Title: Evaluation after Sudden Death in the Young: A Global Approach

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# Evaluation after Sudden Death in the Young: A Global Approach

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**Abstract**

Sudden cardiac death (SCD) is defined as a death occurring usually within an hour of onset of symptoms, arising from an underlying cardiac disease. SCD is a complication of a number of cardiovascular diseases, and is often unexpected. In individuals aged less than 35 years, unexplained SCD is the most common presentation. A significant proportion of SCD in the young (≤ 35 years, SCDY) events may be precipitated by underlying inherited cardiac conditions (ICCs), including both heritable cardiomyopathies and inherited arrhythmia syndromes (also known as cardiac channelopathies). Tragically, sudden death may be the first manifestation of the disease in a family and therefore clinical and genetic evaluation of surviving family members forms a key role in diagnosing the underlying ICC in the family. This is particularly relevant when considering that most ICCs are inherited in an autosomal dominant manner meaning that surviving family members have a 50% chance of inheriting the same disease substrate.

This review will outline the underlying causes of SCDY and outline our universal approach to familial evaluation following a young person's sudden death.

**Non-Standard Abbreviations and Acronyms**

sudden cardiac death (in the young) - SCD[Y]

inherited cardiac conditions - ICC

hypertrophic cardiomyopathy - HCM

dilated cardiomyopathy - DCM

arrhythmogenic cardiomyopathy - ACM

arrhythmogenic right ventricular cardiomyopathy - ARVC

sudden unexplained death - SUD

sudden arrhythmic death syndromes - SADS

long QT syndrome - LQTS

Brugada syndrome - BrS

catecholaminergic polymorphic ventricular tachycardia - CPVT

short QT syndrome - SQTS

progressive cardiac conduction disease - PCCD

exercise stress test - EST

cardiac MRI - CMR

American College of Medical Genetics and Genomics - ACMG

variants of uncertain significance - VUS

sudden unexplained death in epilepsy - SUDEP

## Introduction

Sudden cardiac death (SCD) is defined as a death due to an underlying cardiac disease occurring within an hour of the onset of symptoms, or unwitnessed deaths where the decedent was well 12-24 hours prior to death.[1, 2] A recent prospective, population-based study from Australia and New Zealand has estimated the prevalence of SCD in the young (SCDY) < 35 years old to be 1.3 cases per 100,000 persons, with males comprising 70% of the decedents.[3] This is lower than previous estimates that were as high as 8.5 per 100,000 persons but similar to a population-based Danish study.[4-8] SCDY is most likely to occur during sleep or at rest but may also occur during exertion or stress.[3, 9] Although SCDY is unexplained often, SCDY may stem from an underlying inherited cardiac condition (ICC) including primary arrhythmia syndromes (cardiac channelopathies).[3] SCDY, whilst rare, is a tragic and often unexpected event, particularly if this is the sentinel manifestation of the disease in a family. These conditions are inherited typically in an autosomal dominant manner meaning that first degree relatives have a 50% chance of inheriting the same condition.[10, 11] Comprehensive familial evaluation in a specialized multidisciplinary ICC clinic is essential for optimal assessment and management of these families.[12, 13]

## Causes of Sudden Cardiac Death in the Young (SCDY)

SCDY can be caused by a number of underlying cardiac conditions which are categorized broadly into structural and arrhythmogenic causes (**Table 1**). Guidelines recommend that an autopsy should be performed after an unexpected sudden death in the young (SDY) case to determine whether the cause of death was cardiac in origin (SCDY) or non-cardiac. If the death is deemed to be cardiac, the autopsy should attempt to clarify whether the death occurred secondary to an arrhythmic or non-arrhythmic mechanism (**Figure 1**). The cardiac-focused

autopsy includes examination of the heart, aorta and coronaries as well as histopathology of both left and right ventricular myocardium.[2] An expert cardiac autopsy by a cardiac pathologist can improve diagnostic accuracy after a SDY case.[14] A recent update of the guidelines for autopsy investigation of SDY highlights the importance of specialist histopathological investigation and a multidisciplinary approach to ensure preventative strategies for families.[15]

The structural causes of SCDY are usually identified during a conventional gross and histopathologic autopsy, and include the cardiomyopathies (hypertrophic cardiomyopathy [HCM], dilated cardiomyopathy [DCM], arrhythmogenic cardiomyopathy [ACM] which also covers arrhythmogenic right ventricular cardiomyopathy [ARVC]), as well as myocarditis, coronary artery disease (including coronary artery anomalies), aortic disease, and congenital heart disease. Cases, where no cause of death has been established following a comprehensive autopsy that incorporates a cardiac-specific autopsy and negative toxicology, have been regarded as autopsy negative sudden unexplained death (SUD) (**Figure 2**). These deaths are also termed sudden arrhythmic death syndromes (SADS) because they may be due to arrhythmias secondary to underlying primary arrhythmogenic disorders.[1, 14] Causes include long QT syndrome (LQTS), Brugada syndrome (BrS), catecholaminergic polymorphic ventricular tachycardia (CPVT), short QT syndrome (SQTS), and progressive cardiac conduction disease (PCCD). These cardiac conditions may leave no clues to be found during a conventional autopsy. Most of these disorders are associated with mutations in genes encoding ion channel pore-forming alpha subunits and/or their interacting/auxiliary proteins, responsible for sodium-, potassium-, and calcium- channels. Therefore, comprehensive clinical evaluation of surviving family members as well as post-mortem genetic testing of the decedent (aka, the 'molecular autopsy'), form an integral part of the diagnostic pathway.[1, 9, 16-19] Occasionally, despite no evidence of structural

change at autopsy, cardiomyopathy may be diagnosed clinically in a family or genetically on molecular autopsy, indicating that lethal arrhythmias could occur prior to any evidence of overt structural changes.[19, 20] If the SDY case remains unexplained after a thorough post-mortem evaluation that includes conventional autopsy, toxicology, and a molecular autopsy (post-mortem genetic testing), then the case can be designated as autopsy negative, genetically elusive SUD/SADS.

**Diagnostic Approach Following SDY**

The following diagnostic approach is for those SDY cases where initial evidence supports a cardiac cause (i.e. SCDY). In cases where a specific cardiac cause of death has been identified following a conventional autopsy (e.g. HCM), the investigation of surviving family members can be tailored to that particular diagnosis in the family. However, when the autopsy is negative and the current designation for the decedent is autopsy negative SUD/SADS, familial evaluation requires a methodical approach to ensure all family members are investigated thoroughly with appropriate cardiologic tests in a timely manner.

We recommend a two-stage approach to the clinical/cardiologic evaluations in family members of the decedent searching for evidence of an ICC. All first degree blood relatives are recommended stage one investigations, and those in whom investigations remain negative are then offered the more complex or invasive investigations either on another occasion or the same day (**Figure 2**). Symptomatic and/or obligate relatives should also be prioritized. We recommend a broad approach to ensure that no subtle phenotypic features are missed. Furthermore, there is increasing evidence of phenotypic cross-over within genotype-associated syndromes.[21]

Baseline assessment includes a focused history and examination, with specific 'red flags' including a history of syncope or palpitations and a family history of other premature and/or

unexplained sudden deaths or any cardiac disease.[1] Baseline investigations include electrocardiogram (ECG) with both standard and high precordial lead positions: V1 and V2 in the 2nd and 3rd intercostal spaces.[22] High precordial leads assess for the presence of an underlying spontaneous, type 1 Brugada ECG pattern and increase the sensitivity without compromising specificity by recording over the whole right ventricular outflow tract in all patients.[23] Cardiac imaging with a baseline echocardiogram assesses left and right ventricular size and function as well as left ventricular wall thickness to exclude a cardiomyopathy.[1]

An exercise stress test (EST) is recommended for assessment of the behaviour of the QT interval from lying to standing, during exercise and in the recovery phase which may assist in unmasking otherwise concealed LQTS.[24-27] The EST is also a key investigation for detecting CPVT, which is characterised by a normal baseline ECG and echocardiogram but ventricular ectopy, bidirectional couplets and ventricular tachycardia (VT) as well as rarely polymorphic VT on exercise.[1] A standard 24-hour ambulatory Holter monitor may assess ventricular ectopy or arrhythmias, however the yield is typically low in the absence of symptoms such as palpitations.[19]

If these level/tier-one investigations fail to yield a diagnosis, then level/tier-two investigations may be pursued in a further attempt to uncover evidence for an underlying ICC. If there is any suspicion of an ACM in general (or ARVC in particular) or any other structural abnormalities on baseline level 1 investigations, then advanced cardiac imaging with cardiac MRI (CMR) using gadolinium contrast is recommended.[1] Signal-averaged ECG (SAECG) is recommended as a tier 2 investigation with ≥1 abnormal parameter satisfying one minor diagnostic criteria for ARVC.[2, 28] If no other diagnosis is established and the characteristics of the sudden death may be consistent with Brugada syndrome, we recommend proceeding to drug

provocation testing with a class I antiarrhythmic agent for first-degree relatives with a structurally normal heart. Options include Ajmaline 1mg/kg (maximum 100mg) over 5 minutes (or Flecainide 2mg/kg (maximum of 150 mg) over 10 minutes; Procainamide 10mg/kg over 10 minutes; Pilsicainide 1mg/kg over 10 minutes) using standard and high precordial ECG lead positions is recommended.[2] However, drug potency for inducing the type 1 pattern varies: for example, Ajmaline results in significantly more positives results than Procainamide or Flecainide.[29, 30] This results in an increased diagnostic yield for BrS.[31] A 12-lead Holter monitor with high precordial lead positions may then be used to assess for a dynamic spontaneous Brugada pattern over a 24-hour period for prognostic utility.[32] Ideally, if performed beforehand, detection of a dynamic type 1 pattern may obviate the need for drug provocation testing. Invasive electrophysiology study is utilized rarely and is not included currently in a standard diagnostic pathway.

When evaluating children of decedents or young siblings, we recommend offering a clinical review with level/tier-one investigations around the time of the SCDY event. Due to age-related penetrance of a number of ICCs, we recommend that children be re-evaluated at the clinic in adolescence or early adulthood.[33] We do not recommend drug-provocation testing until early adulthood as a negative test can be found, even in individuals hosting a familial *SCN5A* variant.[34, 35] Fever is an important trigger for arrhythmia in children with BrS and we suggest an ECG is performed at the time of fever (if possible) on at least one occasion if there is a suspicion of BrS in the family.[36] Predictive genetic testing in children can be beneficial, particularly for LQTS and CPVT to allow initiation of potentially life-saving medication, however it also carries significant ethical and legal considerations that are best managed by an experienced genetic cardiologist and/or cardiac genetic counsellor.[37, 38]

Occasionally there may be equivocal structural findings on autopsy such as left ventricular hypertrophy without disarray or unexplained fibrosis where diagnostic criteria for cardiomyopathy are not met. These may represent an early cardiomyopathic phenotype, findings that predispose to risk of SCDY, or incidental findings that are unrelated to the cause of death which may be an arrhythmia syndrome. As such, decedents should be treated as SADS/SUD until proven otherwise and their family evaluated accordingly.[20, 39] Similarly, SADS family members may have equivocal clinical results suggestive but not diagnostic of cardiomyopathy. We advise standard assessment (figure 2) with additional imaging as required and then follow-up for any evolution of phenotype.

It is important, therefore, to acknowledge that there is a risk of overdiagnosis of family members, particularly in the absence of symptoms. Investigation following a SCDY should be performed in an experienced multidisciplinary genetic heart disease/ICC clinic[2].

**Molecular Autopsy After SCD**

Either preceding the evaluation of the decedent's surviving first degree relatives, or more often concurrently, a second tier autopsy with post-mortem genetic testing should be performed. International guidelines recommend that a sample of DNA-rich tissue (either blood, thymus, spleen, or liver) be retained at the time of autopsy, in order for post-mortem genetic testing ("the molecular autopsy") to be performed in the decedent.[1] In cases where a structural cardiac cause of death (for example HCM) has been identified, a disease/phenotype-focused molecular autopsy should be performed. If the decedent's disease-causative or 'pathogenic' variant is found, then cascade testing of the first degree relatives can be performed. Family members, who test negative for that implicated variant AND have a negative cardiac evaluation as well, can then be

dismissed. Obviously, those living relatives who test positive will require ongoing disease-specific surveillance and therapy.

In cases provisionally classified as autopsy negative SUD or SADS following conventional post-mortem examination, the molecular autopsy can be performed to elucidate evidence for a primary inherited arrhythmia syndrome as the cause. A standard molecular autopsy panel will typically include the 4 main genes accounting for majority significant number of previously unexplained SCDs, including *KCNQ1* (LQT1), *KCNH2* (LQT2), *SCN5A* (LQT3/BrS1), and *RYR2* (CPVT1). The diagnostic yield is reported at 15-30% across many studies using Sanger sequencing or next generation cardiac panels.[3, 9, 40, 41] Successful exome based molecular autopsy has been reported.[42, 43] The earliest exome-based case series of 50 sudden deaths from Australia showed a yield of 10% in known LQTS genes as well as 21% yield in other cardiomyopathy and channelopathy genes.[44] A study of 59 cases using targeted exome sequencing of 135 genes, also including cardiomyopathy genes, was positive in 29%.[45] These findings suggest that a malignant arrhythmic phenotype leading to sudden death may pre-date cardiomyopathy phenotype, particularly ARVC and DCM. Molecular autopsy may therefore be a vital diagnostic tool in permitting accurate family evaluation even where the conventional autopsy is negative.[46-48]

However, rapid expansion of high throughput genetic testing has also led to increased identification of variants of uncertain significance (VUS). Widespread sequencing in normal controls has improved considerably our understanding of "normal" genetic variation.[49, 50] For example, Refsgaard et al, identified 33 rare variants previously associated with LQTS in the Exome Sequencing Project database.[51] If these variants were all truly disease causing, then the prevalence of LQTS would be 1 in 31 rather than 1 in 2000. Large public genetic databases (e.g.

gnomAD, ExAC) have confirmed the important issue of increased background noise and the increased burden of VUS first suggested over 15-years ago.[52] Of the canonical SCD-predisposing channelopathy genes, this is especially true for variants identified in *SCN5A* which has a high rate of background noise of 2% in Caucasians and 4-5% in non-Caucasians with a signal-to-noise ratio of 4.5-11.[52]

With increased stringency on variant calling using the spectrum of pathogenicity across ACMG criteria (from benign to pathogenic), many variants which may have been labelled pathogenic in the past are downgraded ("reclassified") to benign or left in "genetic purgatory" as a VUS.[53] This is reflected in a recent study of over 300 SADS cases finding a yield of only 13% of pathogenic and likely pathogenic variants, but a 42% VUS yield.[9] Pathogenic and likely pathogenic variants can be used in first-degree family member testing to assess their underlying risk, and potentially discharge gene-negative patients from ongoing clinical screening. The identification of a VUS cannot, however, be used for predictive testing in family members nor should it be ignored and treated merely as an innocent bystander.[51] Importantly, some VUS can be promoted easily and quickly to likely pathogenic variants after review by the primary sudden death investigative team while other VUS remain stuck indefinitely in ambiguity. To reclassify a VUS requires significant work-up in a family or laboratory in order to accurately determine benign or pathogenic status. This may include functional testing in vitro or in vivo, expansion of family pedigrees, identification of other families with similar phenotypes and the same genotype, or identification of the genotype in unaffected individuals. There is some evidence that community-based data aggregation and sharing may improve genetic variant classification; large registries of sudden death in the young may also help in the future.[54, 55] Unfortunately, these

variants will often remain a VUS for many years.[53] These families should be managed as though they are genotype-negative and the VUS should not influence clinical management decisions.

In addition, interpretation of genetic results in individuals with suspected BrS warrant careful review. There is an emerging body of evidence that, BrS is more likely to have an oligogenic, rather than monogenic basis.[56] Therefore, the negative predictive value for a family member who tests negative for the family's presumed-causative *SCN5A* variant is not 100%. New data show that single gene associations beyond *SCN5A* lack sufficiently robust evidence to be used clinically.[47] Therefore, careful interpretation of any potentially causative variant is vital and all previously published BrS-susceptibility genes other than *SCN5A* should be viewed more conservatively as a gene of uncertain significance (GUS).[56-58]

Historically, molecular autopsy has relied on the successful storage of blood or another DNA-rich medium such as frozen spleen, liver, or thymus. However, recently, successful exome-sequencing based molecular autopsy from formalin-fixed tissue has also been reported.[59, 60] This has opened up a larger resource of diagnostically useful post-mortem tissue for genetic testing, when more reliable DNA sources such as blood are unavailable.

Exome sequencing in cardiac, epilepsy, and neurological control genes following sudden unexplained death in epilepsy (SUDEP) has shown that 7% of these cases in fact have LQTS-associated variants. This suggests that a proportion of these deaths may be SCDY cases.[61] Therefore, it may be appropriate for these families to also undergo a baseline cardiac assessment with an ECG in certain circumstances, such as an antemortem history of exertional or poorly controlled *grand mal* seizures or a family history of multiple events.

**Implications for Surviving Family Members**

Channelopathies and cardiomyopathies are most commonly inherited in an autosomal dominant manner. There is therefore a 50% chance that other family members may have inherited the same variant as the deceased and are therefore also at risk of SCD.[37] By using a combined approach of clinical screening in first degree family members and post mortem genetic testing in the decedent, there has been an increased diagnostic yield of 40% compared with using either of these approaches in isolation.[9]

Guidelines recommend predictive ("mutation/pathogenic variant-specific") genetic testing in first degree family members if a pathogenic/likely pathogenic variant is identified in the decedent.[37] As stated above, it is critical that any variant identified is reviewed carefully for pathogenicity to ensure that the variant is "causal" prior to being used in any predictive testing in surviving family members.[62] It can then be used to inform further follow up in family members and potential therapeutic recommendations.

Being able to establish a diagnosis following SCDY, provides important benefits to the surviving family, including psychological closure for some.[2] Relatives identified with the same disease as the decedent may also be at risk of SCD.[1, 2, 37] Early identification prompts important interventions including lifestyle advice (e.g. avoidance of triggers such as QT prolonging medications in LQTS, prompt treatment of fever in BrS) as well as commencement of potentially life-saving medications (e.g. beta blockers in LQTS and CPVT). Recent data suggests up to 42% of individuals with a LQTS-causative variant, have a QTc within the normal range (< 460 ms) and therefore, a precise molecular diagnosis is integral in this setting.[63] The greatest benefit in establishing a genetic diagnosis may be for those who do not possess the causative variant and are therefore able to be discharged from further follow-up in the cardiac genetic clinic.[10] Pre- and

post-test genetic counselling is critical to ensure that these families understand the potential implications and limitations of cardiac genetic testing.[64]

**Multidisciplinary Genetic Heart Disease Clinic**

Managing the individuals and families after SCDY requires specific expertise. The families experience a range of emotions from grief to hopelessness to anger. It is important that when they attend the clinic they feel supported through the investigation and assessment procedure. There is often a feeling of guilt amongst surviving family members, particularly if a parent is identified to have the genetic condition that may have caused the death of their child.[12] Studies have shown almost 1 in 2 family members experience prolonged psychological difficulties following SCDY, particularly prolonged grief and post-traumatic stress. This is worse in those who have poor adaptation to a molecular autopsy result.[65] On the other hand, being able to provide the families with an answer by finding a cause for the SCD can also help to provide closure and improve psychological well-being in surviving relatives. Therefore, it is important that the families are reviewed in a multidisciplinary setting and team including genetic cardiologists, pediatricians, nurses, genetic counsellors, pathologists, psychologists/psychiatrists, and physiologists who are all specifically trained in the field.[38]

**Conclusion**

SCDY is a rare but tragic outcome arising from certain cardiac diseases including the heritable cardiomyopathies and channelopathies. While SCD can occur in a patient previously diagnosed with and treated for their ICC, SCD is often the first presentation of disease in a family. Approximately 40% of SCDY cases remain unexplained after autopsy and toxicology. Because of the genetic basis of most underlying cardiovascular causes, timely and accurate assessment of surviving family members, in conjunction with a molecular autopsy in the decedent are the

mainstays for trying to establish a definitive cause in order to provide the kind of closure and clarity that these families deserve. Managing families following SCDY is best performed in a multidisciplinary genetic heart disease clinic.

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**Table 1:** Causes of SCDY are categorized broadly into either structural or primary arrhythmic causes. Conditions in bold are predominantly genetic in their causation. Conditions are listed in alphabetical order.

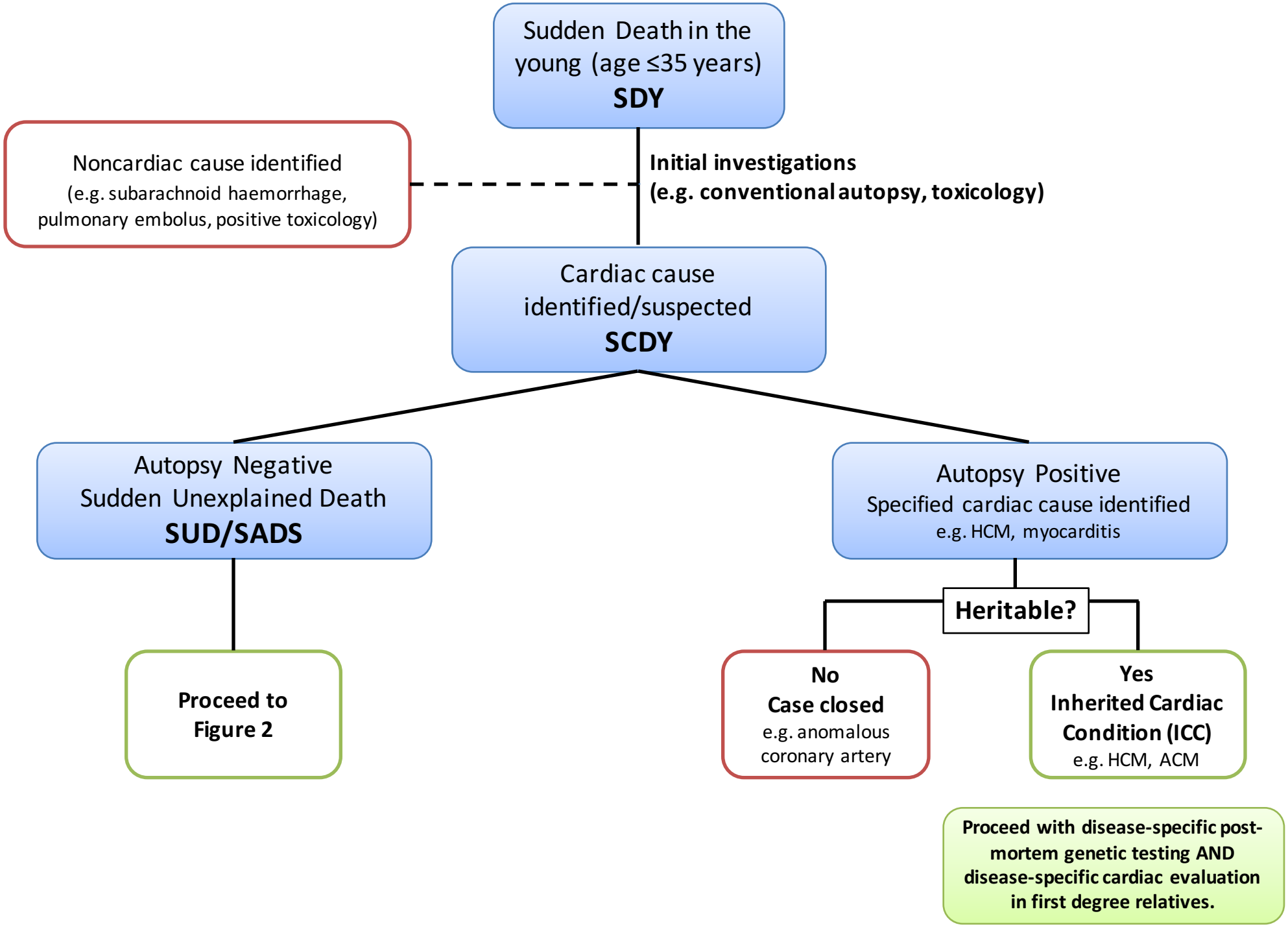
| **STRUCTURAL CAUSES** | **ARRHYTHMOGENIC CAUSES**<br><br>**(Primary Electrical Diseases, AKA Cardiac Channelopathies)** |
|---|---|
| • **Aortic disease**<br>• **Arrhythmogenic cardiomyopathy (ACM)**<br>• **Arrhythmogenic right ventricular cardiomyopathy (ARVC)***<br>• Congenital heart disease<br>• Coronary anomalies<br>• Coronary artery disease* (secondary to **familial hypercholesterolemia**)<br>• **Dilated cardiomyopathy (DCM)***<br>• **Hypertrophic cardiomyopathy (HCM)** *<br>• Myocarditis*<br>• **Restrictive cardiomyopathy (RCM)** | • **Brugada syndrome (BrS)***<br>• **Catecholaminergic polymorphic ventricular tachycardia (CPVT)***<br>• **Early repolarization syndrome (ERS)**<br>• **Long QT syndrome (LQTS)***<br>• **Progressive cardiac conduction disease (PCCD)**<br>• **Short QT syndrome (SQTS)**<br>• Wolff-Parkinson-White Syndrome (WPW) |

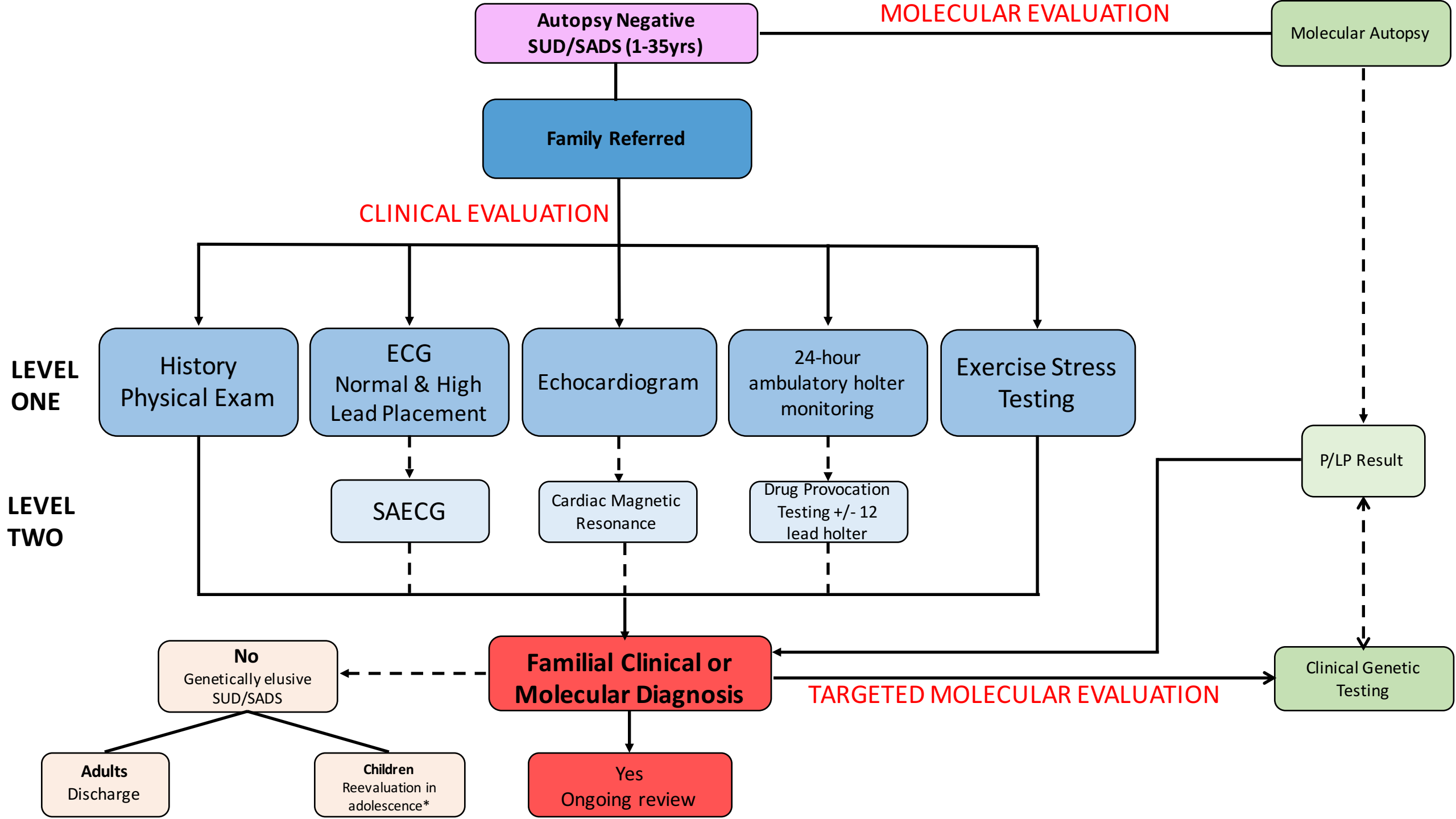
*denotes most common causes of SCDY[3]

**Figure Legends:**

**Figure 1**: Flow chart for investigation and management of a family following a sudden death in the young. SDY- sudden death in the young, SCDY- sudden cardiac death in the young, SUD- sudden unexplained death, SADS- sudden arrhythmic death syndrome, ICC- inherited cardiac condition, HCM- hypertrophic cardiomyopathy, ACM- arrhythmogenic cardiomyopathy

**Figure 2**: Comprehensive two-tier clinical and molecular diagnostic pathway for at least first degree relatives of an autopsy negative SUD/SADS victim. *recommend re-evaluation in late adolescence/early adulthood, with consideration of drug provocation testing at this time. ˜ 12-lead Holter may be used after provocation testing for risk evaluation or beforehand in case a dynamic type 1 pattern obviates the need for drug provocation. SUD- sudden unexplained death, SADS- sudden arrhythmic death syndrome, ECG- electrocardiogram, SAECG- signal-averaged electrocardiogram, P/LP- pathogenic/likely pathogenic


Sudden Death in the young (age ≤35 years)
SDY
Noncardiac cause identified
(e.g. subarachnoid haemorrhage, pulmonary embolus, positive toxicology)
Initial investigations
(e.g. conventional autopsy, toxicology)
Cardiac cause identified/suspected
SCDY
Autopsy Negative
Sudden Unexplained Death
SUD/SADS
Autopsy Positive
Specified cardiac cause identified
e.g. HCM, myocarditis
Heritable?
Proceed to Figure 2
No
Case closed
e.g. anomalous coronary artery
Yes
Inherited Cardiac Condition (ICC)
e.g. HCM, ACM
Proceed with disease-specific post-mortem genetic testing AND disease-specific cardiac evaluation in first degree relatives.


Autopsy Negative
SUD/SADS (1-35yrs)
MOLECULAR EVALUATION
Molecular Autopsy
Family Referred
CLINICAL EVALUATION
LEVEL ONE
History
Physical Exam
ECG
Normal & High
Lead Placement
Echocardiogram
24-hour
ambulatory holter
monitoring
Exercise Stress
Testing
LEVEL TWO
SAECG
Cardiac Magnetic
Resonance
Drug Provocation
Testing +/- 12
lead holter
P/LP Result
Familial Clinical or
Molecular Diagnosis
TARGETED MOLECULAR EVALUATION
Clinical Genetic
Testing
No
Genetically elusive
SUD/SADS
Adults
Discharge
Children
Reevaluation in
adolescence*
Yes
Ongoing review