, and if there is a personal history of cardiac disease, unexplained syncope, presyncope, or seizures (26). Toxicological deaths from compounds that are novel and cannot be screened as part of typical drug testing may still be the best explanation for a particular death given the investigative information available, particularly if there is a significant history of drug abuse. Each case should be considered carefully to ascertain the best interpretation of the findings. However, if the circumstances are unclear and no anatomical or toxicological cause for death is identified, saving a source of DNA and discussing the possibility of a primary arrhythmia syndrome as part of the autopsy report is appropriate.

Prior to the autopsy, the pathologist will receive a detailed account of the scene and circumstances of the death and from such information recognize that a sudden cardiac death is a real differential diagnostic consideration. Subsequent to this, he or she will characterize the cardiac disease present through the autopsy and determine if it represents acquired disease, a congenital anomaly, or a first significant manifestation of possible genetic disease. However, when it comes to the medicolegal investigation of a sudden death in a young person, these are ideal circumstances. Unfortunately, this is often not how cases present to the forensic pathologist, who more often receives limited scene and historical information as well as past medical history. Due diligence is necessary to obtain information about how the decedent was discovered at the scene, what the environmental conditions were, and review all medical records that may be available. For example, pathologists encounter cases of intoxicated individuals who are discovered in positions that may lead to a determination that positional asphyxia was a significant contributor to death. However, if the position of the decedent was not provided to the pathologist, the autopsy findings may be interpreted as negative and the possibility of a sudden cardiac event may be entertained. This sometimes happens when paramedics have extracted and moved the decedent to attempt resuscitation before investigators can document the initial position of the decedent. Alternatively, if the decedent is starting to exhibit significant putrefactive decompositional changes, then this too can limit what may be adequately evaluated at autopsy. This is not to say that a sudden cardiac death was not possible, but the ability to exclude other, subtle causes of death becomes more difficult. This decreases the pre-test probability that one is dealing with a sudden cardiac death, particularly if the death is unwitnessed and the decedent is not found for some time after their death (**Tables 2 and 3**). These cases require great care and a discussion in the autopsy report regarding the limitations to any opinions proffered. Other factors such as the age of the individual or other comorbid conditions (e.g., infants or those with a well-recognized seizure disorder) can also confound cause of death determination by introducing other "syndromic" classifications for consideration, such as sudden infant death syndrome and sudden unexplained death in epilepsy.

A common source of diagnostic error when considering genetic cardiovascular disease is misinterpreting arti-

facts or mimics of cardiac pathology (**Table 4**). This may lead one to believe that there is a genetic condition when there is not or one may miss a possible genetic condition when overinterpreting the artifact or mimic as acquired disease. The two most common scenarios we see are overinterpretation of increased epicardial fat in someone who is overweight or obese as evidence of arrhythmogenic cardiomyopathy and overinterpretation of hypertensive heart disease as hypertrophic cardiomyopathy. In our death investigation system, we have put quality assurance processes in place to catch such cases once the autopsy reports are available for review; however, instances of failing to recognize a potential genetic condition are somewhat harder to evaluate after the autopsy has been completed. It is our hope that ongoing educational efforts will minimize such cases.

Limit Issues

If a young person dies suddenly and unexpectedly and no anatomical or toxicological cause for death is identified outside of the heart, then the following scenarios

Table 2: Strength of Inference That a Death is Likely a Sudden Cardiac Death (SCD) At the Time of Medicolegal Autopsy – Common Forensic Scenarios

Scenario*	Likelihood of SCD
Previously well with witnessed sudden collapse at sporting event, shoveling driveway, during sex, or any other physical activity	High
Found dead (unwitnessed) when only seen a few minutes to an hour or so earlier	Good
Found dead and not seen for many hours	Possible
Found dead and not seen for days with decompositional changes	Unclear

* Baseline assumption: no anatomical or toxicological cause for death is identified at autopsy and the scene is benign.

Table 3: Questions to Consider When Evaluating a Sudden Death and Considering the Need For Genetic Studies

Issue	Factors to Consider
Is it actually a sudden cardiac death?	Scene, history, full autopsy, ancillary studies (toxicology and consider microbiology and biochemistry if relevant to history and postmortem findings) – generates both positive and exclusionary information
What is the postmortem interval?	The longer the postmortem interval with increasing decompositional changes, the less confidence one may have in excluding other conditions
Do the historical circumstances cohere with a sudden arrhythmic death?	Detailed history needed
Has the individual died "of" cardiac disease or died "with" cardiac disease?	Consideration of cardiac pathology within the context of the entire death investigation*
Is an arrhythmia a possible contributing factor to the circumstances of death?	e.g., Unexpected drowning in someone who can swim and no other external influence identified? Motor vehicle collision with insufficient trauma to cause death? Fall down stairs with insufficient trauma to cause death? Homicide by heart attack? Sudden death during police restraint?
Are other "syndromic" diagnoses under consideration?	Sudden unexplained death in infancy, bed-sharing with infants, sudden unexplained death in epilepsy**, excited delirium
Are the cardiac changes you are evaluating a possible artifact or mimic?	See **Table 4**

* Even if death is not due to a cardiac cause, if a likely primary cardiomyopathy is identified at autopsy then this needs to be fully evaluated, DNA retained, and family encouraged to be assessed in a heritable heart disease clinic.

** It is recognized that a subset of individuals diagnosed with a seizure disorder may in fact have an underlying primary arrhythmia syndrome; cases of suspected sudden unexpected death in epilepsy should be evaluated carefully and consideration given to retaining DNA if there is also a family history of sudden death or arrhythmia.

may become difficult to evaluate. In a young person, how much atherosclerotic luminal stenosis is enough to ascribe death to advanced atherosclerotic coronary artery disease (when the degree of stenosis is borderline) and not due to some other process? How much macroscopic and microscopic ventricular remodeling is enough to call something a cardiomyopathy? How often is unexplained interstitial fibrous tissue deposition due to remote myocarditis or due to a remote history of cocaine usage? When should one consider that unexplained fibrous tissue deposition may represent evidence of arrhythmogenic cardiomyopathy (27)? How does one manage true left ventricular hypertrophy that is unexplained? How much myxomatous mitral valve disease and associated scar tissue within the posterior-inferior wall of the left ventricular myocardium is enough to attribute to death (28)? How much left ventricular hypertrophy is enough to significantly increase the risk of an arrhythmia (29)? How does one manage findings that are of uncertain significance in the setting of an apparent sudden cardiac death (30)? The common factor in each of these scenarios is the issue of how to interpret the pathology present when it is neither clearly lethal nor clearly benign. These issues are also difficult for cardiac pathologists and come to the forefront when trying to ascertain if one may be dealing with a possible genetic cardiac disease, or not.

Genotype-Phenotype Correlation

The emergence of pathological overlap syndromes has made interpreting cardiovascular phenotypes a challenge for a small subset of cases. It has been recognized for some time that end stage hypertrophic cardiomyopathy can present with a dilated phenotype (so called "burnt-out" HCM); however, there can also be phenotypic overlap between dilated cardiomyopathy and arrhythmogenic cardiomyopathy and there is emerging evidence of mutations in genes classically attributed to cardiomyopathic disease (such as

Table 4: Common Artifacts and Mimics of Cardiac Pathology

Artifact or Mimic	Factor to Consider
Increased epicardial fat misconstrued as arrhythmogenic cardiomyopathy	Increased epicardial fat may be normal in obese individuals as well as some with diabetes mellitus – in general, must have fibrofatty tissue replacement to consider pathological diagnosis of arrhythmogenic cardiomyopathy – occasional fibrofatty tissue associated with severe atherosclerotic heart disease is also sometimes over-ascribed to a primary cardiomyopathy
Hypertensive heart disease overinterpreted as hypertrophic cardiomyopathy	This is often a problem with nomenclature. An enlarged and hypertrophied heart does not diagnose hypertrophic cardiomyopathy – requires marked cardiomyocyte hypertrophy and geographic regions of myofibre disarray microscopically
Mildly increased thickness of left ventricular myocardium (> 1.5 cm) when heart weight normal	The left ventricular thickness may be mildly increased at autopsy and not represent evidence of left ventricular hypertrophy (postmortem artifact) – be sure to only measure compact layer of myocardium, correlate with weight of heart and correlate with expected histological changes for internal consistency
"Dilated cardiomyopathy" with putrefactive decompositional changes	The cardiac chambers can enlarge somewhat with increased decompositional changes – must be cautious not to over-interpret dilated cardiomyopathy when considering heart under such circumstances
Occasional minute foci of lymphocytic inflammation	Occasional minute foci of interstitial chronic inflammation is not uncommon in any heart and could be over-interpreted as myocarditis – distinct foci of myonecrosis in the appropriate clinical context is generally helpful
Contraction band change	Contraction bands can occur following vigorous resuscitative efforts, following direct trauma to the heart or following any significant acute myocardial injury (e.g., reperfusion injury). Contraction band change is not synonymous with acute ischemic injury. One can also see significant hemorrhage associated with vigorous chest compressions, which can cause epicardial and intramuscular hemorrhage
Mild, single leaflet mitral valve prolapse	Myxomatous mitral valve disease when moderate to severe, involving both leaflets and associated with increased fibrous tissue within the papillary muscles and posterior-inferior wall of the left ventricle may cause mitral valve prolapse and sudden death – if these findings are not present, be very cautious in ascribing mild pathological findings as evidence of mitral valve prolapse causing sudden cardiac death
Coronary artery tunneling	Be cautious of over-ascribing a mild degree of tunneling as potentially lethal – a modest degree of intramuscular tunneling of the left anterior descending artery is a common finding at autopsy
Focal myofibre disarray overinterpreted as hypertrophic cardiomyopathy	Small foci of myofibre disarray is commonly seen where the free ventricular walls meets the muscular interventricular septum and where larger trabeculae carnae and papillary muscles meet the free wall

for arrhythmogenic cardiomyopathy) also present in individuals with structurally normal hearts who die suddenly and unexpectedly (31-35). We also know that different mutations in a particular gene can lead to different phenotypic presentations (i.e., pleiotropy) such as with *SCN5A*, where different mutations are associated with Long QT Syndrome, Brugada Syndrome, conduction system disease, and dilated cardiomyopathy (36). Knowledge of what genetic variants are present or what class of gene products is mutated could potentially provide prognostic information (37). All of this is to say that there is not a perfect correlation between genotype and phenotype when considering genetic cardiovascular disease and, thus, great care is needed for interpretation.

We do not know the full spectrum of genes associated with many of the primary arrhythmia syndromes or cardiomyopathies. Thus, the lack of genetic variants at the time of genetic testing does not exclude the possibility that a particular pathological phenotype may have an underlying genetic basis. In addition, not all of the genetic variants elucidated are necessarily significant for the phenotype identified, so it is imperative that when evaluating a case of sudden death in a young person, first establishing that the cause of death is likely cardiac in nature and then carefully interpreting the correct cardiac phenotype is critical for heritable heart disease clinics to make any sense of genetic information that may arise from subsequent testing. This not only has implications for individual families, but also impacts epidemiological studies of various forms of heart disease in the community.

Case Types Where Molecular Autopsy May Support the Death Investigation

There are multiple cases that present to the forensic pathologist where one may consider employing genetic testing to augment the information that flows from a death investigation and potentially support regional genetics clinics (**Table 5**). In each of these instances, careful consideration of the benefits of adding genetic testing to supplement what can be learned from the autopsy must be weighed against the potential for confusion if the phenotype is not well characterized or the circumstances of death are not clear; thus, each case must be considered individually.

Table 5: Common Case Types Where the Molecular Autopsy Might Be Considered to Assist Forensic Pathologists

Case Type
Sudden cardiac death (structural and non-structural heart disease) – genetic panels to assess primary arrhythmia syndromes and / or cardiomyopathies may be helpful (Comment: if considering an unexplained dilated cardiomyopathy in a person under 30 years, also examine skeletal muscle to assess for a generalized myopathic process)
Vascular disorders (aortopathy / arteriopathy) – connective tissue disease panels (Marfan Syndrome, Ehlers-Danlos Syndrome, Loeys-Dietz Syndrome, familial thoracic aortic aneurysm and dissection, syndromic aortopathies)
Sudden and unexpected death in infants and young children (SIDS / SUDI, SUDC) – a subset of these cases likely involve genetic cardiovascular disease – much still unknown about the pathophysiology of the SUDI cohort
Sudden unexpected death in epilepsy (SUDEP) – some preliminarily evidence to suggest that primary arrhythmia syndromes may present as a seizure disorder. There are also familial forms of true seizure disorders.
Other complex neurological disorders – based on clinic-pathological phenotype and family history
Some complex congenital disorders – based on clinic-pathological phenotype and family history
Thrombophilias and bleeding disorders – both disorders of platelet function and coagulopathies may have a genetic basis
Triad cases in infants – dependent on history, clinical and pathological findings – may consider bleeding disorders, connective tissue disorders, or metabolic disorders
Deaths in the setting of positional restraint, excited delirium, unexplained death in the setting of electronic control device usage – careful consideration of primary arrhythmia syndromes or cardiomyopathies
Sudden and unexpected death in the setting of a criminal act (i.e., homicide by heart attack) – careful consideration of primary arrhythmia syndromes or cardiomyopathies
Other uncommon genetic syndromes identified at autopsy that may be incidentally identified at autopsy, such as autosomal dominant polycystic kidney disease, hereditary spherocytosis, connective tissue disorders, genetic cancer syndromes, etc.

Where such testing may be of great value is in areas where the underlying cause and mechanism of death are contentious. One example may be sudden and unexpected deaths in individuals with prone positional restraint at the time of death and/or excited delirium (38). Anecdotal evidence from our death investigation system has already identified cases where a potentially relevant mutation is identified, that may potentially contribute to the mechanism of death. While genetic factors that increase the susceptibility for injury or sudden death need not change any of the legal considerations in a case (akin to the classical "thin skull" argument), in an inquest hearing they may help to explain, at least in part, why a person may have died under such circumstances and what underlying vulnerabilities may have been present and not initially recognized. These results are very preliminary and much needs to be evaluated before a clear understanding develops of the real incidence of genetic anomalies in such cases and when genetic variant assessment may be relevant to help explain such deaths (39).

Another example where genetic testing has facilitated understanding of pathological findings has been in the area of traumatic, basal, subarachnoid hemorrhage that occurs following an assault. Some of these cases are thought to be caused by disruption of either the extracranial segment of the vertebral artery (with an associated dissection that tracks intracranially, leading to bleeding around the brainstem) or laceration of an intracranial vertebral artery or a primary branch thereof. In some of these cases, careful histological analysis of the vertebral arteries has revealed evidence of underlying arterial disease such as segmental arterial mediolysis or other forms of degenerative arteriopathic disease (40). Genetic testing for connective tissue disorders has revealed *COL3A1* mutations that are associated with Ehlers-Danlos Syndrome and correlate with preexisting vascular disease histologically. We have also employed genetic testing with good results under circumstances where idiopathic vertebral artery dissections have been identified. As with the heart, determining if a particular genetic variant is relevant to a particular case is dependent on correlating genotype with the relevant disease phenotype.

Recommendations for Further Consideration

The nature of forensic pathology is such that forensic pathologists commonly investigate uncommon conditions. This includes deaths of individuals with occult genetic disease in which the first clear manifestation of disease was sudden death. Forensic pathologists also encounter instances where subtle, early forms of disease are identified at autopsy that do not necessarily exhibit the full repertoire of phenotypic features for a given genetic condition, making their diagnosis challenging. Nevertheless, pathologists have a rich tradition of being at the forefront in identifying and defining emerging disease through the autopsy. There are still many poorly defined pathological entities and these require new tools to investigate their significance. Molecular genetics provides such a tool.

In the era of the vanishing hospital autopsy, forensic pathologists are being asked more often to investigate complex medical disease. The nature of forensic pathology practice has changed to accommodate this need, honing the skills classically seen in the hospital setting. Thus, forensic pathologists are ideally situated to apply the principals of molecular medicine to pathological work. Multiple forensic pathology fellowship programs have already started to incorporate additional training regarding common genetic entities that may be identified at autopsy. This may be viewed as a continuation of the molecular pathology training that has already been assimilated by anatomical pathology fellowship programs for many years.

Genetic Testing Should Never Replace Carefully Conducted, High Quality Autopsies

Ultimately, genotypic information is only of value if a clear understanding of phenotype is available. In part, this involves accurate pathological characterization of disease or, in the case of primary arrhythmia syndromes, confidence that an alternate cause for death is not present. Forensic pathologists must be comfortable with the full spectrum of issues related to a negative autopsy. Of course, any information that is obtained from the postmortem examination, including the lack of any specific pathological findings, must

be interpreted within the historical circumstances and scene information available.

Any Work to Develop a Molecular Autopsy Program Should be Considered Within an Ethics Framework, Including Medical Ethics Consultation and Periodic Reevaluation

If carefully managed, a molecular autopsy program is ethically sound and has an opportunity to enhance death investigations and outcomes for families. Some of the ethical challenges to consider could include informed consent, privacy issues, and the regional availability of healthcare services for affected families. Our experience over more than six years is that families are keen to have such testing performed and there is often great pressure to consider testing if not already performed. Families often readily identify literature online that advocates genetic testing under a variety of settings. If the genetic testing does not appear warranted by the circumstances or pathological findings in the case, then often meeting with the family helps to alleviate anxiety. Furthermore, offering to bank a sample of DNA on the family's behalf from remaining toxicology blood permits genetic testing in future if the circumstances change.

Be Wary Not to Overinterpret Genetic Results

Interpretation of the relevance of a given genetic anomaly is very complicated. In cases of sudden natural deaths, pathologists are often working with a partial dataset and close collaboration with other specialists such as clinicians, geneticists, and genetic counselors is recommended. This requires the establishment of good working relationships with specialists in the region. We do not have a genetic counselor or geneticist on staff at our facility, however we have ready access to such specialists when needed as the result of building such relationships. Nevertheless, the forensic pathologist may consider genetic information when evaluating criminally suspicious or contentious cases. Forensic pathologists will be asked to testify in public forums, either at inquest or criminal justice proceedings. The historical circumstances of these deaths are often better understood that for sudden, natural deaths given the resources typically devoted to their investigation and deal with issues that forensic pathologists are readily familiar, particularly those issues that are controversial. Such cases require care and a familiarity with the literature available as these cases may enter criminal justice or inquest proceedings. Overall, the pathologist needs to recognize his or her limits and when necessary, work as a team with other subspecialists.

Do Not Use Genetic Cardiovascular Disease as a Catchall Explanation For All Negative Autopsies

When there is insufficient scene and circumstantial information to ascertain how an individual died, or if postmortem decompositional changes have obscured appropriate analysis of a case, then great caution should be exercised prior to ascribing genetic disease as a possible explanation for the death. At the very least, within the postmortem report the pathologist should provide a sense of the quality of the opinions that may be proffered and state the limitations of postmortem examination itself. Or, if the findings are unclear, then they should be expressed in descriptive terms and not stated in a way that falsely provides certainty. Ethically, effective disclosure to families of the possibility of a genetic condition is important if the pathologist believes this to be a serious diagnostic consideration. Furthermore, appropriate characterization of the quality of the pathological and investigative evidence for other specialists to evaluate is also important.

Consideration of What Cases May or May Not Have a Genetic Underpinning is Changing How We View Cardiovascular Pathology, Thus Careful Cardiovascular Examinations by a Cardiovascular Pathologist are Recommended

Not all types of structural heart disease fit into easily defined diagnostic categories. Evaluating difficult diagnostic entities, whether as the result of a pathological overlap syndrome, the result of having findings of uncertain significance, or having findings that are borderline lethal relates to the issue of understanding when one has a reasonable prospect of having a genetic condition. An appreciation of these challeng-

ing diagnostic issues will improve the likelihood that the pathologist will recognize them at the time of the postmortem examination, ideally seek support from a cardiovascular pathologist, and flag the case by recommending further evaluation of relevant family members by other specialists. Evaluating cardiovascular pathology through the lens of molecular genetics will surely change how we practically manage sudden death investigations and our understanding of the pathophysiology of cardiovascular disease.

CONCLUSION

The transition of forensic pathology into the realm of molecular medicine holds great promise in advancing death investigation, our understanding of contentious pathological entities, and assisting families if this process is managed carefully and collaboratively with other specialists. It has become a core part of our own pathology practice; however, the fact that not all jurisdictions can feasibly support a full molecular autopsy program as outlined is recognized. Moreover, some jurisdictions have restricted mandates to focus largely on nonnatural deaths and workforce limitations as well as other constraints that can complicate the development of a molecular autopsy program. Yet, any efforts to improve the recognition of genetic disease by pathologists that encourages collection of a source of DNA and disclosure to families will greatly assist. Furthermore, helping to establish a process whereby local heritable heart disease clinics or genetics clinics can assess appropriate relatives is also of great advantage to families, even if genetic testing cannot be accommodated by the death investigation system itself. Sometimes families will be willing to pay for genetic testing themselves and, if relevant, the courts may inquire if samples of blood or tissue are available for further assessment to adjudicate inquest proceedings or criminal matters. Forensic pathologists may be asked to provide guidance on how best to manage genetic testing and who may be in the best position to interpret the results.

Molecular medicine is transforming healthcare and will transform forensic medicine. Regardless of the death investigation mandate, the molecular autopsy could play a direct role in understanding why some individuals die suddenly and unexpectedly under "forensic" circumstances. One may consider the problem of sudden death in the young from a public heath perspective and develop a staged approach to support this health concern. Developing the expertise, basic infrastructure, and experience to support the investigation of sudden natural deaths will allow forensic pathologists to take advantage of this technology when needed for their forensically oriented case work.

More recent consensus guidelines for cardiologists who investigate surviving family members recognize the importance of the work forensic pathologists and coroners do and recommend that sources of DNA and detailed autopsy reports be sought from death investigation systems. This trend will only grow and will require forensic pathologists and coroners to engage the issues outlined above.

With respect to sudden cardiac deaths, forensic pathologists and coroners can also align their efforts with regional or national organizations, such as Hearts in Rhythm Organization (HIRO: https://heartsys.org/5/hiro/?m=home) in Canada or the SADS Foundation (Sudden Arrhythmia Death Syndrome: www.sads.org) to stay connected with their clinical and genetics colleagues at the national level.

While the field of pharmacogenetics it is beyond the scope of this manuscript, applying molecular genetic analysis to questions of drug metabolism and individual therapeutic or adverse responses to drugs is also an evolving discipline. This is an area of forensic toxicology that forensic pathologists investigating drug-related deaths may need to consider. Like the investigation of sudden deaths due to disease, forensic pathologists will need to be aware of the promise and limitations of pharmacogenetics (41-44). Finally, as molecular genetics is being incorporated into autopsy practice, we are also witnessing the assimilation of postmortem radiology as a diagnostic tool. Forensic pathologists are now better able to address investigative problems more effectively than they could even just five to ten years ago. The forensic autopsy now truly extends from the scene to the gene. We live in exciting times.

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