REVIEW ARTICLE

The role of genetic testing in unexplained sudden death

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Most sudden deaths are because of a cardiac etiology and are termed sudden cardiac death (SCD). In younger individuals coronary artery disease is less prevalent and cardiac genetic disorders are more common. If sudden death is unexplained despite an appropriate autopsy and toxicologic assessment the term sudden arrhythmic death syndrome (SADS) may be used. This is an umbrella term and common underlying etiologies are primary arrhythmia syndromes with a familial basis such as Brugada syndrome, long QT syndrome, and subtle forms of cardiomyopathy. The first clinical presentation of these conditions is often SCD, which makes identification, screening, and risk stratification crucial to avert further deaths. This review will focus on genetic testing in the context of family screening. It will address the role of the "molecular autopsy" alongside current postmortem practices in the evaluation of SADS deaths. We describe the current data underlying genetic testing in these conditions, explore the potential for next-generation sequencing, and discuss the inherent diagnostic problems in determination of pathogenicity. (Translational Research 2015;■:1–15)

Abbreviations: AHA = American Heart Association; ARVC = arrhythmogenic right ventricular cardiomyopathy; BrS = Brugada syndrome; ChIP = channel interacting protein; CPVT = catecholaminergic polymorphic ventricular tachycardia; DCM = dilated cardiomyopathy; EHRA = European Heart Rhythm Association; ERS = early repolarization syndrome; HCM = hypertrophic cardiomyopathy; HRS = Heart Rhythm Society; IVF = idiopathic ventricular fibrillation; LQTS = long QT syndrome; PCCD = premature cardiac conduction disease; SADS = sudden arrhythmic death syndrome; SCD = sudden cardiac death; SNR = signal-to-noise ratio; SQTS = short QT syndrome; SUDS = sudden unexpected death syndrome; VUS = variant of unknown significance

INTRODUCTION

The estimated incidence of sudden cardiac death (SCD) in the general population in Europe and the United States (US) is between 50 and 100 per 100,000 per annum.[1-3] SCD claims 300,000–400,000 deaths per annum in the US[4,5] and there were an estimated 70,000 SCDs in the UK in 2010, most because of ischemic events.[6] SCD is usually defined as an unheralded witnessed instantaneous death but it may also be described as being preceded by a prodrome of acute cardiac symptoms up to 1 hour before death.[5] Unwitnessed cases without a prior deterioration in the preceding 12–24 hours may also be included. Estimates vary, however, because of a dependence on the presence

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of autopsied vs nonautopsied cases and the variability of definitions and the duration of prodromal symptoms before the terminal event. A national prospective survey of English coroners' cases for more than a 20-month period in the late 1990s evaluated sudden deaths in Caucasians aged 4–64 years with no history of cardiac disease, negative toxicology, and last seen alive within 12 hours of death. A normal coroner's autopsy and subsequent evaluation of the heart by a cardiac pathologist established these as sudden arrhythmic death syndrome (SADS) cases with an estimated incidence of up to 1.38 per 100,000 per annum.

SCD in the young. In individuals aged <35 years inherited cardiac diseases are more prevalent and estimates of the incidence of SCD vary depending on definitions and study site. For example, a Danish nationwide analysis of deaths from 2000 to 2006 revealed an annual incidence of young SCD of 2.8 per 100,000,[7] whereas a retrospective US study of >6 million military recruits reported an incidence as high as 13 per 100,000 per annum.[8] Puranik et al[9] retrospectively reviewed pathologic reports from 427 autopsied sudden death cases aged 5–35 years at a forensic medical facility from 1995 to 2004 in Sydney. The most common cardiac cause of death was presumed arrhythmia in those with no (or minimal) structural heart disease (29%), that is, SADS. Retrospective analysis of death certification, autopsy reports, and registry data estimated that 31% of autopsied SCD cases in Danes aged 1–49 years were unexplained and attributed to SADS.[10]

Conversely, in 79% of 197 cases of young SCD in Italy, histologic analysis yielded a structural diagnosis such as cardiomyopathy or focal myocarditis; 6% were unexplained and attributable to SADS.[11] In the Veneto region of Italy, studies have implicated arrhythmogenic right ventricular cardiomyopathy (ARVC) in 20% of sudden deaths among athletes and the young.[12] Maron et al[13] described sudden deaths in young competitive athletes for more than a 27-year period in the US: 56% were because of cardiovascular disease, the most common cause (36%) being hypertrophic cardiomyopathy (HCM).

In the UK the incidence of cardiac death in the young (≤35 years) as determined by analysis of Office for National Statistics data was 1.8 per 100,000 in England and Wales (2002–2005). Critical appraisal estimated the incidence of SADS as 0.24 per 100,000 per annum, significantly higher than the 0.1 per 100,000 reported by the Office for National Statistics as instantaneous unexplained sudden death, but still less than most other estimates.[14] This is likely to be because of misclassification of the cause of death as mortality data are largely derived from death certificate documentation that may under-report the true incidence of cardiac arrhythmia. For example, in one study a significant proportion (23%) of unexplained drowning cases carried mutations associated with arrhythmia syndromes,[15] and certification of sudden death in epilepsy may overlook cases, which result from a primary arrhythmic cause.[16,17]

DEFINING SADS

A consensus statement from the Heart Rhythm Society (HRS), European Heart Rhythm Association (EHRA), and the Asia Pacific HRS defines SADS as a pathologic diagnosis of exclusion after postmortem cardiac investigation and toxicologic analysis.[18] Even if nondiagnostic pathology is detected, cases should still be considered as SADS because of the high chance of underlying inherited ion channel disease.[19] Expert autopsy is also recommended as general pathologists may misdiagnose cases, over diagnosing ARVC, and under diagnosing SADS.[20] Guidelines for autopsy practice exist and include detailed description of postmortem sampling techniques with integration of specialist skills in the evaluation of possible familial disorders.[21,22] In the US, the state-wide Sudden Death in the Young Registry collates young SCD case data, using DNA analysis in a subset of cases for the purpose of further evaluation and future research.[23] Unfortunately, access to expert cardiac pathology is very much limited internationally.

DIAGNOSTIC APPROACHES AND GENETIC TESTING

Two approaches may be taken to make a diagnosis in a family: familial clinical evaluation with genetic testing targeted to phenotype and postmortem genetic testing "the molecular autopsy." The overall aim is to identify cardiac genetic disease were present and institute preventative treatment were necessary to avert further SCD.[24] The clinical role of genetic testing is therefore a diagnostic one and dependent on identifying mutations, that is, disease-causing or pathogenic rare genetic variants. The rapid development in sequencing technology has, however, led to the identification of frequent rare genetic variation in both healthy and affected individuals. Rare variants are often private to a specific family and therefore may be unknown in the literature. Their associated risk for disease causation and therefore their clinical significance is often uncertain and a major challenge as incorrect inferences of causality can have serious implications for diagnosis and management of families. If pathogenicity of a rare variant remains uncertain then it is termed a "variant of unknown significance" (VUS).[25]

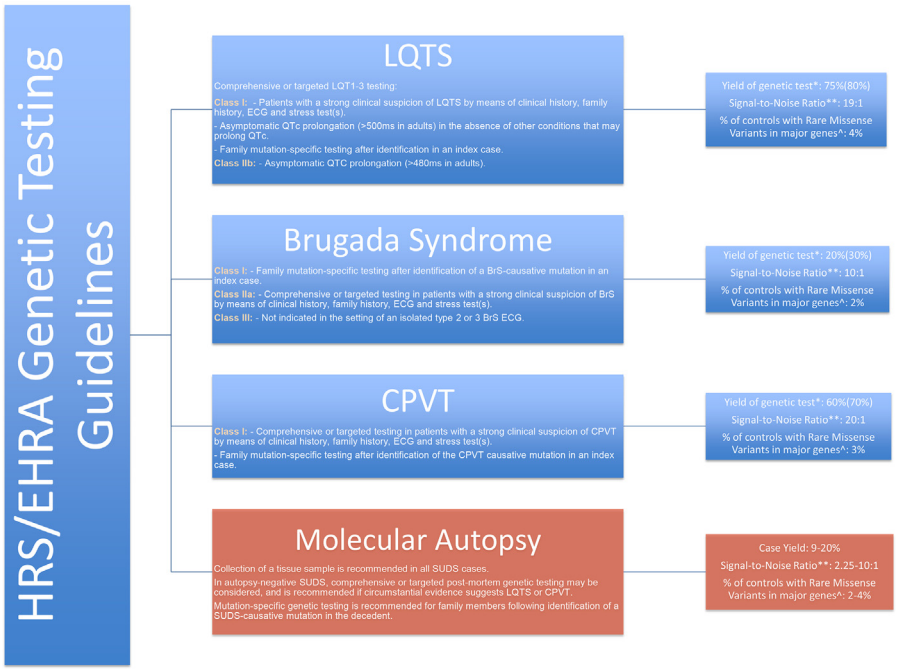

Fig 1. Guidelines for genetic testing in the channelopathies and diagnostic utility based on the signal-to-noise ratio. Estimates for molecular autopsy are based on the studies analyzed in this review. Adapted from the *HRS and EHRA Genetic Testing Guidelines*.[27] *Yield of diagnostic test—proportion of patients with positive genotyping, derived from unrelated cases. The first number is the yield when a major gene has been targeted. The number in parenthesis is the total when including all disease-associated genes. **Signal-to-noise ratio—estimate of positive predictive value, devised by dividing the case yield of rare variants by the background control rate of rare missense variants among major genes. Rare missense variants—possible disease-causing mutations determined by a percentage of rare amino acid substitutions in the major disease-associated genes. BrS, Brugada syndrome; CPVT, catecholaminergic polymorphic ventricular tachycardia; ECG, electrocardiogram; EHRA, European Heart Rhythm Association; HRS, Heart Rhythm Society; LQTS, long QT syndrome; SUDS, sudden unexpected death syndrome.

The presence in healthy normal people of rare variants, usually missense, is described as genetic "background noise" (ie, the frequency of rare variants within a particular gene in the healthy population).[26] The ratio of rare variant yield in cases (also mainly missense) to background noise is known as the "signal-to-noise ratio" (SNR) (Fig 1). Various methods for assessing likelihood of pathogenicity of rare variants have evolved as follows.[28,29]

Absence of the variant in a healthy control population. Before the extent of rare genetic variation in the genome was appreciated, most research studies and laboratory reports presumed pathogenicity on the basis of absence in a control population. Although the presence of a rare variant in the general population is helpful in reducing the chance of it being directly causative, novelty no longer carries much weight. Nonetheless, it is the first step in assessing likelihood of pathogenicity and increasingly large reference databases are available such as the Exome Aggregation Consortium with exome data from more than 60,000 unrelated individuals.[30]

Cosegregation of phenotype with genotype in large families. This is the process of associating a variant with disease in a family by identification of the variant in all affected individuals. This assists in determining the likelihood of causality, although if one affected relative does not carry the variant then it cannot be used as a clinical test. In practice, however, this approach is often not feasible because of small family sizes and the possibility of association by chance. The detection of a de novo rare variant in an associated gene in an affected child with unaffected parents does, however, support strongly the likely pathogenicity of the variant.

Severity of the type of mutation, that is, nonsense vs missense. In general, the likelihood of a functional effect of a variant on a protein is increased the more "radical" the associated DNA change is. Nonsynonymous single nucleotide changes, that is missense mutations, are common and are found in both healthy unaffected and affected individuals and are most difficult to assess (see aforementioned). Mutations because of deletions or insertions and some splice-site mutations may lead to abnormal protein products and

protein truncations. These "nonsense" mutations are less common and less likely to be present in unaffected individuals. Causation is therefore less difficult to determine. For example, premature truncation *SCN5A* mutations have been linked to severity of disease and are highly likely to be pathogenic because of haploinsufficiency.[31] However, although truncating variants of the gene titin (*TTN*) are associated with dilated cardiomyopathy (DCM),[32] they are also present in unaffected controls and represent a diagnostic challenge.[33]

Prior and reliable description in the literature. It can be helpful if a variant has previously been associated with disease in another proband and their family. Unfortunately not all prior reports are robust. Previously published rare variants postulated to be mutations have since been determined to be present in population databases at too high a frequency to be causative of disease.[34,35] They may represent functionally active variants that modify disease expression or otherwise are nonfunctional population variants. For example, one study examined an established exome database and compared it with a catalog of 197 variants reported as causative of DCM; 16.8% of the variants reported as causative were also present in the exome database, although functional data suggested that a significant proportion may have been lower penetrance disease-causing alleles.[36] Another study of childhood recessive diseases suggested 27% of mutations previously cited as pathogenic are in fact common polymorphisms or misinterpreted variants.[37]

Amino acid conservation and in silico methods. Missense mutations result in an amino acid changes that may have varying differences of side chain size, polarity, and lipophilicity compared with the wild-type protein. The greater the difference is the more likely that a structural and/or functional change will occur. In addition, the amino acid may be at a critical part of the protein that is conserved in other human proteins with similar functional domains (paralogs) or the same protein in other species (orthologs). Measures of conservation of amino acids and therefore likelihood of disease causation because of an amino acid change have been assessed by algorithms such as the Grantham conservation score.[38] These methods have also been incorporated into a number of different in silico tools that can also use predicted protein structural changes to infer causation. In combination, they can be effective in assisting assessment of pathogenicity of rare variants in *KCNQ1* and *KCNH2* in long QT syndrome (LQTS)[39] and to lesser extent *SCN5A* variants in LQTS and Brugada syndrome (BrS).[40] They are, however, unreliable as sole evidence of pathogenicity in the clinical setting.

Functional expression studies of the variant's biophysical effect. The consequences of mutations can be studied in RNA expression systems and functional models such as ion channel expression studies, animal models, and induced pluripotent stem cell–derived cardiomyocytes. Unfortunately, these are generally costly and are not feasible for routine evaluation of genetic findings.

To optimize and standardize assessment, Campuzano et al have recently proposed a scoring system to determine the pathogenicity of genetic variants associated with the arrhythmia syndromes combining data from the previously mentioned methods. A scoring range from 0 to 15 categorized variants into separate groups, including benign, VUS, and pathogenic.[41] This sort of approach may enable better classification of variants in the future.

Determining the causation of disease in families can be further complicated by other genetic variants that can influence phenotype may also be responsible, known as digenic or oligogenic inheritance. For example, 5%–10% of LQTS patients have mutations in 2 or more of the known genes, which can influence disease expression.[42]

CAUSES OF SADS AND THE ROLE OF GENETIC TESTING

Arrhythmia syndromes (or cardiac channelopathies) are a heterogeneous group of conditions that are usually hereditary and are associated with an apparently structurally normal heart. They are often caused by mutations in genes encoding cardiac ion channel subunits and channel interacting proteins (Table I). The channelopathies include LQTS, BrS, catecholaminergic polymorphic ventricular tachycardia (CPVT), short QT syndrome, idiopathic ventricular fibrillation, early repolarization syndrome, premature cardiac conduction disease, and others. These are described in Table I. LQTS has the highest yield from testing (approximately 75%), with wide variation among the other channelopathies (20% in the BrS).[25] In some cases, despite a normal heart being found at autopsy, cardiomyopathy is detected during clinical evaluation of the family. Thus, disease insufficient to cause histopathologic changes detectable even at expert autopsy may still cause or predispose toward SCD.[70] We focus on the main phenotypes of interest subsequently.

Long QT syndrome. Congenital LQTS has a prevalence of approximately 1:2000[71] and is characterized by prolongation of the QT interval on the electrocardiogram (ECG) and increased risk of the characteristic ventricular arrhythmia, Torsades de Pointes, and SCD. Hundreds of mutations in 14 genes[25] predominantly encoding components and channel interacting proteins of repolarizing potassium currents and the sodium channel current (Nav1.5) have been identified (Table I).

Table I. Genes associated with ion channel diseases and their associated phenotypes and frequencies

Clinical syndrome	Gene	Ion channel component	Effect of mutation	Ion channel disease	Frequency in phenotype (+reference)
Brugada syndrome	*SCN5A*	α Subunit *I*Na	Loss of function	BrS1	20%–30%[40]
	GPD1L	*I*Na ChIP	Loss of function	BrS2	<1%[43]
	CACNA1C	α Subunit *I*Ca	Loss of function	BrS3	BrS 2%–12%[43-45]
	CACNB2	β Subunit *I*Ca	Loss of function	BrS4	BrS 2%–12%[43-45]
	SCN1B	β Subunit *I*Na	Loss of function	BrS5	<1%[43]
	KCNE3	β Subunit *I*Ks/*I*to	Gain of function	BrS6	<1%[43]
	SCN3B	β subunit *I*Na	Loss of function	BrS7	<1%[43]
	HCN4	*I*f	Loss of function	BrS8	NA[46]
	KCNJ8	α Subunit *I*KATP	Gain of function	BrS9	<1%[43]
	CACNA2D1	α2d subunit *I*Ca	Loss of function	BrS10	BrS 2%–12%[43-45]
	KCND3	α subunit *I*to	Gain of function	BrS11	<1%[43]
	MOG1	*I*Na ChIP	Loss of function	BrS12	<1%[43]
	SLMAP	*I*Na ChIP	Loss of function	BrS13	NA[47]
Long QT syndrome	*KCNQ1*	α Subunit *I*Ks	Loss of function	LQT1	40%–55%[48]
	KCNH2	α Subunit *I*Kr	Loss of function	LQT2	35%–45%[48]
	SCN5A	α Subunit *I*Na	Gain of function	LQT3	2%–8%[48]
	ANK2	*I*Na,K, *I*NCX ChIP	Loss of function	LQT4	<1%[48]
	KCNE1	β Subunit *I*Ks	Loss of function	LQT5	<1%[48]
	KCNE2	β Subunit *I*Kr	Loss of function	LQT6	<1%[48]
	KCNJ2	α Subunit *I*K1	Loss of function	LQT7 (CPVT-like)	<1%[48]
	CACNA2D1	α2d Subunit *I*Ca	Gain of function	LQT8	<1%[48]
	CAV3	*I*Na ChIP	Gain of function	LQT9	<1%[48]
	SCN4B	β Subunit *I*Na	Gain of function	LQT10	<0.1%[48]
	AKAP9	*I*Ks ChIP	Loss of function	LQT11	<0.1%[48]
	SNTA1	*I*Na ChIP	Gain of function	LQT12	<0.1%[48]
	KCNJ5	α Subunit *I*KAch	Loss of function	LQT13	NA[49]
	CALM1/CALM2	SR Ca regulation	Loss of function	LQT14	NA[50]
CPVT	*RYR2*	α Subunit	Loss of function	CPVT1	50%[51]
	CASQ2	SR Ca regulation	Loss of function	CPVT2	NA[52]
	TRDN	SR Ca regulation	Loss of function	CPVT4	NA[53]
	CALM1	SR Ca regulation	Loss of function	CPVT5	NA[54]
Other syndromes	*KCNQ1*	α Subunit *I*Ks	Gain of function	SQT2, FAF	NA[55,56]
	KCNH2	α Subunit *I*Kr	Gain of function	SQT1	NA[57]
	KCNE2	β Subunit *I*Kr	Gain of function	FAF	NA[58]
	KCNJ2	α Subunit *I*K1	Gain of function	SQT3, FAF	NA[57,59]
	KCNJ8	α Subunit *I*KATP	Gain of function	ERS	NA[60,61]
	DPP6	*I*to	Gain of function	IVF	NA[62]
	HCN4	*I*f	Loss of function	SSS	NA[63]
	CACNA1C	α Subunit *I*Ca	Loss of function	ERS	NA
	CACNB2	β Subunit *I*Ca	Loss of function	ERS	NA
	CACNA2D1	α2d Subunit *I*Ca	Loss of function	ERS	NA
	SCN5A	α Subunit *I*Na	Loss of function	PCCD	NA[64]
				ERS	±6%[65]
				SSS	2%–3%[66]
			Mixed effect	Overlap	NA[67,68]
			Gain of function	MEPPC	NA[69]

Abbreviations: BrSX, Brugada syndrome subtype X; *ChIP*, channel interacting protein; *CPVT*, catecholaminergic polymorphic ventricular tachycardia; *ERS*, early repolarization syndrome; *FAF*, familial atrial fibrillation; *ICa*, depolarizing inward calcium current (slow); *If*, funny current; *IK1*, Kir2.1 inward rectifying current; *IKACH, acetylcholine-sensitive inward rectifying potassium current; IKATP*, ATP-sensitive potassium channel; *IKr*, repolarizing outward rapid rectifying potassium current; *IKs*, repolarizing outward slow rectifying potassium current; *INa*, depolarizing inward sodium current (fast) Nav1.5; *INCX*, sodium-calcium exchanger-associated current; *Ito*, transient outward potassium current; *IVF*, idiopathic ventricular fibrillation; *LQTX*, long QT syndrome subtype X; *MEPPC*, multifocal ectopic Purkinje-related premature contractions; *NA*, not ascertained or applicable; *PCCD*, premature cardiac conduction disease; *SQTX*, short QT syndrome subtype X; *SR Ca*, sarcoplasmic reticulum calcium; *SSS*, sick sinus syndrome.
Adapted from Wilde and Behr.[25]

Approximately 75% of patients with the syndrome have mutations in 1 of 3 "major" LQTS genes, the potassium channel genes *KCNQ1* (LQT1, 35%) and *KCNH2* (LQT2, 30%) and the sodium channel gene *SCN5A* (LQT3, 10%).[72] With a background genetic "noise" level of 2%–4% for major genes, this gives a

relatively favorable SNR. The 2011 HRS and EHRA Expert Consensus Statement therefore recommends comprehensive or LQT1–3 targeted LQTS genetic testing for any patient meeting diagnostic criteria (Fig 1).[28] The 2013 diagnostic guidelines include carriers of a pathogenic mutation as being affected, regardless of clinical features.[27] Genotype can be associated with phenotypic differences in clinical presentation and may be important in the SADS victim's presentation: LQT1 with exercise (especially swimming) and high emotional states; LQT2 with sudden arousal and acoustic triggers; and LQT3 with rest or sleep.[72]

The less common or "minor" LQTS genes have been associated with phenotype in small studies with low yields and small control groups. In some cases linkage is not available and causality rests on functional data and absence in a control. For example, the original description of *CAV3* as the cause of LQT9 has been questioned in subsequent follow-up studies.[73] Background rare variation increases when the minor genes are included in testing panels but with little increase in yield. This makes the SNR less favorable and result less readily interpretable.

Brugada syndrome. BrS is diagnosed by the presence of at least 2 mm of coved ST increase with T wave inversion in at least one of the standard and high intercostal space right ventricular ECG leads: the type 1 ECG pattern.[18,74] This pattern can be concealed and often dynamic, unmasked by factors such as sleep[75] and pyrexia,[76] as well as provoked by sodium channel blocking drugs such as ajmaline, procainamide, and flecainide.[77] It can manifest with syncope because of polymorphic ventricular tachycardia, ultimately predisposing to ventricular fibrillation and SCD, which is commonly the first presentation.[78] The prevalence of a BrS type 1 ECG is higher in Asian countries, approximately 0.15% in adults. This is compared with <0.02% in a Western population.[79]

BrS is a heterogeneous condition with reduced genetic penetrance. Recent evidence has suggested variable clinical phenotypes, where the syndrome may overlap with structural conditions such as ARVC.[80] Inheritance can be autosomal dominant with variable expressivity or sporadic,[81] as such, most individuals can remain asymptomatic throughout their lifetime. Recent evidence has also supported an oligogenic inheritance pattern.[82] The most commonly affected gene is the *SCN5A* gene (approximately 20% of BrS cases, BrS1[83]). More than 300 mutations of *SCN5A* have been described[84] leading to loss of function because of a reduction in the amplitude of the sodium channel current by reduced expression and/or altered voltage-gating properties.[85] Conversely, although gain-of-function *SCN5A* mutations are found in LQT3, an overlap in phenotype exists between BrS and LQTS, both having been described in large families.[67] In addition, there are large *SCN5A* positive families that have affected individuals who are noncarriers, suggesting a modifier role rather than direct causation in these pedigrees.[86]

Many other genes have been associated with the syndrome (Table I). One study identified mutations of the L-type calcium channel (encoded by *CACNA1C*, *CACNB2B*, and *CACNA2D1*) in 10%–15% of BrS cases.[44] The clinical phenotype also demonstrated a short QT interval. This has not been replicated by a follow-up study and the rest of these candidate genes account for very small numbers of cases.[43] Other than *GPD1L* and *SCNB1*, these have not demonstrated genetic linkage in pedigrees.[22] A recent study has also implicated *SCN10A*, which encodes the neuronal current Nav1.8, as being responsible for a significant proportion of BrS cases.[87] This has not been confirmed in a similar study of *SCN5A* negative BrS patients. Great caution must therefore be used in assessing rare variants in these low frequency genes.

Thus, the overall SNR level and potential for oligogenic inheritance are relatively unfavorable for diagnostic testing and recommendations are not as strong as in LQTS. Genetic testing can be useful in known or suspected cases of BrS, but a positive test adds little to the on-going clinical management. It is, however, recommended for assessing relatives (Fig 1).[27]

Catecholaminergic polymorphic ventricular tachycardia. CPVT is a rare inherited arrhythmic disorder first described in 1975,[88] typically presenting within the first decade of life. Prevalence is estimated in 1:10,000 and individuals are at risk of SCD by means of catecholamine-induced polymorphic and bidirectional VT.[89] The mechanism of arrhythmia in CPVT is chiefly because of intracellular calcium mishandling, where mutations in the *RYR2* gene cause uncontrolled calcium release from the sarcoplasmic reticulum during diastole.[90] The initial clinical manifestation is typically syncope associated with exercise or acute stress; however, SCD can be its sole presentation. The heart structure and resting ECG is often unremarkable in CPVT, necessitating use of exercise or catecholamine stress testing to capture arrhythmias, most commonly bidirectional VT.[27]

CPVT principally arises from mutations in 2 genes: CPVT1-*RYR2* (encodes the cardiac ryanodine receptor channel) and CPVT2-*CASQ2* (encodes calsequestrin, responsible for buffering calcium in the sarcoplasmic reticulum).[91] CPVT has been described in small numbers of *KCNJ2*, *ANKB*, and *CALM1/2* carriers, genes that have also been associated with LQTS.[92] The condition is inherited with high penetrance in either

an autosomal dominant (*RYR2*) or recessive (*CASQ2*) fashion, the former being more common affecting 55%–65% of probands, with CASQ2 only affecting a small number of cases but increasing the likelihood of earlier symptom onset.[91] More than 100 mutations of *RYR2* have been linked to CPVT1, the autosomal dominant form, which affect certain regions of the protein (mainly the FKBP12.6 binding domain, covering most *RYR2*).[93] Because of the large size of the gene, targeted screening has been used in the past and can potentially miss causative mutations.[94] Recent studies suggest that mutation location may be associated with severity of the phenotype, suggesting a future role for genetic risk stratification alongside conventional techniques.[95]

Because of the favorable SNR level targeted CPVT genetic testing is recommended for any patient with clinical suspicion of CPVT based on the clinical findings and electrocardiographic phenotype during stress testing. Mutation-specific genetic testing is also recommended for family members and appropriate relatives after the identification of the CPVT-causative mutation in an index case.[27] Like with LQTS, the 2013 diagnostic guidelines include carriers of a pathogenic mutation as being affected, regardless of clinical features.[18]

Cardiomyopathies. The cardiomyopathies are diseases of the heart muscle associated with mechanical failure of myocardial performance. The American Heart Association (AHA) describes a heterogeneous group of diseases usually exhibiting ventricular hypertrophy or dilatation, caused by a variety of etiologies that frequently are genetic.[96] They can broadly be divided into HCM, DCM, and ARVC.

HCM, the most common cardiomyopathy, has a prevalence of approximately 1:500 of the general population.[96] It is characterized by unexplained cardiac hypertrophy, often in an asymmetric septal pattern, but not always. Mutations involving sarcomere proteins and other proteins involved in cardiac contraction are responsible. Familial HCM is mostly (>90%) inherited in an autosomal dominant fashion and most mutations involve cardiac β-myosin heavy chain (*MYH7*) and cardiac myosin binding protein C (*MYBPC3*). Genes encoding mitochondrial enzymes have also been identified.[97] HRS and EHRA guidelines recommend genetic testing after diagnosis, with mutation-specific testing indicated for family members after identification.[27]

In idiopathic DCM, the heart is dilated and impaired leading to symptoms and signs of congestive cardiac failure but without an apparent underlying cause. The estimated prevalence is approximately 1:2500, affecting both adults and children; inherited forms make up to 50%, where there is a family history in one or more relatives.[98] Mutations in >30 genes have been associated with DCM, most following an autosomal dominant inheritance pattern.[99] The titin gene (*TTN*) probably accounts for approximately 20% of cases in recent studies but the high frequency of rare variants has limited the clinical utility of assessing risk; other genes have a low yield.[100] Extensive or targeted (*LMNA* and *SCN5A*) testing is recommended (class I indication) for those patients with significant conduction disease with or without overt DCM as mutations in these genes can indicate an increased risk of SCD.[27]

ARVC is characterized by fibro-fatty replacement of the right and/or left ventricular myocardium with associated risk of SCD because of ventricular arrhythmias. Most cases are inherited in an autosomal dominant pattern with incomplete penetrance and variable expressivity. Its overall prevalence is approximately 1:10000, often presenting between the second and fourth decade of life.[101] Seven genes have been associated with ARVC: *JUP*, *DSP*, *PKP2*, *DSG2*, *DSC2*, *TGFβ3*, and *TMEM43*.[102] HRS and EHRA guidelines suggest that comprehensive or targeted genetic testing can be useful in those that meet existing 2010 task force diagnostic criteria (which include evaluation of right ventricular structure and repolarization abnormalities), but is not helpful in those with an uncertain diagnosis.[27]

FAMILY EVALUATION AND PHENOTYPE TARGETED GENETIC TESTING

The first study of families of SADS victims reported a limited investigative protocol followed by targeted gene testing in 32 families.[103] Inherited cardiac disease, mainly LQTS, was diagnosed in 22% of families although testing of the LQTS genes (*KCNQ1*, *KCNH2*, *SCN5A*, *KCNE1*, and *KCNE2*) did not contribute to any of the clinical diagnoses. A follow-up study of 57 families used more comprehensive clinical assessment and targeted genetic evaluation.[70] In addition, where available, DNA extracted from formalin-fixed paraffin blocks from 24 SADS cases was used for molecular autopsy of the LQTS genes. Twenty-five families with phenotypic features of cardiac genetic disease underwent targeted mutation analysis. Overall, 53% of families had features of inherited cardiac disease based on the clinical and genetic evaluation, where 70% were diagnosed with LQTS or BrS. Genetic evaluation alone contributed to only 1 (2%) case (identifying a likely LQTS-causing mutation). Other studies have also examined the yield of clinical evaluation and are described in Fig 2. The mean yield of these various studies is 32% with a range of 18%–53%. This variability is very much dependent on the population, the availability of autopsy and expert

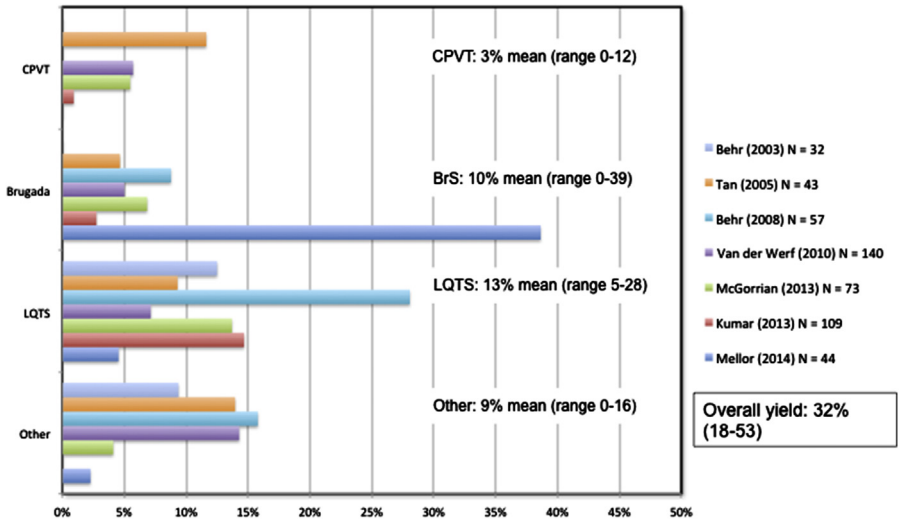

Fig 2. Causes of sudden arrhythmic death syndrome as determined by various studies of familial evaluation.[70,103-108] BrS, Brugada syndrome; CPVT, catecholaminergic polymorphic ventricular tachycardia; LQTS, long QT syndrome.

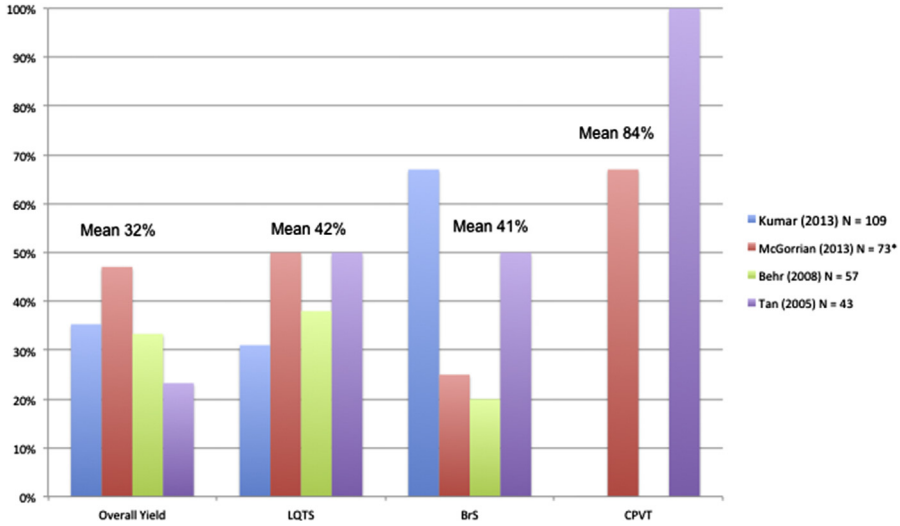

Fig 3. Proportion of molecular diagnoses made in studies of clinical evaluation when testing is targeted to those with a clinical phenotype.[70,105-107] LQTS, long QT syndrome. *1 CPVT and 1 BrS mutation were exclusively identified via post-mortem genetic testing.

autopsy, and the rigor of the investigative protocol used. Once a diagnosis is made the actual proportion of molecular diagnoses in those with a clinical phenotype ranges from 23%−47% overall (Fig 3).

Clinical role. The importance of systematic and comprehensive clinical and genetic evaluation in SADS families is reinforced by recent guidelines.[18] Familial evaluation always begins with a detailed analysis of the index case that may give clues to the underlying cause. Pertinent background information includes prior symptoms and clinical encounters (including any previous ECGs) as well as the circumstances of death and pathologic reports. First-degree relatives are then offered full clinical cardiological assessment. Relatives with worrisome symptoms such as syncope, seizures, or palpitations are prioritized for assessment and obligate carriers in a pedigree will also be targeted. This includes history and examination, resting 12-lead ECG (with and without high right ventricular leads), echocardiography, exercise, and ambulatory ECGs, and where indicated signal averaged ECG, provocative testing with sodium channel blockers and/or epinephrine, cardiac imaging, and genetic testing. This

is typically conducted in a multidisciplinary environment with access to genetic counseling in a dedicated inherited cardiac disease clinic.[109] After comprehensive evaluation, if no abnormalities are detected in first-degree relatives, the risk of future cardiac events related to inherited cardiac disease is generally low.[110]

In the context of familial assessment, gene testing of SADS relatives is targeted to the phenotype identified in the affected members of the family as described in the previous section. Cascade genetic testing is then performed when a positive clinical diagnosis is made in a family member, enabling identification in other relatives by testing for a specific mutation.[111] Coordination of clinical cardiological evaluation and mutation analysis can also help to clarify whether a VUS is likely to be causative that is segregation analysis.

The lack of 100% sensitivity of genetic testing must be addressed by appropriate counseling as well as exploration of relatives' concerns and any psychological implications. In 2014, Erskine et al[112] conducted interviews with 50 individuals who had a personal or family history of an arrhythmic syndrome or SCD. Seventy-four percent pursued genetic testing for LQTS or another channelopathy, motivated by factors such as relieving uncertainty surrounding a potential diagnosis, seeking an explanation for a family member's death, and guiding future medical management. Expectations concerning the scope of genetic testing and individual efficacy in establishing a diagnosis are varied. The main reasons against pursuing testing were fear, denial, and lack of information.

Unfortunately a universal and structured approach to deal with the families of young SCD victims is lacking. Indeed, in many countries no formal local guidance exists for evaluating relatives of SADS cases, and an autopsy is often not required. Van der Werf et al[104] conducted a community-based intervention study to try to increase autopsy rates of young SCD victims in the Netherlands. Increased awareness in 1 intervention group was promoted via a dedicated helpline for the use of community general practitioners and coroners; information provided by educational meetings and correspondence was also used in another group. The autopsy rate was not significantly improved in either intervention and few families went on to have targeted evaluation in an inherited cardiac disease clinic. This study highlights the difficulties faced in establishing access to suitable services for relatives. Assessment of the cause of death in young SCD cases beyond just exclusion of foul play should be mandatory, as should the onward notification of families to health services. Steps have been taken in Australia, New Zealand, Canada, and the UK, but large gaps in practice remain even in these countries.[113-115]

MOLECULAR AUTOPSY

Postmortem genetic testing involves the collection of tissue suitable for DNA extraction at autopsy and mutation analysis for specified genes, often described as the "molecular autopsy."

Postmortem sample collection. Postmortem genetic testing has traditionally relied on formalin-fixed paraffin-embedded (FFPE) tissue because of ease of storage and transport. More amenable sample media, such as fresh frozen tissue or EDTA-preserved blood, have not been routinely archived postmortem. In 2008, Carturan et al evaluated different DNA extraction protocols and the feasibility of mutational analysis from archived FFPE tissue. A total of 35 sudden unexpected death syndrome (SUDS) cases were studied; using a number of DNA extraction techniques, nearly one-third of the regions of interest could not be examined.[116]

In case of SADS, HRS guidelines recommend collection of a tissue sample for subsequent DNA analysis.[18] Tissue samples include heart, liver, spleen, and whole EDTA tube or blood spot testing. The National Association of Medical Examiners suggests circumstances in which such specimens should be saved, detailing DNA preservation standards and appropriate counseling of relatives in the context of SCD.[117] FFPE should therefore be avoided to allow consistent DNA sampling and more favorable diagnostic yield.

Results in research. The first documented report was in 1999 where LQTS was diagnosed from an autopsy specimen of a 19 year-old girl who died after a near-drowning.[118] Early work by Tester et al involved mutational analysis of LQTS-associated genes (*KCNQ1*, *KCNH2*, and *SCN5A*)[119] and *RyR2*, implicated in CPVT, in 49 SADS cases.[120] Targeted analysis of 18 exons of RyR2 revealed potential CPVT1-causing mutations in 1 of every 7 cases, whereas the yield in LQTS cases was 1 in 5 (20%), giving an overall yield of 35%.[27]

Further research into molecular autopsy has, however, indicated lower yields. In 2011, Skinner et al[121] performed postmortem genetic testing for LQTS in young sudden unexplained death cases. LQT1-6 gene analysis was performed using direct sequencing or denaturing high-performance liquid chromatography. Thirty-three cases underwent testing, and missense mutations in LQTS genes were found in 15%. A more conservative yield was obtained by Winkel et al,[122] who examined a young Danish SUDS population of 44 between 2000

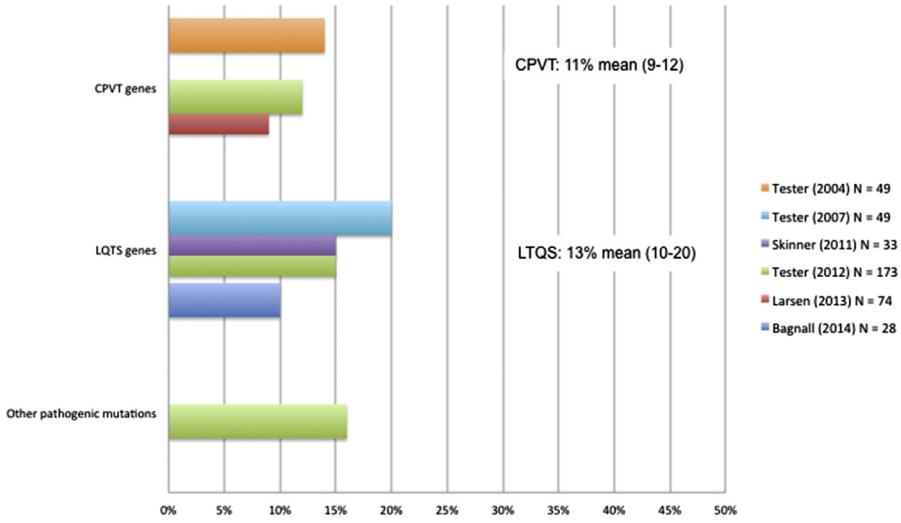

Fig 4. Causes of sudden arrhythmic death syndrome as determined by various molecular autopsy studies.[119-121,124-126] CPVT, catecholaminergic polymorphic ventricular tachycardia; LQTS, long QT syndrome.

and 2006. *KCNQ1*, *KCNH2*, and *SCN5A* were sequenced; 11% carried a mutation in at least 1 of the 3 genes. In a UK study, a molecular diagnosis was made in 14% of 45 cases, and a higher uptake of cascade screening in relatives where a molecular diagnosis had been made at autopsy was also reported.[123]

The yield of testing LQT and CPVT genes was re-examined by Tester et al[124] more recently in an extended series of 173 autopsy-negative SUDS cases that include their original 49 strong cohort. Forty-five likely pathogenic mutations were identified, but the overall yield (25%) was lower than their initial cohort. However, the yield was significantly higher (45%) in those aged <50 years with a family history of premature SCD. Fig 4 describes the yield of molecular autopsy from this and other recent studies. The mean yield of LQTS and CPVT gene testing was 13% and 11%, respectively, in keeping with more recent conservative estimates.

Clinical role. Thus, if the role for the molecular autopsy is to attempt to make a genetic diagnosis in a family then it cannot supplant clinical evaluation given the lower yield. It may complement evaluation by identifying sporadic mutations and focusing evaluation on any offspring of the victim. It may help to confirm or exclude a putative genetic cause in a family especially if a VUS had been found. Occasionally, it may provide a clear well recognized causative variant and direct family evaluation. However, the SNR in molecular autopsy is already as low as 2.25 for the major LQTS and CPVT genes because of the relatively low yields and frequency of rare variation in the general population (see Fig 1). It is therefore not a stand-alone tool at this time. Nonetheless molecular autopsy may have implications for the prevention of additional deaths in other relatives, and HRS and EHRA guidelines currently recommend genetic testing if circumstantial evidence points toward a clinical diagnosis of LQTS or CPVT[70] (Fig 1). This has been reinforced by the more recent clinical testing guidelines that state that an arrhythmia syndrome focused gene panel can be useful.[18]

NEXT-GENERATION SEQUENCING AND THE FUTURE

Genetic testing for the channelopathies has traditionally involved Sanger sequencing for single gene mutations.[127] This method is limited by potential scalability, as more genes with susceptibility to channelopathy have been identified. Advances in technology, massive parallel sequencing or next-generation sequencing, have led to the ability to simultaneously sequence protein coding exons of all genes nucleotide by nucleotide ("the exome," which is approximately 1% of the genome) from much smaller quantities of DNA.[128]

Bagnall et al[125] performed exome sequencing on a subgroup of 28 SUDS cases referred for autopsy in Australia between 2005 and 2009. Three rare variants were discovered in the 3 most common LQTS genes when a narrow panel of the 4 major genes (*KCNQ1*, *KCNH2*, *SCN5A*, and RYR2) was studied. The scope of the molecular autopsy was also expanded to include a wider selection of >70 arrhythmia and cardiomyopathy genes, subsequently revealing a variant in the *CACNA1C* gene of a 20 year-old man who died in his sleep. This variant had previously been identified in a LQTS family,[129] highlighting that screening additional genes may offer causality beyond the "usual suspects." Unfortunately two-thirds of the other putative mutations that were identified were VUSs.

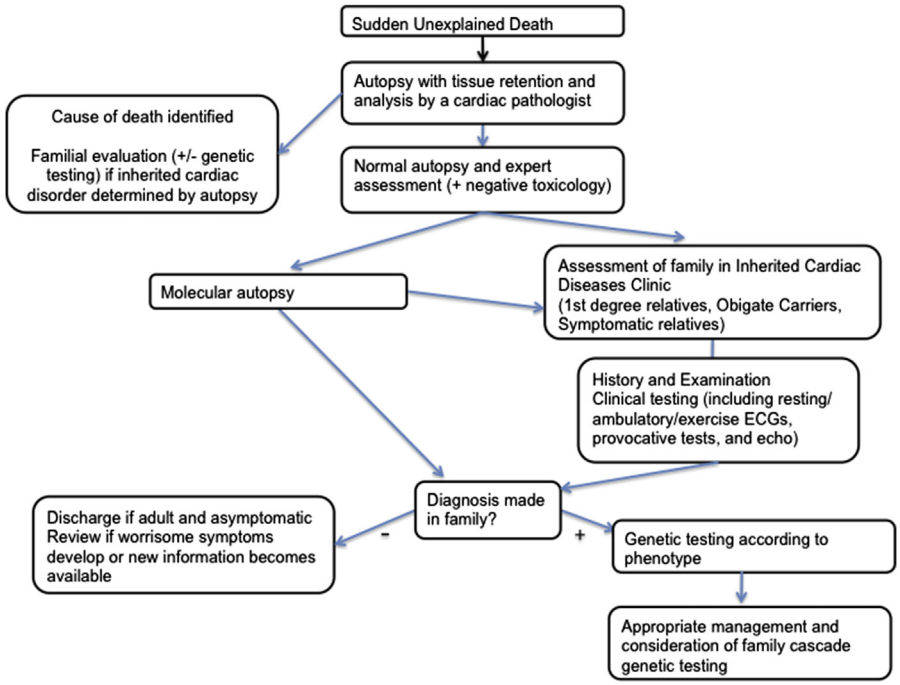

Fig 5. Recommended diagnostic pathway after a sudden unexplained death (adapted from Wong and Behr[130]). ECG, electrocardiogram.

This highlights the concern over increasing gene panel sizes. The SNR will become even lower for the expanded "next-generation" arrhythmia syndrome and cardiac genetic disease panels because of the enhanced background variation and minimal increase in yield. The family context is currently the only way to extract additional value from the high yield of VUSs by molecular autopsy. This situation will only improve when more is understood about how to assess the likely functional effects of mutations detected in these disorders.

CONCLUSIONS

The molecular autopsy can only occasionally diagnose disease in an index case and direct subsequent family evaluation. Because of methodological issues surrounding determination of variant pathogenicity, the comprehensive next-generation sequencing panel will have to complement careful phenotypic evaluation of family members to inform a targeted genotyping strategy (see Fig 5). Indeed, the utility of genetic testing is only really appreciated in such a context and represents the best chance of developing clinically meaningful results. Only once robust methods are in place for assessing the pathogenicity or disease susceptibility of putative mutations from a large range of potential genetic causes can the molecular autopsy be a truly diagnostic tool. In the interim most of its clinical utility will be provided by analyzing the 4 major LQTS, BrS, and CPVT genes that we already know and understand best.

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