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ACMG STATEMENT

Points to consider in the practice of postmortem genetic testing: A statement of the American College of Medical Genetics and Genomics (ACMG)

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Introduction

A traditional autopsy involves both histopathological examination of tissues and toxicology studies and is often used to help obtain a postmortem diagnosis in cases of sudden death. More recently, molecular technologies including next-generation sequencing are being used to assist in establishing or supporting a diagnosis when traditional autopsies fail to uncover a cause. Next-generation sequencing methods can also be used to more fully characterize a variety of conditions identified at autopsy that are suspected of having a heritable cause. For specific clinical indications such as sudden arrhythmic death syndrome, postmortem genetic testing has a relatively high diagnostic yield, leading

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to a molecular diagnosis in approximately 30% of traditional autopsy-negative cases.[1,2] As "molecular autopsies" involving postmortem genetic testing become more common, there is a need to address the unique set of challenges and issues inherent in postmortem testing.

Challenges with postmortem genetic testing include difficulties in obtaining appropriate specimens for testing, a lack of insurance reimbursement for genetic testing of deceased individuals, the often limited availability of complete phenotypic information to help guide interpretation of genetic test results, and concerns around obtaining appropriate consent for the individual and/or family members.[3] In addition, there is no consensus guidance for laboratories regarding how to approach variant reporting for postmortem diagnostic testing (including whether it should be treated differently than routine genetic testing). This new points to consider statement will address these and other concerns related to the use of "molecular autopsies" from the perspective of laboratories, genetic counselors, and clinicians.

General Considerations

- Although the term "molecular autopsy" is commonly used, the term "postmortem genetic testing" may be more appropriate. Other terms such as "immunohistochemical autopsy" and "toxicologic autopsy" are not routinely used, and postmortem genetic testing is typically only 1 component of a more comprehensive autopsy/postmortem evaluation of a case involving sudden death.
- The cost of genetic testing is dependent on the type of testing ordered (eg, exome/genome sequencing is often more expensive than a gene panel) and postmortem testing is typically not covered by insurance providers. Therefore, out-of-pocket and/or institutional payments may be the only options available to cover the cost of postmortem genetic testing. However, some insurance providers may cover the cost of testing (using the deceased individual's sample) if they are insuring an at-risk family member and the at-risk family member meets criteria for insurance coverage based on familial risk.
- The long-term storage of postmortem specimens (including DNA) through biobanks and other services may be beneficial for the future evaluation of the deceased individual and the decedent's family members; however, logistics and costs can be difficult to manage.
- Postmortem genetic testing may be clinically beneficial for the decedent in that it can (1) assist in generating a diagnosis if no other cause was identified through autopsy, and (2) confirm or more fully characterize a suspected diagnosis based on autopsy findings.
- Postmortem genetic testing may be clinically beneficial for the decedent's family. If a pathogenic or likely pathogenic variant is identified in the deceased individual, at-risk family members may be tested to guide the surveillance and/or medical management of those individuals. Family members testing negative can forego additional surveillance.
- Postmortem genetic testing may be clinically beneficial in cases of sudden death in infants, children, and adults as well as in cases of fetal demise or stillbirth[4] as part of a fetal autopsy.
- In addition to laboratory geneticists and molecular pathologists, medical examiners/coroners (ME/Cs) and clinical geneticists/genetic counselors are important for the success of postmortem genetic testing. ME/Cs are responsible for collecting and storing the specimens as well as ordering the testing. Clinical geneticists/genetic counselors help the family, and ME/Cs navigate the postmortem genetic testing process (provide information about postmortem genetic testing, coordinate sending samples, discuss costs, etc) and provide interpretation of results including risk assessment for the family. For the purposes of this document, where "ME/C" is mentioned, it should be considered to be more broadly applicable to all pathologists who perform postmortem examinations.
- Providing the laboratory with complete and accurate phenotypic information is critical for the success of postmortem genetic testing. This information assists the laboratory with gene prioritization and determining which variants should be reported.
- Maintaining appropriate chain of custody for postmortem specimens may be necessary to determine whether the evidence may be admissible later in a legal proceeding.[5] Specimen requirements must be in place for shipping, handling, and receipt from collection to reporting. Not all laboratories have the infrastructure or workflows to allow for this; thus, advanced coordination with the shipper and receiving laboratory is required to ensure chain of custody, if necessary. There is also the potential for ME/Cs or other laboratory professionals to be called as expert witnesses if they are involved in postmortem genetic testing cases.
- If genetic testing results are to be used for diagnostic purposes in family members, testing must be performed in a CLIA-certified laboratory or, if performed in a research or other non-CLIA laboratory, results must be confirmed in a CLIA laboratory before using for clinical purposes.

Considerations for Ordering, Consenting, and Communication (for the Clinician and Genetic Counselor)

- If postmortem genetic testing is ordered by a court of law through the ME/C for a minor after a suspicious or unexpected death, in most states, the results/report do not have to be shared with the family; sharing results may interfere with the ME/C's ability to determine the cause and manner of death.

- Postmortem genetic testing may be ordered by an ME/C at the request of the family or as part of a death investigation when a traditional autopsy fails to identify a cause of death, in which case it should ideally be ordered after appropriate discussion with the family and counseling of next of kin has taken place.
- In the United States, a significant proportion of coroners are not physicians. Coroners who are not physicians lack the training and credentialing required to carry out a clinical autopsy; thus, their ability to order, interpret, and synthesize postmortem genetic findings may be limited and consultation with a clinical geneticist/genetic counselor may be beneficial.
- In the United States, an ME/C may elect to pursue genetic testing without family consent according to statutory authority in cases of public or medicolegal autopsies.[6,7] Autopsy reports may also be subject to public disclosure, because the Health Insurance Portability and Accountability Act Privacy Rule may not apply.
- If the ME/C suspects an underlying genetic cause, they should reach out to the family so that a decision can be made in consultation with the primary care provider or medical team. Because genetic testing has the potential to affect not only the decedent but also other family members, there may be a "duty to warn" component that adds a layer of nuance as compared with other postmortem investigations.
- Although consent generally is not legally required before postmortem genetic testing, unless it would compromise the death investigation, reasonable efforts should be made by relevant parties to notify the decedent's family (at a minimum, next of kin or first-degree relatives) before conducting genetic testing as an aspect of a death investigation or when a positive test result is obtained. A reasonable effort should also be made to solicit consent to obtain samples from biological relatives, if available, for confirmatory testing.[6]
- Informed consent, if pursued, should be obtained by a genetic counselor, clinical geneticist, or in their absence, by other appropriately trained clinical providers. These providers are encouraged to remain regularly updated regarding the standards of care and best practices for obtaining informed consent for genetic testing, including risks, benefits, and alternatives of currently available testing platforms.
- Samples saved for clinical testing should be prioritized for clinical testing; however, if clinical testing is negative or a family cannot afford clinical testing, enrollment in research is encouraged. Research on deceased individuals requires family consent, ideally involving both next of kin and first-degree relatives who may directly benefit from any findings. Currently, the National Association of Medical Examiners, the National Institutes of Health's Genotype-Tissue Expression Project and Genomic Data Sharing (GDS) Policy, and the Swiss Academy of Medical Sciences all recommend obtaining consent from the family before proceeding with secondary research on biological samples from decedents.[8]
- If a pathogenic or likely pathogenic finding is identified, the next of kin should be referred to a qualified genetic counselor or clinical geneticist to explain the results and recommend follow-up testing, including genetic testing and surveillance. The potential risk to biological relatives should be clearly communicated. If genetic testing is negative, families should be counseled on the limitations of current technology and knowledge about genetic variation because it relates to sudden death and the possibility of DNA banking for future analysis. Phenotype-guided clinical screening of relevant individuals may also be appropriate.[9]

Considerations for Testing and Interpretation (for the Laboratory)

Appropriate specimen types, collection, and storage

- ME/Cs should maintain policies and procedures regarding when dedicated samples, typically purple top EDTA tubes for DNA preservation, should be collected for potential postmortem genetic testing. The National Association of Medical Examiners recommends collecting a dedicated blood or tissue sample on every autopsy so that it can be saved and stored appropriately in case genetic testing is needed later; these samples could then be discarded by the ME/C if the traditional autopsy uncovers a definitive diagnosis without genetic testing.[7]
- If blood samples are not available for genetic testing, other organ tissues may be used and fresh tissue or cultured cells such as fibroblasts may be more successful in yielding a result. However, the transport of fresh tissue is difficult and may require dry ice if the tissue has previously been frozen. Alternatively, formalin-fixed paraffin-embedded tissue can be transported at room temperature, although formalin-fixed paraffin-embedded tissue is not accepted by many genetic testing laboratories because of low success rates, possibly related to longer periods of fixation in autopsy settings. Specific indications (such as stillbirth) may also have unique specimen requirements to consider.
- Hair, vitreous fluid, synovial fluid, and urine (except for certain mitochondrial cases) are not good candidates for testing because of the low amount of DNA obtained, contamination, etc.
- Dried blood spots and buccal swabs are additional sources of DNA that are easy to obtain, store, and ship, although the quantity of DNA obtained from these specimens may be less than that obtained from other sources and may not be accepted by all genetic testing laboratories.
- Specimens such as dried blood spots, cerebrospinal fluid, bile, urine, skin tissue, and fibroblasts may also

serve as sources of metabolites, which may be beneficial in generating or confirming a postmortem diagnosis.
- Sample type and sample collection information (date, time of collection after death, storage conditions, etc) must be provided to the laboratory to determine acceptability.
- Laboratories may consider postmortem-specific retention guidelines (unless prohibited by federal/state regulations), because specimens for postmortem testing are typically irreplaceable.[7]
- A guarantee of long-term storage requires a DNA banking facility, which is typically beyond the services provided by ME/C offices or clinical laboratories.

Appropriate tests and variant types

- Although gene panels may be suitable in many situations, exome or genome sequencing for postmortem genetic testing may be more appropriate depending on the clinical indication.[10,11] Limitations of gene panel content, the potential availability of secondary findings, and differences in test costs should be considered when making these decisions. An appropriate gene panel followed by reflex exome/genome sequencing may be appropriate.
- A negative test result from a gene panel, exome, or genome analysis does not exclude a genetic cause or contribution for a case of sudden death.
- If a pathogenic or likely pathogenic variant is observed in the deceased individual, variant-specific or targeted deletion/duplication analysis using an orthogonal methodology, when appropriate, can be used for any additional familial testing. The decedent's DNA sample can serve as a positive control to validate the accuracy of the variant-specific or targeted test.
- Laboratories should maintain policies and procedures regarding the variant types that can be reliably detected and reported for postmortem genetic testing (eg, single-nucleotide variants [SNVs], copy number variants [CNVs]), and the detectable/reportable variant types should be based on the laboratory's ability to achieve acceptable validation results for that variant type.[12] Variant confirmation practices in the postmortem setting should follow established laboratory policies and procedures.
- Laboratories should consider, when appropriate, offering CNV analysis along with SNV/insertion/deletion analysis for postmortem cases, because many of the genes currently associated with causes of sudden death can have CNVs, which may be missed if only SNVs/insertions/deletions are evaluated.[13]
- Laboratories should consider, when appropriate, offering mitochondrial genome analysis in addition to nuclear genome analysis for postmortem cases, because some of the genes currently associated with causes of sudden death are present in the mitochondrial genome and may be missed if only the nuclear genome is evaluated.[14]

Considerations for Reporting (for the Laboratory)

- Variant classification for postmortem genetic testing should be performed according to the same standards that have been established for genetic testing of samples from living individuals as outlined by professional guidelines;[15,16] laboratories should maintain policies and procedures regarding their reportable variant classifications in this setting.
- Pathogenic and likely pathogenic variants in primary genes that are clinically relevant for the decedent should be reported. Benign and likely benign variants should not be reported.
- If feasible, laboratories should consider reporting variants of uncertain significance in genes that are related to the cause of death for postmortem genetic testing cases that are ordered by an ME/C, at the request of the family, or as part of a death investigation when a traditional autopsy fails to identify a cause of death. Segregation testing in family members may assist the laboratory in upgrading or downgrading the classification of certain variants.
- Postmortem genetic testing should be used solely as a component of the autopsy/death investigation to determine or confirm the cause of death; thus, only variants that are directly related to the cause of death should be reported. Incidental/secondary findings that are unrelated to sudden death should not be reported unless consent was obtained from a family member according to standard practices. Heterozygous variants for recessive genetic conditions that are unrelated to sudden death should not be reported.
- Laboratories should maintain policies and procedures regarding variant re-evaluation, and those policies and procedures should follow the established recommendations for the testing of living individuals.[17] If the postmortem genetic testing is ordered by an ME/C, at the request of the family, or as part of a death investigation when a traditional autopsy fails to identify a cause of death, laboratories should communicate any updated variant classifications to the individuals who initiated testing or other appropriate providers of the involved family members through established means (eg, report addendums, updated reports).
- Consumers of postmortem genetic testing reports include forensic pathologists, general pathologists, nonpathologist physicians, coroners (not necessarily medically trained), lawyers, and the general public. Therefore, if feasible, postmortem genetic testing reports should ideally be succinct and free of jargon, because they will be read by individuals with a wide spectrum of understanding of genetics. However, interaction with a genetics professional (clinical geneticist or genetic counselor) is also important, because they are specifically trained to communicate genetic testing results.

- Because some postmortem genetic testing reports will be part of the public record, sensitivity needs to be given to inadvertently including any health history for surviving family members.
- If feasible, reports should clearly explain the variant classifications, outlining the relevant criteria used to arrive at each classification. Laboratories may want to consider including an appendix that defines each variant classification in simple terms as well as any actions that are typically taken by individuals with such a diagnosis.
- Because postmortem samples are often irreplaceable, if the initial genetic testing results are suboptimal or inadequate, laboratories may want to consider accepting and testing other available samples from the deceased individual either to avoid depletion of the primary specimen or if other specimens were handled differently and may be more likely to yield a successful result. The laboratory may also want to consider releasing incomplete reports under specific situations (eg, cases in which the SNV analysis is successful but the CNV analysis is not).

Conflict of Interest

The authors declare no conflicts of interest.

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