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Molecular diagnostics of cardiovascular diseases in sudden unexplained death

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ABSTRACT

The most challenging type of sudden cardiac death is sudden unexplained death. The etiologies for sudden unexplained death are diverse and not necessarily confined to the cardiovascular system. Nevertheless, certain cardiovascular diseases, particularly cardiac channelopathies and cardiomyopathies, are known to play significant roles in sudden deaths. The purpose of the review is to provide autopsy pathologists with an actionable guide through illuminating the clinically relevant molecular basis of cardiac channelopathies and cardiomyopathies, as well as the changing landscape of molecular diagnostics.

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1. Introduction

Diseases of the heart are the leading cause of death (COD) in the United States accounting for an estimated 2150 deaths each day [1]. On average, one person in the United States dies from cardiovascular disease every 40 s [1]. The vast majority of sudden cardiac deaths are due to ischemic heart disease, hypertensive heart disease, and cerebrovascular disease [2]. These entities are fairly straightforward for a pathologist to diagnose through characteristic anatomic findings and a review of the relevant history when all other competing causes have been ruled out.

The most challenging type of sudden cardiac death is when life suddenly and unexpectedly ends in an apparently healthy, often young, person who lacks a medical history and significant findings after a comprehensive medicolegal investigation. Typically, the "autopsy negative" death requires the most detailed level of study including investigation of the circumstances leading up to death, the scene in which death occurred, gross and microscopic examination of important organs (brain, heart, lungs, liver, and kidneys), various ancillary laboratory studies (such as toxicology assays, microbiologic cultures, and metabolic screening tests in infants) as well as review of any prior medical records. The COD is unexplained and commonly certified as "cardiac arrhythmia of unknown etiology."

The etiologies for sudden unexplained death (SUD) are diverse and not necessarily confined to the cardiovascular system [3]. Nevertheless, certain cardiovascular diseases, particularly cardiac channelopathies and cardiomyopathies, are known to play significant roles in sudden deaths [4]. Through the advances of molecular testing technologies, the breadth and depth of our understanding of SUD-contributing cardiovascular diseases have been drastically improved over the past decade. These discoveries are directly impacting the practices of autopsy pathologists and medical examiners. Molecular diagnostics is of particular utility in SUD investigation since the heart of the decedent cannot be functionally evaluated. The purpose of the review is to provide autopsy pathologists with an actionable guide through illuminating the clinically relevant molecular basis of cardiac channelopathies and cardiomyopathies, as well as the changing landscape of molecular diagnostics.

2. Molecular basis of cardiac channelopathies and cardiomyopathies

Cardiac channelopathies (also known as ion channelopathies) primarily affect heart rhythm and cardiac electrical conduction and comprise of a group of inheritable cardiac arrhythmia syndromes such as long QT (LQT) syndrome, catecholaminergic polymorphic ventricular tachycardia (CPVT), Brugada syndrome, short QT syndrome (SQTS), and familial atrial fibrillation (AF). The prevalence of the cardiac channelopathies as a group is estimated to be approximately 1 in 3000 to 5000 individuals in the general population [4]. The majority of individuals affected by cardiac channelopathies may be asymptomatic or have clinical complaints such as palpitations or syncope that require medical assistance [5]. Only a small percentage of the affected individuals die suddenly. The known genes for cardiac channelopathies are summarized in Table 1. These genes primarily encode cardiac ion channel proteins or subunits as well as a small number of proteins involved in ion channel protein processing, trafficking, or cell membrane targeting [6]. Mutations in cardiac ion channel genes alter the action potentials of cardiac myocytes, either

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Table 1
Cardiac channelopathy genes

Diseases	Genes	Functions	Testing sensitivity & limitation
LQTs	*KCNQ*1 (LQT1), *KCNH*2 (LQT2), *SCN*5A (LQT3), *ANK*2 (LQT4), *KCNE*1 (LQT5), *KCNE*2 (LQT6), *KCNJ*2 (LQT7), *CACNA*1C (LQT8), *CAV*3 (LQT9), *SCN*4B (LQT10), *AKAP*9 (LQT11), *SNTA*1 (LQT12), and *KCNJ*5 (LQT13)	Cardiac ion channels (Na^+, K^+, Cal^{2+}) or regulatory proteins	Approximately 75% of LQT patients are positive; 5% patients have large deletions/duplications (indels)
Brugada Syndrome/ J-Wave Syndromes	*SCN*5A, *GPD*1L, *CACNA*1C, *CACNB*2, *SCN*1B, *SCN*3B, *KCND*3, *KCNE*3, and *KCNJ*8	Cardiac ion channels (Na^+, K^+, Cal^{2+}) or regulatory proteins	Approximately 25–40% of Brugada patients are positive
CPVT	*RYR*2, *CASQ*2, *ANK*2, and *KCNJ*2	Cardiac ion channels (Ca^{2+}, K^+) or regulatory protein	Approximately 50%–70% of CPVT patients are positive
SQTS	*KCNH*2 (SQT1), *KCNQ*1 (SQT2), and *KCNJ*2 (SQT3)	Cardiac potassium channels	Approximately 15–20% of SQT patients are positive
Familial AF	*KCNQ*1, *KNCE*2, *KCNJ*2, *KCNE*1L, *SCN*5A, *NPPA*, *GJA*5, and *KCNA*5	Cardiac ion channels (K^+, Na^+) or regulatory proteins	Unknown

LQTs, long QT syndrome; CPVT, catecholaminergic polymorphic ventricular tachycardia; SQTS, short QT syndrome; AF, atrial fibrillation.

by producing functionally abnormal ion channels or the aberrant processing and/or targeting of ion channel proteins to the proper intracellular or cellular membrane locations. Interestingly, mutations in the same cardiac ion channel gene account for different channeloapthies. For example, mutations in *SCN*5A gene have been reported in LQT syndrome Type 3, Brugada syndrome, as well as AF, largely depending on various effects of the mutations to the encoded voltage-gated sodium channel [7]. It has been estimated that approximately 15 to 20% of sudden cardiac deaths are due to the presence of the disease-causing variants in six major cardiac ion channel genes (*KCNQ*1, *KCNH*2, *SCN*5A, *KCNE*1, *KCNE*2, and selective exons of *RyR*2) [4,8–10]. The significance of cardiac channelopathies in SUD is expected to be greater when molecular testing is expanded to include other channelopathy-causing genes.

Along with the cardiac channelopathies, it is also useful to test for cardiomyopathies, particularly hypertrophic cardiomyopathy (HCM), arrhythmogenic right ventricular cardiomyopathy (ARVC), idiopathic dilated cardiomyopathy (DCM) and, occasionally, left ventricular noncompaction cardiomyopathy (LVNC), when investigating SUDs of young children and adults [11]. It is important to keep in mind that the pathognomonic gross and histopathologic changes of cardiac tissue for cardiomyopathies may be concealed (particularly in the developing hearts of children or in the hearts of athletes), making these entities especially difficult to accurately diagnose at autopsy. Collectively, cardiomyopathies are common in the general population, with the prevalence ranging from approximately 1:500 [12] for HCM to 1:1000–1:1250 [13] for ARVD. It is estimated that approximately 1% of people with HCM and half of ARVC patients die suddenly [12,13]. The majorities of HCM genes encode different components of the cardiac sarcomere [12] and the ARVC genes primarily encode components of the plasma membrane desmosome complex [13]; in contrast, the DCM genes products are involved in more diverse biological pathways, such as Z-disk, nuclear lamina, and intermediate filaments [14] (Table 2). The molecular basis for HCM, DCM, and LVNC are not clearly separated: certain HCM genes, such as *MYH*7, *MYBPC*3, *TNNT*2, *ACTC1*, are also known for DCM and LVNC [4].

Both cardiac channelopathies and cardiomyopathies share similar molecular characteristics that are important for our understanding of molecular testing approaches and results: (a) There are no common, recurrent variants reported in large patient cohorts: among the numerous mutations reported responsible for cardiac channelopathies and cardiomyopathies, the majority occurred in a single or a few families with dominant effect (gain-of-function, haploid insufficient, or dominate negative); (b) reduced penetrance and variable expressivities are common for the variants identified in both group of diseases, which makes family segregation studies challenging to interpret. The presence of genetic and epigenetic modifiers, along with exposure to various environmental triggers, as well as developmental changes in children and young adults, have all been implicated in the variability in the relationship between the complex molecular characteristics and disease expression. However, there are reports of "lethal variants" such as some *LMNA* variants that have been implicated as high risk for sudden death in DCM [15], as well as mutations located in the pore/transmembrane regions of the *KCNQ*1, *KCNH*2, and *SCN*5A genes associated with a more severe outcome [16]. (c) Not all clinically suspected patients have identifiable mutations in the known disease-causing genes. This is possible since there likely are other, presently unidentified genes responsible for the disease, a patient manifests the phenocopy of the disease with different underlying etiology, or limitations of current testing technologies cannot identify the mutation.

3. Changing landscape in molecular diagnostics

Until recently, comprehensive molecular diagnostics of cardiac channelopathies and cardiomyopathies had been laborious and cost prohibitive in a clinical laboratory. Because there are no screenable, common, recurrent mutations, sequencing the encoding regions of the disease genes becomes the choice of testing. Laboratories have

Table 2
Cardiomyopathy genes

Diseases	Genes	Functions	Testing sensitivity & limitation
HCM	*ACTC*1, *GLA*, *LAMP*2, *MYBPC*3, *MYH*7, *MYL*2, *MYL*3, *MYOZ*2, *PRKAG*2, *TNNT*2, *TNNI*3, *TNNC*1, *CSRP*3, *TTN*, *ACTN*2, and *TPM*1, *PLN*	Components of sarcomere, e.g., myosin heavy and light chains	Approximately 50–60% of HCM patients are positive
ARVC	*PKP*2, *DSP*, *DSG*2, *DSC*2, *TGFB*3, *JUP*, *RyR*2, and *TMEM*43	Components of Desmosome, Ca^{2+} regulation, and transforming growth factor	Approximately 50-60% or ARVD patients are positive
DCM	*TTN*, *ANKRD*1, *ACTC*, *LDB*3, *LMNA*, *MYBPC*3, *MYH*7, *MYH*6 *PLN*, *SCN*5A, *TAZ*, *TNNC*1, *TNNI*3, *TNNT*2, *BAG*3, *ANKRD*1, *RBM*20, *TPM*1, *CSRP*3, *CTF*1, *DES*, *EMD*, *LDB*3, etc.	Components of sarcomere, Z-disk, nuclear lamina proteins, and intermediate filaments, RNA-binding proteins, molecular chaperone regulatory proteins	Approximately 25% of DCM patients are positive
LVNC	*ACTC1*, *LDB*3, *LMNA*, *MYBPC*3, *MYH*7, *TAZ*, and *TNNT*2	Components of sarcomere and nuclear lamina proteins	Approximately 25% of LVNC patients are positive

HCM, hypertrophic cardiomyopathy; ARVC, arrhythmogenic right ventricular cardiomyopathy; DCM, idiopathic dilated cardiomyopathy; LVNC, left ventricular noncompaction cardiomyopathy.