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The molecular autopsy: an indispensable step following sudden cardiac death in the young?

Nicole J. Boczek[1,2], **David J. Tester**[2,3], and **Michael J. Ackerman**[2,4,5]

[1]Mayo Graduate School, Mayo Clinic, Rochester

[2]Windland Smith Rice Sudden Death Genomics Laboratory, Mayo Clinic, Rochester

[3]Department of Medicine (Division of Cardiovascular Diseases), Mayo Clinic, Rochester

[4]Department of Molecular Pharmacology and Experimental Therapeutics, Mayo Clinic, Rochester

[5]Department of Pediatrics (Division of Pediatric Cardiology), Mayo Clinic, Rochester

Abstract

Annually thousands of sudden deaths involving young individuals (< 35 years of age) remain unexplained following a complete medicolegal investigation that includes an autopsy. In fact, epidemiological studies have estimated that over half of sudden deaths involving previously healthy young individuals have no morphological abnormalities identifiable at autopsy. Cardiac channelopathies associated with structurally normal hearts such as long QT syndrome (LQTS), catecholaminergic polymorphic ventricular tachycardia (CPVT), and Brugada syndrome (BrS), leave no evidence to be found at autopsy, leaving investigators to only speculate that a lethal arrhythmia might lie at the heart of a sudden unexplained death (SUD). In cases of autopsy-negative SUD, continued investigation, through the use of a cardiological and genetic evaluation of first- or second-degree relatives and/or a molecular autopsy, may pinpoint the underlying mechanism attributing to the sudden death and allow for the identification of living family members with the pathogenic substrate that renders them vulnerable to an increased risk for cardiac events, including sudden death.

Keywords

Molecular autopsy; Sudden death; Channelopathies; Long QT syndrome; Genetic testing

Tragically, thousands of individuals younger than 35 years of age die suddenly each year. While many of these deaths are attributed to sudden cardiac death due to an underlying structural pathology evident at autopsy, a significant number remain unexplained and are termed sudden unexplained death (SUD). In this review, we will explore the epidemiology of sudden cardiac death in the young, the relationship between cardiac channelopathies and SUD, and the indispensible steps of a clinical assessment of surviving relatives and the molecular autopsy of the decedent in the evaluation of SUD. Finally, we will examine some

Corresponding address, Michael J. Ackerman MD, PhD Department of Medicine (Division of Cardiovascular Diseases) Department of Pediatrics (Division of Pediatric Cardiology), Mayo Clinic, Rochester, MN ackerman.michael@mayo.edu.

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of the current recommendations in the evaluation of sudden death in the young as well as provide some indications for a molecular autopsy.

Epidemiology of sudden cardiac death in the young—how common is sudden unexplained death?

It is estimated that 300,000–400,000 individuals die suddenly each year in the United States, with the vast majority being the elderly [40]. In comparison, sudden death in the young is uncommon, with an incidence between 1.3 and 8.5 per 100,000 patient-years [22]. However, annually, thousands of young individuals (<35 years of age) die suddenly. Fortunately, the cause and manner of death can often be identified through postmortem investigations that include an autopsy.

An autopsy may reveal noncardiac forms of death, such as pulmonary embolism, asthma, or epilepsy; however, the most common cause of sudden death is sudden cardiac death (SCD). SCD has been defined by the American Heart Association as the sudden, abrupt loss of heart function in a person who may or may not have been diagnosed with heart disease whereby the time and mode of death are unexpected, and the death occurs either instantly or shortly after symptoms appear [1]. Many SCD cases have structural cardiac abnormalities such as hypertrophic cardiomyopathy (HCM), arrhythmogenic right ventricular cardiomyopathy (ARVC), congenital coronary artery anomalies, or myocarditis, which are typically identifiable through autopsy. However, it is estimated that at least 3 % and up to 53 % of cases of youthful (age 1–35 years) sudden deaths have no morphologic abnormalities that are identified through autopsy (Fig. 1, [7,10,12,15, 16, 23, 25, 29, 41, 42]). These cases are referred to as autopsy-negative sudden unexplained death (SUD).

In a 1996 study by Maron and colleagues, HCM was the most common cause of SCD involving young competitive athletes, where 48/134 (36 %) of SCDs were ascribed to HCM and an additional 10 % had “possible HCM.” Only 3 % of cases were deemed to be autopsy-negative SUD [23]. In a study by Corrado and colleagues, only 6 % of their 273 cases of SCD in young people (≤ 35 years of age) in the Veneto region of northeastern Italy were considered autopsy-negative SUD following detailed histological examination of the 28 % of cases that had macroscopically normal hearts. Histological examination revealed concealed pathologic substrates including myocarditis, regional arrhythmogenic right ventricular cardiomyopathy, and conduction system abnormalities [10].

In 2005, Puranik and colleagues examined a population-based cohort of 427 young sudden unexpected death cases (5–35 years old) in eastern Sydney, Australia. SCD was identified in over half of these cases, and contrary to the previous studies, autopsy-negative SUD (29 %) was the leading cause of SCD [29]. Eckart and colleagues examined 6.3 million men and women, American military recruits, aged 18–35 years over a 25-year period. The sudden nontraumatic death rate was 13 per 100,000 recruit years. Approximately half of these deaths could be attributed to an identifiable cardiac abnormality during autopsy, but 35 % of these sudden deaths were autopsy-negative SUD. Several of the cases had a previous family history of sudden death suggesting a potential heritable lethal arrhythmia [15]. Morentin and colleagues analyzed all sudden, non-violent deaths in persons 1–35 years of age occurring in northern Spain from 1991 to 1998. Among the 107 cases of sudden death, 18 % were considered SUD. Interestingly, antecedent symptoms consistent with cardiac arrhythmia manifestations were evident in five SUD cases [25]. Fabre and colleagues reported that over 50 % of their cohort of 453 United Kingdom sudden death cases, 15–81 years of age, had normal hearts both macroscopically and microscopically. Among cases aged 15–35 years, 53.5 % had normal hearts [16]. In a Swedish study involving 15- to 35-year-old subjects with unexplained death, 21 % had a normal heart [42]. An American study involving an

autopsy series of 14- to 40–year-olds, found 16 % with structurally normal hearts [7]. In 2011, Winkel and colleagues performed a nationwide study of SCD in young Danish individuals aged 1–35 years and determined that 29 % were autopsy-negative SUD [41] (Fig. 1).

The discordance in “structurally normal heart” rates observed in these epidemiology studies is most likely attributed to the extensive variation and lack of standardization in the process in which the forensic pathologists/medical examiners/ coroners, responsible for defining the exact cause of sudden death, approach this increasingly complex task. This heterogeneity makes interpretation of epidemiological data on sudden death difficult. In 2008, the Association for European Cardiovascular Pathology described a minimum autopsy standard required for the assessment of SCD including protocols on heart examination, toxicology, histological sampling as well as molecular investigations [3].

Although the majority of SCDs can be attributed to structural abnormalities, there are an alarming number of sudden deaths in the young that remain unexplained after autopsy and postmortem investigation.

Cardiac channelopathies and autopsy-negative sudden unexplained death

Potentially lethal and heritable channelopathy disorders such as long QT syndrome (LQTS), catecholaminergic polymorphic ventricular tachycardia (CPVT), and Brugada syndrome (BrS) may explain a significant number of these autopsy-negative SUD cases. These disorders lead to electrical disturbances in structurally normal hearts and have the capability of instigating lethal cardiac arrhythmias. The electrical abnormalities are often benign, but can quickly spiral out of control in an unsuspecting individual leading to a sudden, early death in an otherwise healthy young individual. Over the past 15 years, through advances in genetic screening, the underlying genetic basis responsible for many inherited cardiac arrhythmia disorders has been discovered. To date, several studies involving either the cardiological assessment of surviving first-degree relatives or the postmortem genetic analysis (the cardiac channel “molecular autopsy”) of the decedent’s DNA have implicated LQTS, CPVT, and BrS as a clinical/molecular basis for as much as one-third of autopsy-negative SUD [33].

Clinical assessment of those left behind

In 2003, Behr and colleagues completed cardiovascular evaluations of 109 first-degree relatives of 32 SUD victims. This study identified that 22 % of the families had evidence suggesting an inherited cardiac disease with the majority having clinical sequelae suggestive of LQTS [6]. In 2005, Tan and colleagues found that 28 % of families had an identifiable cardiac channelopathy [32]. In a 2008 follow-up study by Behr and colleagues, a diagnosis of heritable heart disease was determined in 53 % of first-degree relatives of SUD victims following a more comprehensive clinical evaluation, with 70 % being diagnosed with either LQTS (53 %) or BrS (17 %). Interestingly, 30 % of the families reported a family history of additional unexplained sudden deaths under the age of 45 years, and 20 % of the decedents had a prior history of syncope [5].

In 2010, Van der Werf and colleagues examined a cohort of surviving relatives of 140 SUD cases aged 1–50 years and identified a certain or probable diagnosis in 33 % following a cardiological clinical assessment, with 96 % of the families diagnosed with an inherited cardiac diseases (21 % LQTS, 17 % CPVT, 15 % BrS, and 15 % ARVC). The diagnostic yield depended significantly on the age of the decedent ranging from a high of 70 % when the decedent was aged 1–10 years to a low of 21 % when the decedent was between the ages of 41 and 49 years. While there was no prior clinical diagnosis for the decedent or any

family member, many of these sudden death victims had warning signs prior to their lethal event, such as previous personal syncope in 15 % or a family history of young sudden death in 29% [39].

Incomplete penetrance and variable expressivity are challenging clinical features of the various cardiac channelopathies that consequently lead to “concealed” forms of these disorders [28]. Therefore, clinical assessment of surviving family members of SUD victims may not adequately detect LQTS, CPVT, or BrS in some unsuspecting individuals. A molecular autopsy involving postmortem cardiac channel genetic testing may provide the much needed utility for the forensic evaluation of an SUD case.

Molecular autopsy series of sudden unexplained death in the young

Owing to the increasing suspicion that autopsy-negative SUD cases may indeed be caused by lethal arrhythmias, investigators have sought to determine the spectrum and prevalence of mutations in ion channels that have been attributed to LQTS, CPVT and BrS in autopsy-negative SUD cases. To date, there have been ten molecular autopsy studies (Tab. 1) [9,11,13,14,17, 26, 31, 34, 36, 37] examining the major LQTS-, BrS-, and CPVT-susceptibility genes.

In 2004, Chugh and colleagues identified 12 cases of SUD following a comprehensive postmortem analysis of a consecutive series of 270 adult (age ≥ 20 years) cases of SCD occurring over a 13-year period. Postmortem genetic analysis of the LQTS-susceptibility genes revealed the identical *KCNH2* mutation in two of 12 (17 %) cases of autopsy-negative SUD [9]. Similarly, Di Paolo and colleagues performed LQTS postmortem genetic testing on ten cases of juvenile (ages 13–29 years) SUD and identified *KCNQ1* mutations in two individuals [13]. In 2006, Creighton and colleagues performed a study on nine cases of autopsy-negative SUD and identified a 33 % yield of putative pathogenic channelopathy mutations [11]. In 2009, Nishio identified channel mutations in 24 % of their 17 SUD case cohort [26], and in 2010, Gladding, using DNA isolated from Guthrie (newborn blood spot) cards, identified LQTS-associated mutations in four of 18 (22 %) cases of SUD, aged 2–39 years [17]. In 2011, Skinner and colleagues examined 33 cases, and identified putative pathogenic mutations in 15 % of the cases [31]. Although these studies are small, a trend begins to emerge that 15–33 % of autopsy-negative SUD may be due to lethal arrhythmias.

Doolan and colleagues performed a molecular autopsy study on 59 cases of SUD and did not identify any mutations following a limited mutational analysis of only *KCNQ1* and a targeted analysis of *SCN5A* using genomic DNA derived from formalin-fixed paraffin-embedded tissue (FF-PET) [14]. The authors concluded that the “hit-rate” of a molecular autopsy in young SUD is low. However, because of their limited mutational analysis and the use of a largely unreliable source (FF-PET) of high-quality DNA, it is not too surprising that their mutation detection yield was low. Important to note, in order to successfully perform postmortem genetic testing, coroners, medical examiners, and forensic pathologist must secure “DNA-friendly” blood or tissue samples at autopsy.

In 2007, we completed a cardiac channel molecular autopsy on 49 cases of autopsy-negative SUD [34, 37]. Since then, we have extended this cohort to now include 173 cases of SUD to provide a more extensive analysis to better define the expected yield of mutation detection and offer possible genotype/phenotype correlations that may assist in guiding phenotype-directed mutation detection efforts in future cases of SUD. In this expanded molecular analysis, 26 % of the 173 SUD cases had a putative pathogenic mutation, with 14.5 % having mutations in the LQTS-associated genes and 11.5 % with mutations in the CPVT-associated *RYR2* gene. Sudden death was the sentinel event in 67 % of the mutation-positive SUD cases in this series. Tragically, however, despite no premortem diagnosis of a

suspected cardiac channelopathy in the decedent or family member, there was either a personal or family history of cardiac events indicative of an underlying disorder in nearly 60 % of the mutation-positive SUD cases that went unheeded prior to the unfortunate early demise of the decedent [36].

Together, these studies provide clinical and molecular evidence suggesting that a significant portion of autopsy-negative SUD indeed stems from an underlying cardiac channelopathy. Continued investigation through cardiological assessment and genetic evaluation of first-degree relatives and a molecular autopsy may be beneficial in elucidating the cause of the unexplained death. Because these potentially lethal arrhythmia syndromes are often familial inherited, determining the exact underlying basis for the SUD can have a great impact on the surveillance and treatment of surviving relatives.

Evaluation of sudden unexplained death in the young

Currently, although there is consensus of the necessity to perform thorough evaluations on SUD in the young, there is a general lack of standardization of this process. However, several societies have published specific guidelines for autopsy investigation of sudden unexpected death in the young. In 2008, Basso and colleagues, on behalf of the Association for European Cardiovascular Pathology, recommended strongly for postmortem genetic analysis in both structural and non-structural genetically determined heart disease, to fulfill the requirements for an *adequate* postmortem assessment of SCD [3]. The *T*rans-*T*asman *R*esponse *Aga*inst Sudden *D*eath in the *Y*oung (TRAGADY), endorsed by the Royal College of Pathologists of Australasia and the National Heart Foundation of New Zealand, have proposed guidelines to standardize autopsy practice in young sudden unexpected deaths, ancillary testing, and procurement of materials for postmortem genetic testing (Tab. 2). TRAGADY emphasizes the importance of skilled postmortem autopsies, especially because there are instances when families will suffer the tragic loss of multiple family members owing to insufficient investigations [38].

In 2011, the Hearth Rhythm Society and the European Heart Rhythm Association (HRS/EHRA) gave a consensus statement regarding genetic testing, providing their expert consensus recommendation for postmortem genetic testing in SUD: “In the setting of autopsy-negative SUD, comprehensive or targeted ion channel genetic testing may be considered in an attempt to establish probable cause and manner of death and to facilitate the identification of potentially at-risk relatives and is recommended if circumstantial evidence points toward a clinical diagnosis of LQTS or CPVT specifically.” HRS/EHRA also recommends that for all SUD cases, “DNA-friendly samples” should be acquired to enable subsequent genetic testing. Finally, HRS/EHRA recommends mutation-specific genetic testing for family members following the identification of an SUD-causative mutation in the decedent [2].

The Canadian Cardiovascular Society and Canadian Heart Rhythm Society gave a similar recommendation, including procurement and storage of tissue and/or DNA after SCD with negative autopsy findings for potential future genetic testing. In the case of an autopsy-negative SCD, “genetic testing of retained tissue is recommended only when there is evidence of a clinical phenotype in family members.” If a clinical history of events or clinical evaluation of family members is suggestive of a lethal arrhythmia, genetic screening on the proband and all identified affected family members was recommended [18].

When an SUD case is evaluated, an interdisciplinary collaboration between a pathologist/medical examiner/coroner, cardiologist, and colleagues with experience in genetic counseling is necessary [20, 24, 30]. Hopefully, such a multidisciplinary approach will limit

misinterpretation of pathology findings, genetic test results, and borderline cardiac clinical test results [31]. Partnering with a genetic counselor will be beneficial in gathering appropriate family history, counseling the family about clinical cardiological assessments, genetic testing in relatives, as well as completion of a molecular autopsy in the decedent (Fig. 2). The genetic counselor will also be helpful in interpreting the genetic testing results and discussing the modes of inheritance and risk-stratification within the families. Finally, the genetic counselor can help living relatives of SUD cases discuss some of the psychosocial impacts of such information [8,19,21].

Despite the previously published guidelines, there is not a clear consensus statement pertaining to the extent and breadth of the clinical evaluation of the living relatives to an SUD victim. Based on the previously described studies, it seems reasonable to advise first-degree relatives of the decedent to undergo cardiovascular evaluation, which would include (at minimum) an extensive personal and family history, a physical examination, a 12-lead electrocardiogram, treadmill stress test, and an echocardiogram. Alternatively, or simultaneously, a molecular autopsy (genetic testing) on the major genes associated with LQTS, CPVT, and BrS should be considered as standard of care in the evaluation of an SUD case, especially when the victim is less than 40 years of age.

Indications for molecular autopsy

Many of the published guidelines have recommended postmortem cardiac channel genetic testing, specifically in cases where there is evidence to suggest that a cardiac channelopathy may be responsible for the sudden death. Unfortunately, given the expensive and time-consuming nature of postmortem genetic testing, it is currently necessary for the medical examiner, coroner, or forensic pathologist to be case selective in pursuing a molecular autopsy [27].

Through our recent molecular autopsy investigations of SUD cases, we have garnered some interesting correlations that may help in selecting cases with the greatest potential for mutation discovery and directing genetic testing efforts [35]. For example, females (39 % yield overall) were significantly ($p < 0.005$) more likely to have a mutation than males (18 % yield overall), especially if the death was during adolescence (48 % yield females vs. 18 % in males, aged 11–20 years). For those cases that were mutation positive, females were more likely to have mutations in LQTS-susceptibility genes while males more often had mutations in the CPVT-associated RYR2 gene. Decedents whose death was associated with exercise had a greater detection rate (35 % overall, 50 % in females, 27 % in males) than those with a nonspecific trigger/circumstance (27 % overall, 36 % in females, 19 % in males), and those who died during a period of sleep (19 % overall, 32 % in females, 13 % in males) [35, 36].

Interestingly, for those decedents who were aged 1–10 years with an exercise-associated death, the mutation detection yield was 71 % for females and 60 % for males, with mutations usually associated with CPVT1 or LQT1. However, the yield dropped significantly to about 15 % for both male and female decedents aged 11–20 years with an exercise-associated death. Conversely, those aged 11–20 years that died during sleep had a much higher yield (75 % in females and 18 % in males) than when the sleep-associated death occurred in a decedent aged 1–10 years (0 % in females and 6 % in males). Decedents with a positive personal or family history of cardiac events had a significantly greater mutation detection yield (40 %) than those with no history (19 %) of events. Mutations were identified in 45 % of SUD cases with a family history of a prior sudden death [35, 36].

Thus, one might a priori expect a higher yield of LQTS-associated mutation detection in an adolescent or young adult female compared to a higher expected yield of CPVT-associated mutations among male children. Because young male and female children with exercise-associated death and adolescent females with death during a period of sleep have the highest mutation detection rate ranging from 60 to 75 %, these types of SUD cases should undergo molecular autopsy [35, 36]. Understanding the effect of sex, age, death circumstance, and/or personal or family history of cardiac events, on the overall mutation detection yield, may assist in guiding both the clinical assessment of surviving relatives and the molecular autopsy for cases of SUD, thereby creating a more cost-effective approach to the evaluation of SUD.

Biological material used in a molecular autopsy

Owing to its ease of storage and transportation, archived FF-PET is the only source of DNA that is typically collected at autopsy. Unfortunately, DNA from FF-PET is often error prone and may be unreliable for comprehensive genetic testing. The best samples to procure high-quality DNA for genetic testing include at least 5–10 ml of autopsy blood collected in EDTA tubes, and/or 5 g of fresh heart, liver, or spleen tissue [4]. These materials should be stored at − 80 °C until DNA can be extracted. If available, 50–100 μl of whole blood on filter paper can also be utilized to extract DNA. However, this tends to provide a very small amount of DNA; therefore, while it is a viable option, it is suboptimal because of the limited amount of genetic analysis that can be performed [33]. It is very important that guidelines for the procurement of tissue suitable for DNA extraction and analysis be implemented in the standard of care for the postmortem analysis of SUD.

Conclusion

The combination of a clinical cardiological assessment of surviving relatives and a molecular autopsy of the decedent are indispensible steps for the accurate diagnosis of an SUD. Such a combined, multidisciplinary approach should enable informed genetic counseling for families and should direct the commencement of appropriate preemptive strategies targeted toward averting another tragedy among those left behind. Because autopsy-negative SUD accounts for such a significant number of sudden deaths in the young and considering that cardiac channelopathies contribute to a large portion of these deaths, clinical cardiological assessment of surviving family members and a cardiac channel molecular autopsy should be viewed as the new standard of care for the postmortem evaluation of SUD.

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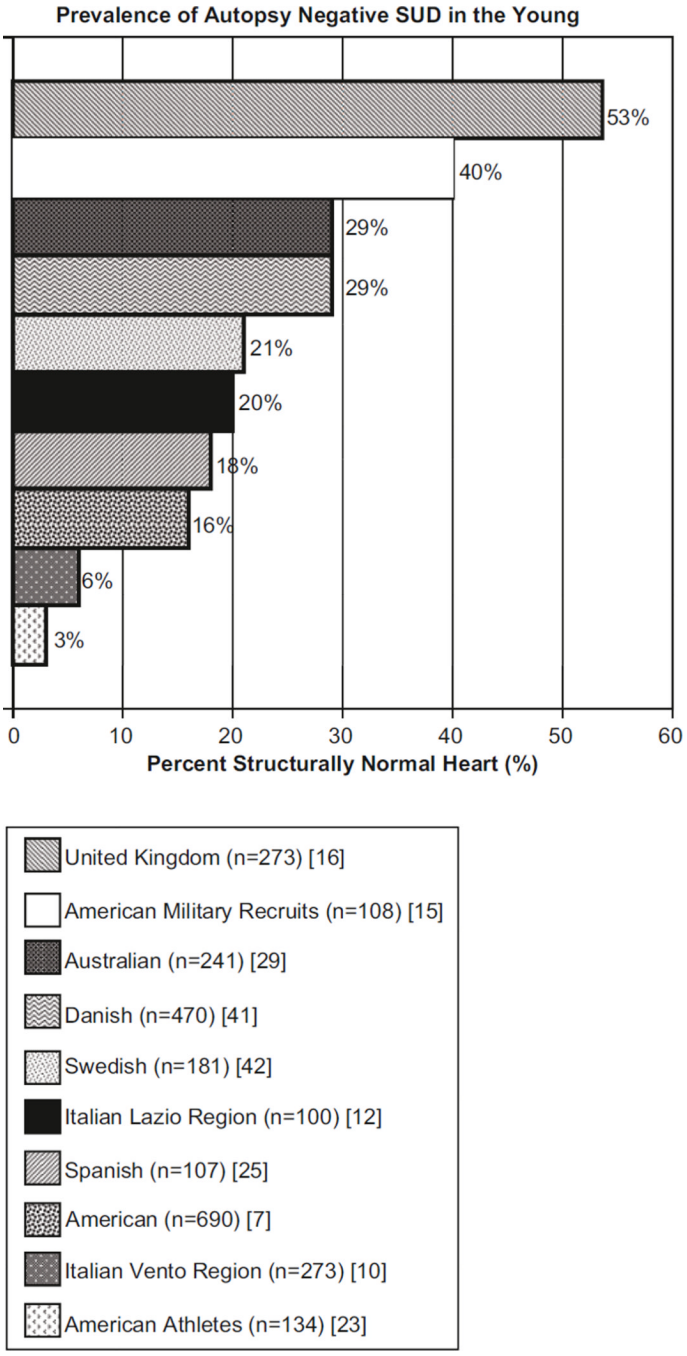

Fig. 1.

The prevalence of autopsy-negative “structurally normal” heart sudden unexplained death in retrospective autopsy series. It is estimated that at least 3 % and perhaps as much as 53 % of sudden deaths involving previously healthy children, adolescents, and young adults have no identifiable morphologic abnormalities found at autopsy (“structurally normal heart”), and the SCD is labeled as autopsy-negative sudden unexplained death (*SUD*). Shown is a bar graph with the percent of autopsy-negative “structurally normal heart” identified during ten retrospective analyses of large population-based autopsy cohorts [33]

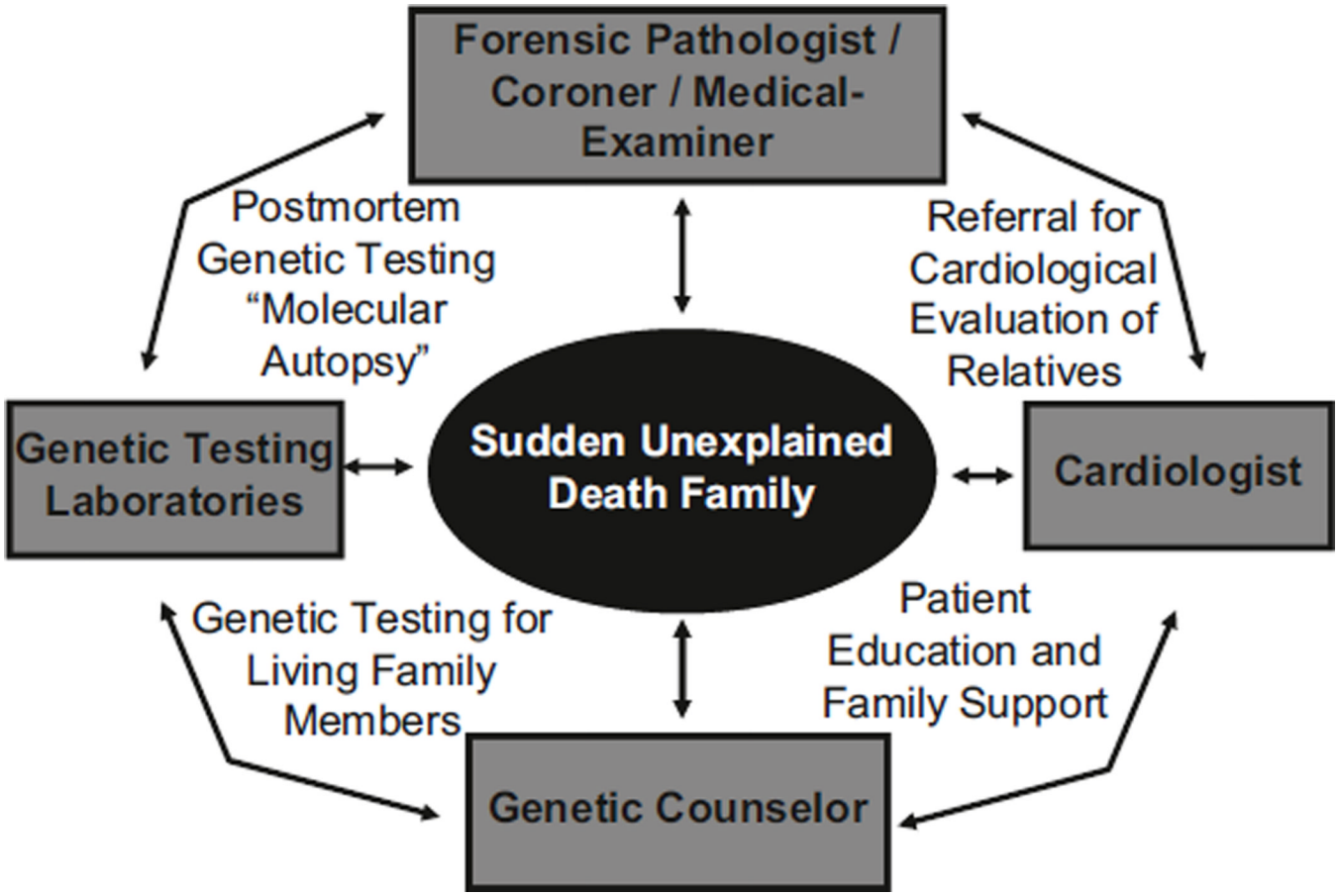

Fig 2.
Multidisciplinary approach to the evaluation of sudden unexplained death (SUD). The evaluation of SUD should be an interdisciplinary collaboration between an expert pathologist/medical examiner/coroner, cardiologist, and colleagues equipped with expertise in genetic counseling, ideally a cardiogenetic counselor

Tab. 1

Summary of molecular autopsy series in autopsy-negative sudden unexplained death (arranged from the smallest to the largest case series published)

Reference	Year published	Number of cases	Age range (years)	Males/ females	Genes analyzed	Number of mutations (% yield)	DNA source	Mutation analysis technique
Creighton [11]	2008	9	1–43	5M/4F	*KCNQ1, KCNH2, SCN5A, KCNE1, KCNE2, RYR2* (target)	1 KCNQ1,2RYR2(33%)	Frozen tissue	Direct DNA sequencing
Di Paolo [13]	2004	10	13–29	5M/5F	LQTS genes	2KCNQ1 (20%)	FF-PET	SSCP
Chug [9]	2004	12	N/A	N/A	*KCNQI, KCNH2, SCN5A, KCNE1, KCNE2*	2KCNH2(17%)	FF-PET	SSCP/direct DNA sequencing
Nishio [26]	2009	17	12–42	13M/4F	*KCNQI, KCNH2, SCN5A, RYR2* (target)	1 KCNQ1,3RYR2(24%)	Autopsy blood	High-resolution melt
Gladding [17]	2010	18	2–39	11 M/7 F	*KCNQI, KCNH2, SCN5A, KCNE1, KCNE2*	2KCNQ1,2KCNH2(22%)	Guthrie (blood spot) card	DHPLC
Skinner [31]	2011	33	1–40	24M/9F	*KCNQI, KCNH2, SCN5A, KCNE1, KCNE2*	1 KCNQ1,1 KCNH2, 1 SCN5A, 2KCNE1(15%)	Frozen tissue or autopsy blood	DHPLC
Doolan [14]	2008	59	1–35	38M/21 F	*KCNQI, SCN5A* (target)	0(0%)	FF-PET	DHPLC
Tester [34,36,37]	2004, 2007, 2011	173	1–43	106M/67F	*KCNQI, KCNH2, SCN5A, KCNE1, KCNE2, RYR2* (target)	11 KCNQ1, 6KCNH2, 6 SCN5A, 2KCNE2, 20RYR2 (26%)	Frozen tissue or autopsy blood	DHPLC

N/A not available, M male, F female, FF-PET formalin-fixed paraffin-embedded tissue, SSCP single-stranded confirmation polymorphism, DHPLC denaturing high-performance liguid chromatography

Tab. 2

Key principles of postmortem investigations of sudden unexpected death in the young (adapted from "Post-mortem in sudden unexpected death in the young: guidelines on autopsy practice,"devised by TRAGADY—Trans-Tasman Response Against Sudden Death in the Young—and endorsed bythe Royal College of Pathologists of Australasia [38])

1. All cases of sudden unexpected or unexplained death in the young (age group of 0–40 years) should have an autopsy
2. A full postmortem examination should be completed
3. The investigation, ideally lead by a pathologist, should involve a team approach **a.** A person designated to liaise with the family **b.** Specialist cardiology involvement with the family when noncardiac causes are excluded **c.** Laboratories with molecular genetics, toxicology, and metabolic expertise
4. A detailed antecedent clinical history must be obtained **a.** Circumstances of the death—detailed review of the date, time, place, and activity surrounding the death (i.e., at home, work, or on the athletic field, at rest or during exercise or emotional excitement). Was the death witnessed? Document any associated seizures, prodromal symptoms **b.** Past medical history—Document the general health status, including previous significant illness or events such as syncope, seizures, epilepsy, palpitations, and respiratory or neurological disease. Retrieve results of any prior investigations (e.g., ECG, EEG, CT, or MRI) **c.** Previous surgical procedures or interventions—Document details of current medications, in particular those that are known to be pro-arrhythmic (see: www.qtdrugs.org)
5. A detailed and relevant family history must be obtained **a.** Family history—Document any family history of premature death (explained or unexplained death, SIDS, unexplained drowning, or unusual motor vehicle accidents), seizures, or syncope. Be sure to include a detailed description of the date, time, place, and activity surrounding such events, if available **b.** Diagnosed disorders—Document any family history of clinical diagnosis of potentially arrhythmic disorders, including LQTS, CPVT, BrS, familial cardiomyopathy, or other cardiac conditions
6. Skilled macroscopic and microscopic examination of the organs is required, particularly of the heart (especially right ventricular muscle) and the brain. This may require some specimens to be examined by other specialists
7. Adequate histological material be obtained for review or, if necessary, referral
8. Tissue or blood suitable for DNA extraction must be obtained
9. Results, including photography, must be documented clearly
10. Results must be described and annotated in a standard fashion which will allow epidemiological data gathering