Sudden arrhythmic death syndrome: familial evaluation identifies inheritable heart disease in the majority of families

Elijah R. Behr[1]*, Chrysoula Dalageorgou[1], Michael Christiansen[2], Petros Syrris[3], Sian Hughes[3], Maria T. Tome Esteban[3], Edward Rowland[3], Steve Jeffery[1], and William J. McKenna[3]

[1]Cardiac and Vascular Division, St George's University of London, Cranmer Terrace, London SW17 0RE, UK; [2]Statens Serum Institut, Copenhagen, Denmark; and [3]The Heart Hospital, University College Hospital, London, UK

Aims	At least 4% of sudden deaths are unexplained at autopsy [sudden arrhythmic death syndrome (SADS)] and a quarter may be due to inherited cardiac disease. We hypothesized that comprehensive clinical investigation of SADS families would identify more susceptible individuals and causes of death.
Methods and results	Fifty seven consecutively referred families with SADS death underwent evaluation including resting 12 lead, 24 h and exercise ECG and 2D echocardiography. Other investigations included signal averaged ECG, ajmaline testing, cardiac magnetic resonance imaging, and mutation analysis. First-degree relatives [184/262 (70%)] underwent evaluation, 13 (7%) reporting unexplained syncope. Seventeen (30%) families had a history of additional unexplained premature sudden death(s). Thirty families (53%) were diagnosed with inheritable heart disease: 13 definite long QT syndrome (LQTS), three possible/probable LQTS, five Brugada syndrome, five arrhythmogenic right ventricular cardiomyopathy (ARVC), and four other cardiomyopathies. One SCN5A and four KCNH2 mutations (38%) were identified in 13 definite LQTS families, one SCN5A mutation (20%) in five Brugada syndrome families and one (25%) PKP2 (plakophyllin2) mutation in four ARVC families.
Conclusion	Over half of SADS deaths were likely to be due to inherited heart disease; accurate identification is vital for appropriate prophylaxis amongst relatives who should undergo comprehensive cardiological evaluation, guided and confirmed by mutation analysis.
Keywords	SADS • Sudden death • Clinical genetics • Long QT syndrome • Brugada syndrome • Cardiomyopathy

Introduction

Previous prospective study of sudden and unexpected deaths in apparently healthy adults in England had identified that in 4.1% of cases, a full coroner's post-mortem, a toxicological screen, and an expert cardiac autopsy failed to reveal any underlying cause of death.[1] A second English prospective population-based survey of victims of unexplained sudden death aged 4–64 years with normal cardiac pathology [the sudden arrhythmic death syndrome (SADS) study] suggested that up to ∼500 such deaths occur per annum,[2] equivalent to 2500 deaths per annum in USA. As part of the study, we evaluated first-degree blood relatives from 32 families of victims of SADS death.[3] A research protocol with a limited clinical evaluation (history, resting and 24 h electrocardiogram (ECG) 2D echocardiography and exercise ECG at the physician's discretion) diagnosed inherited cardiac disease in 7/32 families (22%), four with long QT syndrome (LQTS). Tan *et al.*[4] published results of the comprehensive evaluation of 43 families with a high frequency of unexplained sudden deaths. Seventeen (40%) were identified with inherited cardiac disease including catecholaminergic polymorphic ventricular tachycardia (CPVT), LQTS, Brugada syndrome and arrhythmogenic right ventricular cardiomyopathy (ARVC).

* Corresponding author. Tel: +44 208 725 5939, Fax: +44 208 725 3328, Email: ebehr@sgul.ac.uk
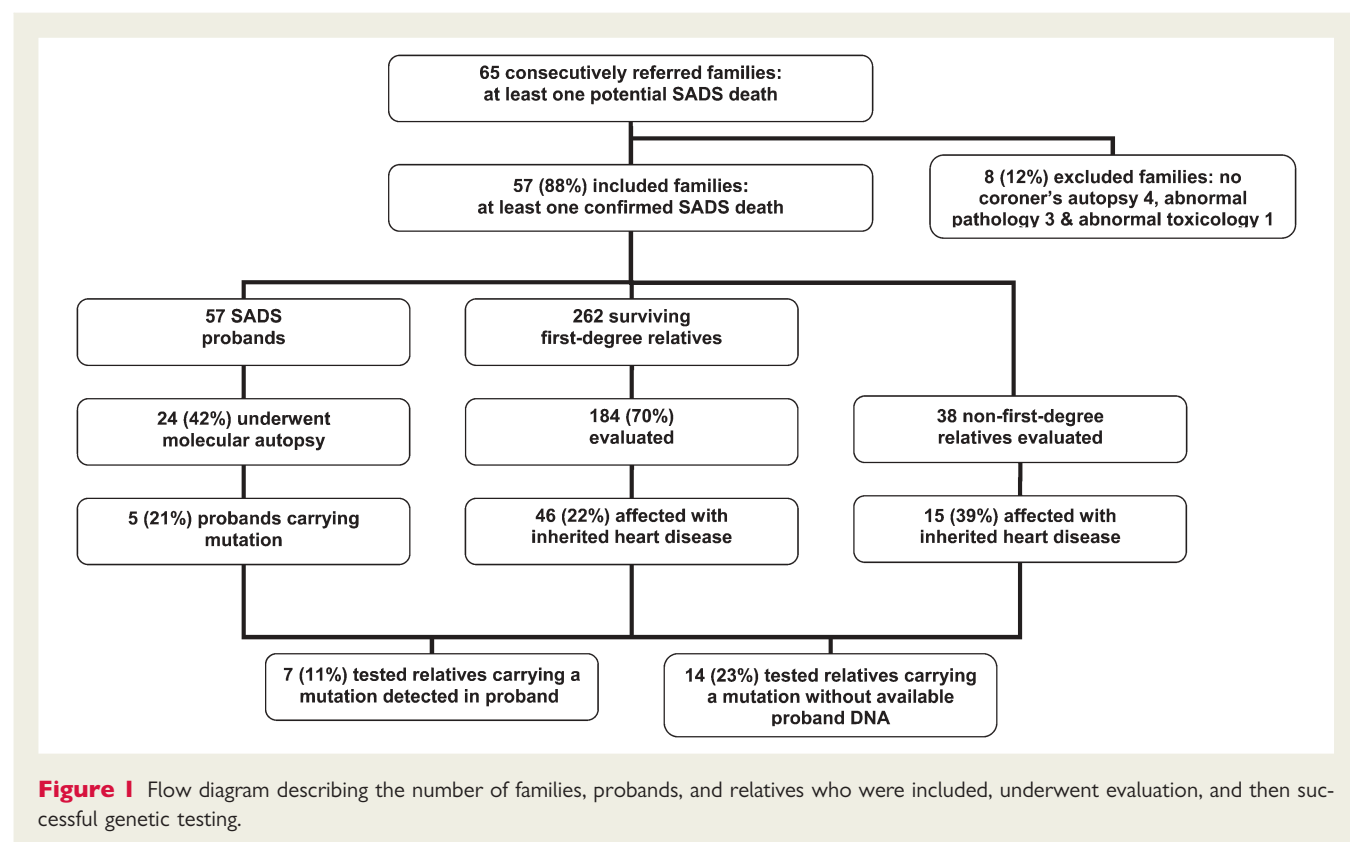
We have undertaken a new study of a second group of unselected families consecutively referred to our institution, in which a SADS death had occurred. We hypothesized that a more comprehensive clinical and genetic evaluation of SADS victims and their families would identify a greater proportion of the probable causes of such deaths and aimed to determine the proportion of relatives at risk of sudden death.
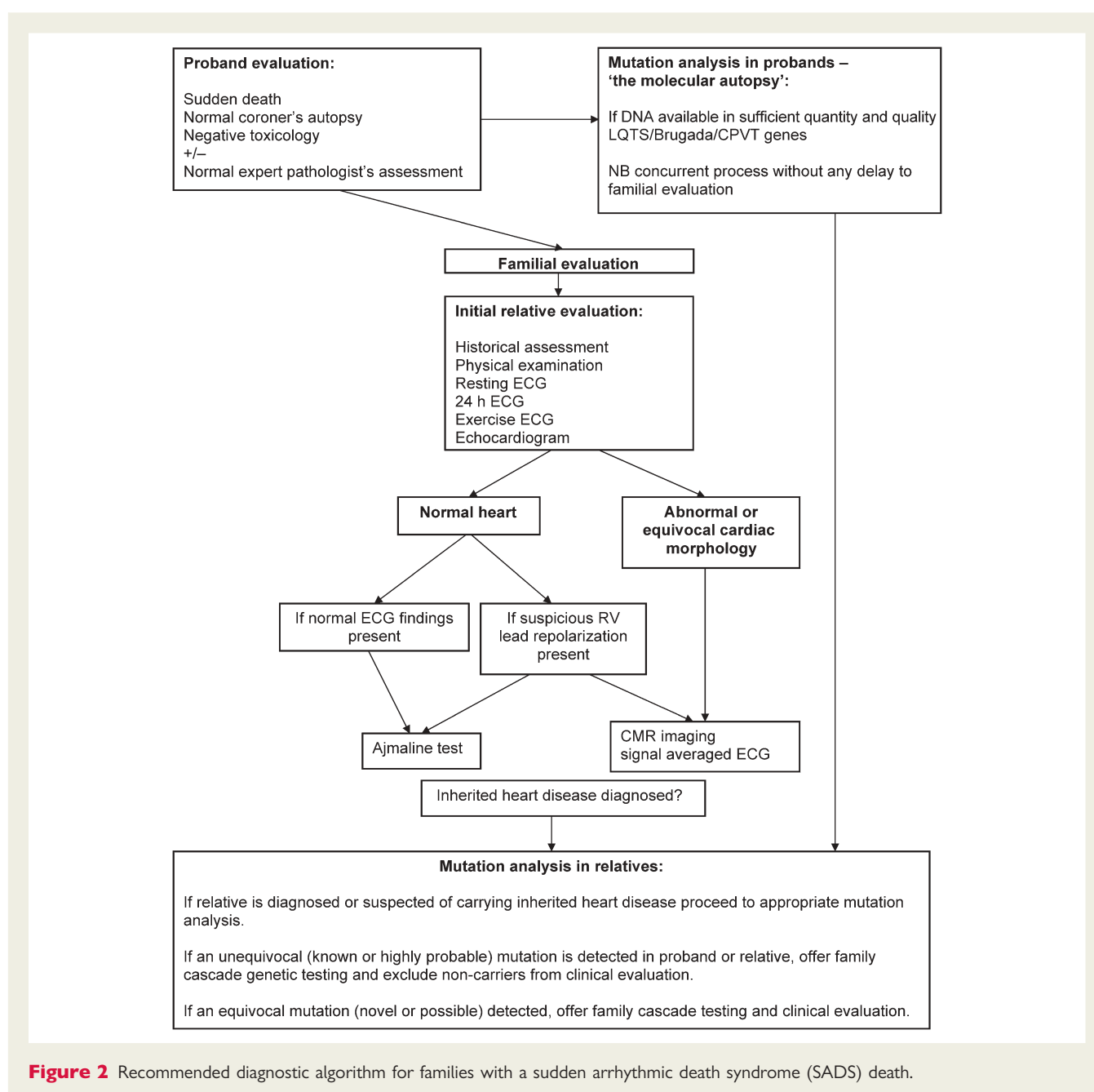
Methods

Sixty-five consecutive families with a possible SADS death, newly referred to St George's Hospital, London, were considered for the study (*Figure 1*). Fifty-seven families were included in the study group having met the inclusion criteria for a SADS death: a sudden unexpected death; age 4–64 years; last seen alive and well within 12 h of being found dead; no prior recorded cardiac disease; a normal full coroner's post-mortem; negative toxicology results; and a normal expert cardiac pathologist's examination of the heart, tissue samples or slides, if available. Autopsy reports were assessed for non-cardiac causes of sudden death and structural heart disease as part of this process. For example, hypertrophic cardiomyopathy (HCM), an important cause of sudden death in the young, can usually be detected at post-mortem and these families were either not referred as having a SADS death or not included.[1] The cohort therefore consisted of families with at least one unexplained sudden death where non-cardiac causes, such as pulmonary embolus, cerebral haemorrhage, and drugs, and structural cardiac causes, such as cardiomyopathy, valvular disease, and myocarditis, had been excluded.

Familial evaluation

A proband's prior history was determined by review of coroner's reports, primary care physicians' records and interview with relatives. The assessment of families then focused upon the initial systematic evaluation of as many first-degree blood relatives as possible: historical review, physical examination, echocardiography and resting, 24 h and exercise ECG. Evaluation was limited to echocardiography and ECG in children was unable to comply with other tests. Blood relatives who were not first-degree (non-first-degree relatives) were evaluated for one or more of the following reasons: (i) familial anxiety prompted a premature referral; (ii) the presence of worrying symptoms; (iii) as part of clinical evaluation/cascade genetic testing after the diagnosis of inherited heart disease in a first-degree relative. Standard criteria for the diagnosis of LQTS (the Schwartz score)[5] were utilized. In addition, during exercise and 24 h ECG recordings, periods of sufficient quality and heart rate stability were examined, commonly during the recovery phase, for accurate measurement of the maximal QTc interval.[6,7] In conjunction with genetic testing results (discussed later), an overall assessment of the likelihood of an individual carrying LQTS (high, intermediate, and low probability) and a family harbouring LQTS (definite or possible/probable) was then made. Standard criteria for the type 1 Brugada ECG pattern and diagnosis of the Brugada syndrome were employed.[8] Additional investigations were performed depending on the results of initial evaluation as summarized in *Figure 2*. If ECG recordings and echocardiography were normal or type 2 and/or 3 Brugada ECG patterns were present then ajmaline testing[8] was undertaken. If there were echocardiographic abnormalities [including equivocal right ventricular (RV) findings] and/or ECG features suggestive of cardiomyopathy, then cardiac magnetic resonance (CMR) imaging and signal averaged ECG (SAECG) were undertaken. Standard criteria for the diagnosis of ARVC in families with a

Figure 1 Flow diagram describing the number of families, probands, and relatives who were included, underwent evaluation, and then successful genetic testing.

Figure 2 Recommended diagnostic algorithm for families with a sudden arrhythmic death syndrome (SADS) death.

history of sudden death were utilized.[9,10] Any further investigations were undertaken as indicated by overall clinical findings. If a relative could not be evaluated at St George's, then data were collected with the assistance of their relatives, relevant physician, and a telephone interview. Investigation results were classified by two of the authors (E.R.B. and W.J.M.) as normal, equivocal or abnormal. In the event of a disagreement a senior colleague (E.R.) was consulted and a consensus agreed. When inherited cardiac disease was diagnosed, the relevant physicians were informed, appropriate management instigated, and follow-up and extended familial evaluation arranged.

Genetic testing

After appropriate counselling, mutation analysis was offered to families using DNA extracted from proband tissues and/or affected relatives' blood samples. The strategy employed is indicated in *Figure 2* and

proceeded alongside clinical evaluation of relatives. Therefore, when proband DNA was available, it was analysed for most genes recognized to cause inherited arrhythmia syndromes – the molecular autopsy (discussed later). Unfortunately, the quality and quantity of this DNA and subsequent mutation analysis was often limited by the nature of retained tissue paraffin blocks. DNA was not available in 33 probands. In 25 families, including 14 where proband DNA eventually became available, mutation analysis was undertaken in a relative with phenotypic abnormalities suggestive of inherited arrhythmia syndromes or ARVC. This was targeted according to phenotypic features and circumstances of death in the proband. If an unequivocal mutation (known or highly probable) was identified in a proband or relative then the family was offered cascade screening. If an equivocal (unknown or possible) mutation was identified co-segregation analysis was performed to determine causality and hence the value of further predictive testing in other family members.

Polymerase chain reaction (PCR) was utilized with primers derived from the published exons and their flanking intronic sequences to amplify the coding regions of genes responsible for cardiac ion channel subunits and their associated proteins implicated in the inherited arrhythmia syndromes (LQTS, short QT syndrome, Brugada syndrome, Andersen's syndrome and CPVT): all exons of KCNQ1, KCNE1, KCNH2, KCNE2, SCN5A,[11–14] ANK2,[15] KCNJ2,[16,17] CAV3,[18] CASQ2,[19] and selected exons (7–9, 13–16, 43–50, 82–84, and 87–105) of hRyR2.[20,21] If ARVC was diagnosed or suspected in relatives then analysis for mutations in the plakophyllin2 (PKP2) and desmoplakin genes was undertaken.[22,23] All PCR products were subjected to single stranded conformational polymorphism (SSCP) screening with polyacrylamide gel electrophoresis to identify non-conforming bands in comparison with an ethnically matched 400-allele control panel. PCR products showing an aberrant SSCP pattern were sequenced bi-directionally to identify any base changes relative to the published genomic DNA sequences, known mutations, and polymorphisms.

Statistics

Data collection and observational analysis employed Microsoft Access 2000 © (version 9). SPSS© (version 12) were utilized for statistical analysis. Pearson's χ^2 or Fisher's exact tests were used to compare proportions. The Binomial test for equal proportions was used to compare dichotomous variables (gender). Student's t-test was used to compare continuous variables. P-values < 0.05 were considered statistically significant.

Results

Figure 1 describes the numbers of families, probands, and relatives who were included, underwent evaluation, and then successful genetic testing.

Proband and family characteristics

Most SADS probands were male and/or died at rest or in sleep although statistical significance was not achieved (*Table 1*). Close to half had previously reported cardiac symptoms (syncope 19%), mainly to their relatives. None had undergone appropriate cardiac assessment as a result of these symptoms. *Table 2* illustrates the characteristics of their families and relatives.

Results of evaluation

In 30/57 (53%) families, definite and possible/probable inherited heart disease was identified (*Figure 3*). In one of these families clinical evaluation did not contribute to a diagnosis but mutation analysis of the proband's DNA identified a likely LQTS disease causing mutation (KCNH2-A80V). A quarter of evaluated first-degree relatives and two-fifths of non-first-degree relatives were diagnosed as affected with likely inheritable heart disease (*Table 3* and *Figure 1*).

Predictors of a diagnosis in an individual and in a family

Affected first degree relatives were similar to unaffected first degree relatives except that they were more likely to report a cardiac symptom especially syncope (*Table 4*). There were practically no differences between diagnosed and undiagnosed SADS probands and their families (including family size and proportion evaluated). The only significant difference was a higher frequency

Table 1 Probands' characteristics

Total number	57
Mean age (range)	26 (6–54)
Male	35 (61%)
Full coroner's autopsy	57 (100%)
Expert autopsy	
Whole heart and histology	20 (35%)
Histology only	23 (40%)
Mode of death: no. (%)	
At rest	36 (63%)
On exertion[a]	17 (30%)
Unknown/others	4 (7%)
Antecedent symptoms	
Present	33 (58%)
Absent	22 (39%)
Unknown	2 (3%)
Syncope	11 (19%)
Palpitations	13 (23%)

[a]Within 15 min of the end of a period of exertion.

of syncope amongst first-degree relatives in a family with a diagnosis (*Table 4*).

Investigations and their utility

Each of the initial investigations undertaken in all first degree relatives contributed either separately or in combination to a diagnosis of inherited heart disease in an individual: 16% of all ECGs, 11% of all exercise ECGs, 9% of all 24 h ECG, and 2% of all echocardiograms. Additional investigations also contributed to a diagnosis of inherited heart disease in an individual: 3/91 of all SAECGs (3%), 6/50 of all ajmaline tests (12%), and 4/25 of all CMR scans (16%). Examples of ECGs are supplied in Supplementary data, online.
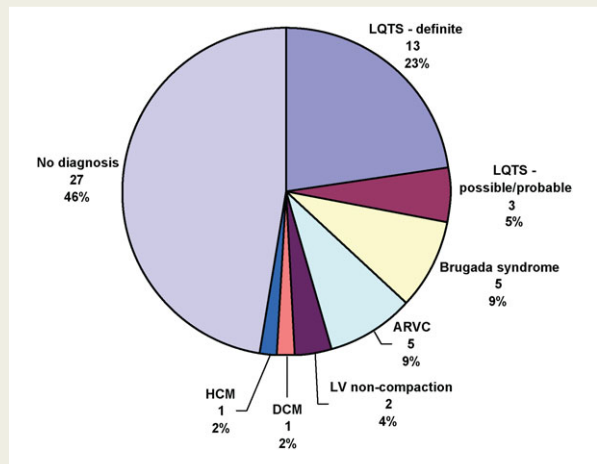
Mutation analysis

DNA was extracted from tissue or post-mortem blood samples from 24 SADS probands and underwent 'molecular autopsy'. This included eight probands whose families later demonstrated structural heart disease. Disease causing mutations that co-segregated with phenotype in a pedigree were detected in 2/6 (33%) probands with a subsequent familial diagnosis of definite or possible/probable LQTS (KCNH2: Q376sp and E544fs49X) and 1/4 (25%) with a familial diagnosis of ARVC (PKP2-S140F). Of 11 relatives who underwent cascade testing, seven carried mutations: four with KCNH2-E544fs49X, one with KCNH2-Q376sp, and two with PKP2-S140F. Two novel mutations SCN5A-P1187L and SCN5A-G1935S were detected in one proband and one, KCNH2-A80V, was detected in another. These could not be assessed further because all relatives evaluated were either unaffected non-carriers or apparently unaffected relatives refused gene testing. In the one family in whom a relative was later diagnosed with HCM, proband DNA was available and underwent testing for inherited arrhythmia syndrome and HCM genes without a mutation being detected. Thirty-three relatives from 15 families whose probands'

Table 2 Characteristics of families and relatives

	First-degree relatives	Non-first-degree relatives
Total	262	–
Total evaluated	184	38
Male:female (%)	77 (42%):107 (58%)[a]	22 (58%):16 (42%)
Total no. of relatives/family: mean (range)	4.60 (2–13)	–
Evaluated no. of relatives/family: mean	3.22	–
Mean proportion (range) of each family's relatives evaluated	68.3% (10–100%)	–
Mean age (range, median)	32.3 (1–72, 32)	19 (2–80)
Relationship to proband		
Parents	69	–
Siblings	85	–
Offspring	30	–
Evaluated relatives ≥ 1 cardiac symptom		
Total	44 (24%)	7 (18%)
Syncope	13 (7%)	3 (8%)
No. of families with history of unexpected premature[b] sudden death		
Any relative(s)	17 (30%)	–
First-degree relative(s)	6 (11%)	–
Non-first-degree relative(s)	14 (25%)	–
No. of unexpected premature[b] sudden deaths (excluding proband)		
First-degree relative(s)	7	–
Non-first-degree relative(s)	28	–

[a]$P = 0.033$.
[b]Age < 45 years.

Figure 3 Pie chart describing the diagnoses made in sudden arrhythmic death syndrome (SADS) families.

DNA were unavailable for analysis also underwent testing. Five relatives with LQTS carried mutations (four with KCNH2-S55L and one with SCN5A-T370M) and nine with Brugada syndrome (SCN5A-E1784K). In one ARVC family no proband DNA was available for mutation analysis and relatives refused testing.

Table 5 summarizes the 11 potential ion channel gene mutations that were detected but not found in controls. SCN5A-E1784K,[24] KCNH2-S55L,[25] and KCNH2-Q376sp[26] had been previously described. The novel mutations, SCN5A-A1680T, hRyR2-V2435I, and SCN5A-L461V failed to segregate with the disease phenotype and were therefore thought to be rare polymorphisms. No KCNJ2, ANK2, CAV3 or CASQ2 mutations were detected. Overall, the systematic genetic study of 31 probands and their families without any evidence of structural heart disease at autopsy or familial evaluation revealed eight ion channel mutations (26%).

Diagnosing long QT syndrome

Definite LQTS was diagnosed in 12/57 (21%) families with 25 affected individuals (17 first degree relatives) and possible/probable LQTS was diagnosed in 3/57 (5%) families with five affected individuals (three first degree relatives). One proband was considered a definite LQTS carrier based on genetic testing results (discussed earlier). The clinical features of probands and families are elaborated in Supplementary material online, *Table S1*. One proband, in whom antemortem data were available, and seven relatives, scored 4 or more on the Schwartz score (high probability LQTS), utilizing the resting ECG, of which four carried a mutation. Twenty-two relatives scored <4 (intermediate or low probability of LQTS) on the presenting ECG but further ECGs and careful assessment of the QTc during 24 h and exercise ECGs as well as

genetic testing results (six mutation carriers) led to 17 of these receiving a definite diagnosis. There were six obligate carriers.

Diagnosing Brugada syndrome

Sixteen relatives (11 first-degree) were diagnosed with Brugada syndrome. Eleven fulfilled clinical diagnostic criteria, five of whom carried the SCN5A-E1784K mutation.[8] Four carried the

Table 3 Numbers of relatives affected by inherited heart disease with associated percentages

Diagnosis	First-degree relatives	Non-first-degree relatives
LQTS – high probability	13 (28%)	8 (53%)
LQTS – intermediate probability	7 (15%)	2 (14%)
Brugada syndrome	11 (24%)	5 (33%)
ARVC	9 (20%)	–
Left ventricular non-compaction	3 (7%)	–
DCM	2 (4%)	–
HCM	1 (2%)	–
Total	46 (100%)	15 (100%)

SCN5A-E1784K mutation without fulfilling criteria, three of whom did not undergo ajmaline testing due to their young age. One was an obligate carrier.

Diagnosing structural heart disease

Of the nine families diagnosed with inherited structural heart disease only one proband's heart (ARVC family) did not undergo an expert cardiac pathologist's assessment. The whole hearts of the majority of these probands [HCM (1), DCM (1), ARVC (2), and left ventricular non-compaction (2) families] underwent expert assessment. There was, however, no evidence of structural heart disease in any proband.

Nine first-degree relatives were diagnosed with ARVC, often requiring a full range of investigations. One relative's echocardiogram revealed left ventricular dilatation and she was subsequently found to carry the known PKP2-S140F mutation.[23] Her father and dead brother's DNA also carried the same mutation and her father's CMR scan demonstrated a non-diagnostic regional wall motion abnormality in the RV free wall.

Left ventricular non-compaction was diagnosed in three relatives, all demonstrating typical echocardiographic morphological features.[27] Two had equivocal ECGs with minor T-wave abnormalities. Dilated and hypertrophic cardiomyopathies were diagnosed in three first degree relatives due to the detection of significant echocardiographic abnormalities in all three and abnormal resting ECGs in two.[28,29]

Table 4 Predictors of a diagnosis of inherited heart disease in evaluated first-degree relatives and families: first degree relatives are considered initially as individuals and then as part of families

	Diagnosis present	Diagnosis absent	Statistical significance?
Evaluated first-degree relatives			
Total no.	46 (25%)	138 (75%)	–
Age (years) mean	31.0	36.8	ns
Male	39%	41%	ns
Symptomatic	46%	18%	$P < 0.001$
Presyncope	7%	1%	$P = 0.035$
Syncope	20%	4%	$P < 0.001$
Families			
Total no. of families	30 (53%)	27 (47%)	–
Total no. of evaluated relatives	102 (55%)	82 (45%)	–
Mean (range) number of first degree relatives/family	4.8 (2–8)	4.4 (2–10)	ns
Mean proportion of each family evaluated	65%	72%	ns
Age (years) mean	32.1	32.5	ns
Male	41%	43%	ns
First-degree relatives reporting symptoms	28 (27%)	16 (20%)	ns
First-degree relatives reporting syncope	11 (11%)	2 (2%)	$P = 0.028$
FH previous premature[a] unexplained SD(s)	11/30 (37%)	6/27 (22%)	ns
Mean no. previous premature[a] unexplained SD(s)/family	0.83	0.37	ns

FH, family history; SD, sudden death.
[a] <45 years old.

Table 5 Results of mutation analysis in sudden arrhythmic death syndrome (SADS) families

Cardiac diagnosis	Number of families with a mutation detected (%)	Mutations detected (unequivocal unless otherwise stated)	Probable novel rare polymorphisms detected
Definite LQTS	5/13 (38%)	KCNH2: Q376sp,[26] E544fs49X, A80V, S55L[25] SCN5A: T370M	
Possible/probable LQTS	0/3		SCN5A: L461V[b] hRyR2: V2435I[b]
Brugada syndrome	1/5 (20%)	SCN5A: E1784K[24]	SCN5A: A1680T
ARVC	1/4 (25%)	PKP2: S140F[23]	
Other structural disease	0/4		
None	1/10 (10%)	SCN5A: P1187L[a], G1935S[a]	

[a]Two equivocal mutations in the same proband.
[b]Two rare polymorphisms in the same relative.

Immediate management

Thirty-one out of 61 affected relatives received therapeutic interventions including betablockers in 30, the majority of whom carried LQTS, and ICDs in seven, three with Brugada syndrome (one asymptomatic) and four with LQTS (three symptomatic and one with other high risk features). All received appropriate life style and drug avoidance advice.

Discussion

Over half of SADS families who underwent cardiac evaluation had features of inherited cardiac disease, approximately one quarter of first-degree relatives being identified as likely to be affected. The conditions detected, and therefore likely to be culpable for the probands' deaths, were LQTS, Brugada syndrome, and subtle structural disease, particularly ARVC. Approximately 7% of relatives had suffered syncopal episodes, a similar prevalence to phenotypically normal relatives in the international LQTS registry.[30] One quarter of families had a history of additional premature sudden deaths. The cohort therefore appears to be at high risk of cardiac events and therapeutic intervention was possible with measures proven to protect individuals carrying genetic cardiac disease from sudden death.[8,31–33]

Two-thirds of SADS victims were young males and the majority died at rest or during sleep. Half suffered antecedent symptoms that were rarely assessed (syncope 19%) and never diagnosed as being due to cardiac disease and there was a family history of premature sudden death in 30%. This raises the possibility that underlying genetic cardiac disease may have been identifiable and therefore treated prior to death if symptoms and family history had prompted evaluation. Indeed one proband had features of definite LQTS during investigation for syncope and seizures yet the diagnosis was only made after her death. It is possible that SADS victims suffered more symptoms than ascertained by this study as the recording of antecedent symptoms relied upon third party reports.

Four out of the 30 families and their probands who were thought to carry inheritable heart disease demonstrated features suggestive of LQTS (possible/probable) that were insufficient to make a 'definite' diagnosis. The possibility of other forms of genetic cardiac disease in these families remains as well as the possibility that their findings represent one end of the spectrum of 'normality'.

The autopsy: how was structural disease missed?

Inherited structural heart disease was detected in relatives yet was not identified at post-mortem despite additional assessment by a specialist cardiac pathologist in the majority of cases. There are three possible reasons for this: (i) Structural genetic myocardial conditions, even when expressed insufficiently at a microscopic level to meet diagnostic criteria, may cause sudden death. (ii) Even thorough expert histopathological evaluation of a macroscopically normal heart may fail to identify areas of myocardium where sufficient abnormality is present to meet diagnostic criteria and cause sudden death. (iii) Insufficient tissue may be available to evaluate the heart thoroughly and satisfactorily, in particular the RV.

Comparison with other studies

This study was undertaken in a tertiary centre. There was therefore a possibility of referral bias, which may have resulted in families with less obvious inherited heart disease being studied. This may have in turn resulted in an underestimate of the frequency of such disease. We therefore compared the results of this study with our previously reported experience in the SADS study, a national population-based prospective survey that recruited via coroners rather than physicians.[3] SADS study probands were older (30 vs. 26 years) and, in addition to a full coroner's autopsy, all underwent expert cardiac pathological evaluation compared with 75% in the current cohort. This reflected that the current study's sample was generated from real world referrals where an expert cardiac pathologist's evaluation may not always be feasible. There were no other significant differences in the characteristics of probands (including the male

predominance), their relatives and their family histories. This suggested that the current cohort is similar to the SADS study's unselected sample.[3]

In comparison with the restricted research protocol of the initial SADS study[3] a comprehensive range of investigations (*Figure 2*) improved the diagnostic yield of both non-structural and structural inherited heart disease amongst first degree relatives (25 vs. 5.5%) and hence SADS families (54 vs. 22%). All investigative tools contributed to individuals' and hence families' ultimate diagnoses. Ajmaline provocation was vital to the diagnosis of Brugada syndrome.[8] A diagnosis of ARVC, however, required detection of a few subtle manifestations, no single test carrying sufficient sensitivity to be the sole diagnostic tool. This may explain why the limited protocol of the SADS study had failed to diagnose ARVC and Brugada syndrome in relatives.

Tan *et al.*[4] utilized a similar comprehensive investigative strategy in families with frequent sudden unexplained deaths and achieved similar diagnostic yields. The overall malignant nature of the histories of those families who were diagnosed with inherited cardiac diseases was consistent, however, with genetic disease even before familial evaluation was undertaken. Indeed the presence of two or more premature sudden unexplained deaths in a family was a predictor of a diagnosis. Our current study had a lower frequency of additional sudden unexplained deaths and such a family history was not of predictive value. This, however, may have been a limitation of the study's sample size. The only significant predictor of a successful diagnosis was the reporting by first-degree relatives of syncope.

Tan's group also differed in that approximately half of index cases had not undergone any pathological examination and the circumstances of death were strongly associated with exertion rather than rest or sleep. This is reflected by 5/43 families being diagnosed with CPVT and carrying mutations in hRyR2 (19;20) and 2/4 LQTS families carrying KCNQ1 mutations.[32] All demonstrated characteristic ventricular arrhythmia and/or sudden death on exercise. Interestingly Tester *et al.*[21,34] have described seven carriers of hRyR2 mutations and five carriers of KCNQ1 mutations in 49 medical examiner cases of sudden unexpected death with normal autopsies and a mixture of exertional, sleep-associated, and unknown circumstances of death.

In the current cohort, no mutations were detected in KCNQ1 or CASQ2 and only one potential variant of hRyR2 was identified although it failed to co-segregate with phenotype. Tan's series appears to be a selected high-risk group without thorough pathological assessment. The SADS population described here and elsewhere[3] may therefore reflect the spectrum of young 'normal heart' sudden death more accurately. In Tester's series a significant proportion of LQT1 and hRyR2 mutations were novel and may have represented uncommon non-pathogenic variants. There remains, however, a difference in genetic composition when compared with the current cohort. This may be explained either by chance or by a difference in methodology when receiving selected autopsy referrals from medical examiners compared with the referral of families as described here.

Nonetheless, a common message does arise from the current study and the three other comparators:[3,4,21,34] that an expert pathological and molecular autopsy in SADS cases is important and that their families warrant evaluation.[35]

Mutation analysis

The presence of potential mutations in SCN5A and KCNH2 amongst SADS families was expected as the characteristic circumstances of death in LQT2, LQT3,[36] and Brugada syndrome[8] include death during sleep, at rest or after sudden arousal from sleep, a feature of the majority of SADS victims. KCNH2 and SCN5A mutations have also been detected in other series of sudden unexpected deaths[34,37] including infants.[38] LQT3 and Brugada syndrome also present more commonly and with greater severity amongst males, another SADS proband characteristic.[8,39] SCN5A-E1784K had been reported previously as causing LQT3,[24] although our family's features were consistent with an overlap of Brugada and LQTS.

The majority of diagnosed cases were not confirmed by genetic testing. Current knowledge means that ~60–70% of patients with LQTS and a minority of those with Brugada syndrome and ARVC can be genotyped.[8,22,23,25,26] The 38% yield in definite LQTS families may be due to the spectrum of LQTS being studied or may be a chance finding. The proportion of families without evidence of structural disease and carrying an LQTS/Brugada syndrome mutation [8/31 (26%)] compares favourably with the proportions reported in two other autopsy series of unexplained sudden deaths described by Chugh *et al.*[37] [2/12 (17%)] and Tester and Ackerman[34] [10/49 (20%)].

Only one (10%) proband from families without a diagnosis, who underwent testing, was identified as carrying mutations (SCN5A-P1187L, SCN5A-G1935S). The yield of testing in this group may have been limited by the difficulty of extracting sufficient quality DNA from paraffin fixed blocks, the commonest form of retained tissue sample.

The family: risk of sudden death and undiagnosed disease

The detection of inherited heart disease in a family appeared to differentiate the family members' level of risk when compared with undiagnosed families due to a higher frequency of syncope amongst relatives. Another marker of inherited heart disease is the presence of a family history of additional sudden premature death(s), but diagnosed families were not significantly different from the undiagnosed. This suggested that those carriers with sufficient expression of inherited disease to merit a diagnosis also tended to be symptomatic. Less penetrant disease that failed to cause symptoms but still caused sudden deaths may therefore be present in some of the 47% of undiagnosed families whose members remain potentially at risk. Consequently an improvement of diagnostic yield is important.

Improving the yield

A greater use of expert cardiac pathologists and retention of the whole heart for their histological review as well as frozen spleen or blood for mutation analysis are vital for improved diagnostics. There was no bias in favour of larger or more thoroughly evaluated families receiving a diagnosis in this study. Nonetheless, encouraging a full evaluation of all first degree relatives (particularly males who attended less frequently than females) or potential obligate carriers may maximize the chances of identifying incompletely penetrant disease. Improvement of the investigative

armamentarium should also be considered. More comprehensive ajmaline testing may increase diagnoses of Brugada syndrome although its sensitivity is not 100%.[8] More comprehensive testing of the RyR2 gene may diagnose non-penetrant CPVT.
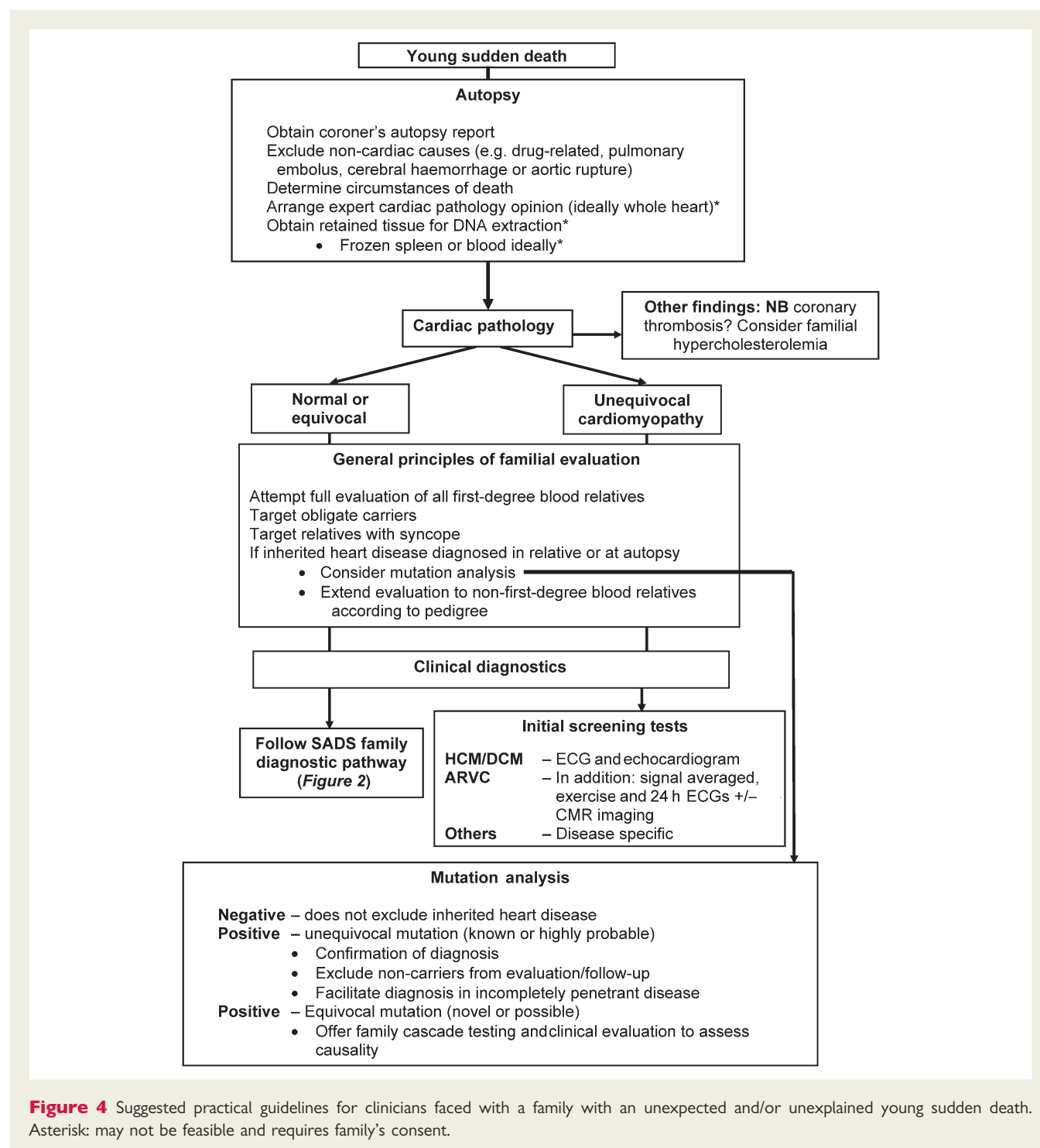
Practical clinical guidelines

Figure 4 provides recommendations for clinicians faced with a family with a young sudden death and places the SADS investigative algorithm (*Figure 2*) in context. Genetic testing in arrhythmia

syndromes or cardiomyopathy should be comprehensive for the major genes involved as compound heterozygosity is prevalent and causes more severe disease.[40]

Summary

These data underscore that SADS deaths are caused by inherited heart disease and more comprehensive clinical and genetic evaluation of families identifies more relatives at risk of sudden death.

Figure 4 Suggested practical guidelines for clinicians faced with a family with an unexpected and/or unexplained young sudden death. Asterisk: may not be feasible and requires family's consent.

The potential number of deaths involved is significant[2] and there are risk stratification algorithms and effective treatments available for these inherited cardiac conditions. This highlights the importance of the prompt recognition of symptomatic young people and the accurate identification of SADS deaths, which should initiate familial evaluation.

Supplementary material

Supplementary material is available at *European Heart Journal* online.

Acknowledgements

References

1. Bowker TJ, Wood DA, Davies MJ, Sheppard MN, Cary NRB, Burton JDK, Chambers DR, Dawling S, Hobson HL, Pyke SDM, Riemersma RA, Thompson SG. Sudden, unexpected cardiac or unexplained death in England: a national survey. *QJM* 2003;**96**: 269–279.

2. Behr ER, Casey A, Sheppard M, Wright M, Bowker TJ, Davies MJ, McKenna WJ, Wood DA. Sudden arrhythmic death syndrome: a national survey of sudden unexplained cardiac death. *Heart* 2007; **93**:601–605.

3. Behr E, Wood DA, Wright M, Syrris P, Sheppard MN, Casey A, Davies MJ, McKenna W. Cardiological assessment of first-degree relatives in sudden arrhythmic death syndrome. *Lancet* 2003;**362**: 1457–1459.

4. Tan HL, Hofman N, van Langen IM, van der Wal AC, Wilde AA. Sudden unexplained death: heritability and diagnostic yield of cardiological and genetic examination in surviving relatives. *Circulation* 2005;**112**:207–213.

5. Schwartz PJ, Moss AJ, Vincent GM, Crampton RS. Diagnostic criteria for the long QT syndrome. An update. *Circulation* 1993;**88**: 782–784.

6. Dillenburg RF, Hamilton RM. Is exercise testing useful in identifying congenital long QT syndrome? *Am J Cardiol* 2002;**89**:233–236.

7. Swan H, Viitasalo M, Piippo K, Laitinen P, Kontula K, Toivonen L. Sinus node function and ventricular repolarization during exercise stress test in long QT syndrome patients with KvLQT1 and HERG potassium channel defects. *J Am Coll Cardiol* 1999;**34**:823–829.

8. Antzelevitch C, Brugada P, Borggrefe M, Brugada J, Brugada R, Corrado D, Gussak I, LeMarec H, Nademanee K, Perez R, Andres R, Shimizu W, Schulze-Bahr E, Tan H, Wilde A. Brugada Syndrome: report of the Second Consensus Conference: Endorsed by the Heart Rhythm Society and the European Heart Rhythm Association. *Circulation* 2005;**111**:659–670.

9. McKenna WJ, Thiene G, Nava A, Fontaliran F, Blomstrom-Lundqvist C, Fontaine G, Camerini F. Diagnosis of arrhythmogenic right ventricular dysplasia/cardiomyopathy. Task Force of the Working Group Myocardial and Pericardial Disease of the European Society of Cardiology and of the Scientific Council on Cardiomyopathies of the International Society and Federation of Cardiology. *Br Heart J* 1994;**71**:215–218.

10. Hamid MS, Norman M, Quraishi A, Firoozi S, Thaman R, Gimeno JR, Sachdev B, Rowland E, Elliott PM, McKenna WJ.

11. Splawski I, Shen J, Timothy KW, Vincent GM, Lehmann MH, Keating MT. Genomic structure of three long QT syndrome genes: KVLQT1, HERG, and KCNE1. *Genomics* 1998;**51**:86–97.

12. Wang Q, Shen J, Splawski I, Atkinson D, Li Z, Robinson JL, Moss AJ, Towbin JA, Keating MT. SCN5A mutations associated with an inherited cardiac arrhythmia, long QT syndrome. *Cell* 1995;**80**:805–811.

13. Brugada R, Hong K, Dumaine R, Cordeiro J, Gaita F, Borggrefe M, Menendez TM, Brugada J, Pollevick GD, Wolpert C, Burashnikov E, Matsuo K, Wu Y, Guerchicoff A, Bianchi F, Giustetto C, Schimpf R, Brugada P, Antzelevitch C. Sudden death associated with short-QT syndrome linked to mutations in HERG. *Circulation* 2004;**109**:30–35.

14. Bellocq C, van Ginneken AC, Bezzina CR, Alders M, Escande D, Mannens MM, Baro I, Wilde AA. Mutation in the KCNQ1 gene leading to the short QT-interval syndrome. *Circulation* 2004;**109**: 2394–2397.

15. Mohler PJ, Schott JJ, Gramolini AO, Dilly KW, Guatimosim S, DuBell WH, Song LS, Haurogne K, Kyndt F, Ali ME, Rogers TB, Lederer WJ, Escande D, Marec HL, Bennett V. Ankyrin-B mutation causes type 4 long-QT cardiac arrhythmia and sudden cardiac death. *Nature* 2003;**421**:634–639.

16. Plaster N M, Tawil R, Tristani-Firouzi M, Canun S, Bendahhou S, Tsunoda A, Donaldson MR, Iannaccone ST, Brunt E, Barohn R, Clark J, Deymeer F, George AL Jr, Fish FA, Hahn A, Nitu A, Ozdemir C, Serdaroglu P, Subramony SH, Wolfe G, Fu YH, Ptacek LJ. Mutations in Kir2.1 cause the developmental and episodic electrical phenotypes of Andersen's syndrome. *Cell* 2001;**105**: 511–519.

17. Priori SG, Pandit SV, Rivolta I, Berenfeld O, Ronchetti E, Dhamoon A, Napolitano C, Anumonwo J, di Barletta MR, Gudapakkam S, Bosi G, Stramba-Badiale M, Jalife J. A novel form of short QT syndrome (SQT3) is caused by a mutation in the KCNJ2 gene. *Circ Res* 2005;**96**:800–807.

18. Vatta M, Ackerman MJ, Ye B, Makielski JC, Ughanze EE, Taylor EW, Tester DJ, Balijepalli RC, Foell JD, Li Z, Kamp TJ, Towbin JA. Mutant caveolin-3 induces persistent late sodium current and is associated with long-QT syndrome. *Circulation* 2006;**114**: 2104–2112.

19. Lahat H, Pras E, Olender T, Avidan N, Ben Asher E, Man O, Levy-Nissenbaum E, Khoury A, Lorber A, Goldman B, Lancet D, Eldar M. A missense mutation in a highly conserved region of CASQ2 is associated with autosomal recessive catecholamine-induced polymorphic ventricular tachycardia in Bedouin families from Israel. *Am J Hum Genet* 2001;**69**:1378–1384.

20. Priori SG, Napolitano C, Tiso N, Memmi M, Vignati G, Bloise R, Sorrentino V, Danieli GA. Mutations in the cardiac ryanodine receptor gene (hRyR2) underlie catecholaminergic polymorphic ventricular tachycardia. *Circulation* 2001;**103**:196–200.

21. Tester DJ, Spoon DB, Valdivia HH, Makielski JC, Ackerman MJ. Targeted mutational analysis of the RyR2-encoded cardiac ryanodine receptor in sudden unexplained death: a molecular autopsy of 49 medical examiner/coroner's cases. *Mayo Clin Proc* 2004;**79**: 1380–1384.

22. Norman M, Simpson M, Mogensen J, Shaw A, Hughes S, Syrris P, Sen-Chowdhry S, Rowland E, Crosby A, McKenna WJ. Novel mutation in desmoplakin causes arrhythmogenic left ventricular cardiomyopathy. *Circulation* 2005;**112**:636–642.

Prospective evaluation of relatives for familial arrhythmogenic right ventricular cardiomyopathy/dysplasia reveals a need to broaden diagnostic criteria. *J Am Coll Cardiol* 2002;**40**:1445–1450.

23. Gerull B, Heuser A, Wichter T, Paul M, Basson CT, McDermott DA, Lerman BB, Markowitz SM, Ellinor PT, MacRae CA, Peters S, Grossmann KS, Drenckhahn J, Michely B, Sasse-Klaassen S, Birchmeier W, Dietz R, Breithardt G, Schulze-Bahr E, Thierfelder L. Mutations in the desmosomal protein plakophilin-2 are common in arrhythmogenic right ventricular cardiomyopathy. *Nat Genet* 2004;**36**:1162–1164.

24. Wei J, Wang DW, Alings M, Fish F, Wathen M, Roden DM, George AL Jr. Congenital long-QT syndrome caused by a novel mutation in a conserved acidic domain of the cardiac Na+ channel. *Circulation* 1999;**99**:3165–3171.

25. Tester DJ, Will ML, Haglund CM, Ackerman MJ. Compendium of cardiac channel mutations in 541 consecutive unrelated patients referred for long QT syndrome genetic testing. *Heart Rhythm* 2005;**2**:507–517.

26. Splawski I, Shen J, Timothy KW, Lehmann MH, Priori S, Robinson JL, Moss AJ, Schwartz PJ, Towbin JA, Vincent GM, Keating MT. Spectrum of mutations in long-QT syndrome genes: KVLQT1, HERG, SCN5A, KCNE1, and KCNE2. *Circulation* 2000; **102**:1178–1185.

27. Murphy RT, Thaman R, Blanes JG, Ward D, Sevdalis E, Papra E, Kiotsekolglou A, Tome MT, Pellerin D, McKenna WJ, Elliott PM. Natural history and familial characteristics of isolated left ventricular non-compaction. *Eur Heart J* 2005;**26**:187–192.

28. Mahon NG, Murphy RT, MacRae CA, Caforio AL, Elliott PM, McKenna WJ. Echocardiographic evaluation in asymptomatic relatives of patients with dilated cardiomyopathy reveals preclinical disease. *Ann Intern Med* 2005;**143**:108–115.

29. Richardson P, McKenna W, Bristow M, Maisch B, Mautner B, O'Connell J, Olsen E, Thiene G, Goodwin J, Gyarfas I, Martin I, Nordet P. Report of the 1995 World Health Organization/ International Society and Federation of Cardiology Task Force on the definition and classification of cardiomyopathies. *Circulation* 1996;**93**:841–842.

30. Priori SG, Napolitano C, Schwartz PJ. Low penetrance in the long QT syndrome: clinical impact. *Circulation* 1999;**99**:529–533.

31. Zareba W, Moss AJ, Daubert JP, Hall WJ, Robinson JL, Andrews M. Implantable cardioverter defibrillator in high-risk long QT syndrome patients. *J Cardiovasc Electrophysiol* 2003;**14**:337–341.

32. Moss AJ, Zareba W, Hall WJ, Schwartz PJ, Crampton RS, Benhorin J, Vincent GM, Locati EH, Priori SG, Napolitano C, Medina A, Zhang L, Robinson JL, Timothy K, Towbin JA, Andrews ML. Effectiveness and limitations of beta-blocker therapy in congenital long-QT syndrome. *Circulation* 2000;**101**: 616–623.

33. Corrado D, Leoni L, Link MS, Bella PD, Gaita F, Curnis A, Salerno JU, Igidbashian D, Raviele A, Disertori M, Zanotto G, Verlato R, Vergara G, Delise P, Turrini P, Basso C, Naccarella F, Maddalena F, Estes NAM, Buja G, Thiene G. Implantable cardioverter-defibrillator therapy for prevention of sudden death in patients with arrhythmogenic right ventricular cardiomyopathy/dysplasia. *Circulation* 2003;**108**:3084–3091.

34. Tester DJ, Ackerman MJ. Postmortem long QT syndrome genetic testing for sudden unexplained death in the young. *J Am Coll Cardiol* 2007;**49**:240–246.

35. Schwartz PJ, Crotti L. Can a message from the dead save lives? *J Am Coll Cardiol* 2007;**49**:247–249.

36. Schwartz PJ, Priori SG, Spazzolini C, Moss AJ, Vincent GM, Napolitano C, Denjoy I, Guicheney P, Breithardt G, Keating MT, Towbin JA, Beggs AH, Brink P, Wilde AA, Toivonen L, Zareba W, Robinson JL, Timothy KW, Corfield V, Wattanasirichaigoon D, Corbett C, Haverkamp W, Schulze-Bahr E, Lehmann MH, Schwartz K, Coumel P, Bloise R. Genotype-phenotype correlation in the long-QT syndrome: gene-specific triggers for life-threatening arrhythmias. *Circulation* 2001;**103**:89–95.

37. Chugh SS, Senashova O, Watts A, Tran PT, Zhou Z, Gong Q, Titus JL, Hayflick SJ. Postmortem molecular screening in unexplained sudden death. *J Am Coll Cardiol* 2004;**43**:1625–1629.

38. Arnestad M, Crotti L, Rognum TOM, Insolia R, Pedrazzini M, Ferrandi C, Vege A, Wang DWM, Rhodes TE, George AL Jr, Schwartz PJM. Prevalence of long-QT syndrome gene variants in sudden infant death syndrome. *Circulation* 2007;**115**:361–367.

39. Priori SG, Schwartz PJ, Napolitano C, Bloise R, Ronchetti E, Grillo M, Vicentini A, Spazzolini C, Nastoli J, Bottelli G, Folli R, Cappelletti D. Risk stratification in the long-QT syndrome. *N Engl J Med* 2003;**348**:1866–1874.

40. Westenskow P, Splawski I, Timothy KW, Keating MT, Sanguinetti MC. Compound mutations: a common cause of severe long-QT syndrome. *Circulation* 2004;**109**:1834–1841.