

Genetic mutations but no phenotype: the use of genetic testing in idiopathic VF and familial
SCD

Dr Yanushi Dullewe Wijeyeratne BMedSci BMBS MRCP

Cardiology Specialty Registrar and Clinical Research Fellow in Cardiology

St George's Hospital and St George's University of London

London SW17 0RE

Dr Elijah R Behr MD, FRCP,

Reader and Honorary Consultant Cardiologist and Electrophysiologist,

Division of Cardiac & Vascular Sciences

St George's Hospital and St George's University of London

London SW17 0RE

ebehr@sgul.ac.uk

Abstract

Approximately 4% of sudden cardiac deaths are unexplained (the sudden arrhythmic death syndrome: SADS), and up to 6-10% of survivors of cardiac arrest do not have an identifiable cardiac abnormality after comprehensive clinical evaluation (idiopathic ventricular fibrillation: IVF). Genetic testing may be able to play a role in diagnostics and can be targeted to an underlying phenotype present in family members following clinical evaluation. Alternatively post-mortem genetic testing (the 'molecular autopsy') may diagnose the underlying cause if a clearly pathogenic rare variant is found. Limitations include a modest yield, and the high probability of finding a variant of unknown significance (VUS) leading to a low signal-to-noise ratio. Next generation sequencing enables cost-efficient high throughput screening of a larger number of genes but at the expense of increased genetic noise. The yield from genetic testing is even lower in IVF in the absence of any suggestion of another phenotype in the index case or his/her family, and should be actively discouraged at this time. Future improvements in diagnostic utility include optimisation of the use of variant-calling pipelines and shared databases as well as patient-specific models of disease to more accurately assign pathogenicity of variants. Studying 'trios' of parents and the index case may better assess the yield of sporadic and recessive disease.

Introduction

Sudden cardiac death (SCD) is a leading cause of mortality worldwide, with coronary artery disease being the major pathology. The prevalence of SCD is 50,000-100,000 pa in the UK, of which up to 4% may be 'unexplained' (1). The finding of a morphologically normal heart on autopsy is common in

most series of young SCD (2–8). Unexplained sudden death of an individual older than 1 year of age with negative pathological and toxicological assessment on autopsy is referred to as sudden arrhythmic death syndrome (SADS) (9). The true incidence of SADS appears to be several fold higher than recorded in official mortality statistics, and is estimated to be up to 1.34/100,000 per annum in the UK (10).

Systematic comprehensive clinical evaluation of patients surviving a cardiac arrest in the absence of overt electrophysiological or structural cardiac abnormalities can lead to identification of a phenotype in 18-53% of cases, with a mean yield of 32% (11). It is estimated that approximately 6-10% of survivors of cardiac arrest, however, do not have an identifiable cardiac abnormality or substrate for arrhythmia following comprehensive clinical evaluation: idiopathic ventricular fibrillation (IVF) (12). The true incidence of IVF is unknown and a longitudinal study of out-of-hospital cardiac arrest survivors suggested a lower prevalence of 1.2% (13). The clinical challenges in understanding and managing idiopathic VF became apparent in the early 1990s (14) and a subsequent expert consensus statement described the hallmark of the condition as the inability to identify a causal relationship between the clinical circumstance and the arrhythmia (15). According to the 2013 HRS/EHRA consensus guidelines an IVF survivor is defined as a resuscitated cardiac arrest victim, preferably with documentation of VF, in whom known cardiac, respiratory, metabolic and toxicological aetiologies have been excluded through clinical evaluation (9). The 2015 ESC guidelines define IVF as an episode of documented ventricular fibrillation following which comprehensive clinical evaluation does not identify an underlying cause. Importantly, as with SADS, it is a diagnosis of exclusion (16).

The approach to investigating these cases combines assessment of the index case and of the family. This incorporates pathological and clinical data but in contemporary clinical practice includes genetic

testing that may be applied in the index case and/or in the relatives. We discuss below the role of genetic analyses in both settings for IVF and SADS.

Common considerations in clinical genetics

Genetic diagnostic yields are never 100%, even for the best characterised diseases, so a negative test does not exclude disease. For example, diagnostic yield of genetic testing in definite cases of the congenital Long QT syndrome (LQTS) is 80-85%, while it is only 20-25% in Brugada syndrome (BrS).

Variant calling can be challenging. Advances in sequencing, including the use of next generation sequencing (NGS), have led to increasing gene panel sizes and enabled simultaneous sequencing of the entire exome, thereby increasing the potential yield of genetic testing. This has also led to the discovery of an increasing number of variants of unknown significance (VUS) compromising diagnostic sensitivity, the so-called 'signal-to-noise' ratio (17,18). The signal-to-noise ratio is the expected yield of rare genetic variants in disease cases divided by the background rate of rare genetic variants in controls. This provides a sense of the positive predictive value of a 'positive' genetic test result (19). For genetic testing targeted to phenotype, it has been estimated that the 'signal-to-noise' ratio for disease-specific genetic testing can be as low as 4:1 for conditions like arrhythmogenic cardiomyopathy, increasing to 19:1 for LQTS. In commercialised disease-specific gene panels that include minor disease-associated genes, where each gene may be responsible for <1% of the disease in question, the signal-to-noise ratio worsens because of the frequency of background variation in these minor genes (19). Rare variants are often missense or private to a specific family and may be VUSs. A VUS or even a likely pathogenic variant can have significant implications for family members if it is used inappropriately to diagnose disease in relatives. Its use should therefore be restricted to clarification of pathogenicity through segregation analysis. This

approach would, however, not be useful for sporadic, *de novo* or germline mutations. The variant should also undergo regular rigorous review in case the scientific literature changes and pathogenicity can be accurately ascribed or excluded.

Consensus guidelines have been developed by the American College of Medical Genetics (ACMG) to set standards on determining the pathogenicity of variants. These are based upon 1) the absence of the variant in a healthy control population, 2) cosegregation of phenotype with genotype in large families, or presence of a *de novo* variant (both paternity and maternity confirmed) in the proband with no family history, 3) severity of the type of mutation (for example nonsense, frameshift mutation or deletion vs. missense mutation) and location of the variant within the genome (for example a critical and well established functional domain), 4) prior description of the variant in the literature, 5) amino acid conservation and evidence from *in silico* modelling tools, and 6) *in vitro* or *in vivo* functional expression studies demonstrating the variant's biophysical effect(s) (17,20).

Miscalling of less common, but not extremely rare, variants as causative adds to problems with interpretation of genetic results. This is most apparent when historical studies utilising small controls have labelled certain variants as causative but when examined in modern data sets appear to be too common to be truly pathogenic of a monogenic disorder. For example, marked overrepresentation of variants previously associated with several inherited cardiac ion channelopathies have been identified in a large exome database provided by the Exome Sequencing Project (21–23). These may represent predisposing genetic factors or benign background variation that may be ethnically specific, and must be taken into account before assigning pathogenicity (24–26). In order to minimise the chances of incorrectly assigning pathogenicity, we suggest that the number of ethnically matched controls in such studies should exceed as much as possible the prevalence of the disorder in the population studied. Thus if a VUS has close to this prevalence in a healthy control population it is highly unlikely to be pathogenic in its own right. Collaborative datasets including the ClinGen and ClinVar partnership, the Exome Variant Server and ExAC databases attempt to address

these issues (27,28). ClinVar and ClinGen aim to centralise the data available on genomic variation and pathogenicity whilst ExAC provides exome data for over 60,000 individuals of different ethnicity.

Figure 1 summarises the current recommendations for genetic testing in the channelopathies, SADS and IVF based upon the expected diagnostic utility. The data behind these recommendations are discussed below.

[FIGURE 1]

Figure 1. Guidelines for genetic testing in the channelopathies, SADS and IVF (17,19).

Genetic testing in idiopathic VF

Concealed arrhythmia syndromes may represent the hidden substrate for IVF but the yield from testing has been low historically (12). Mutations in the cardiac sodium channel gene *SCN5A* affecting channel function were first linked to IVF in 1998 (29). Since then there have been several studies reporting genetic associations with IVF, with *KCND3*, *KCNJ8*, *CALM1*, *RYR2*, and *SCN3B* being implicated. These are, however, mostly case series of isolated findings with a number of limitations (30–37). These include: variability in the definition of IVF and therefore the possibility of other incompletely penetrant conditions; the absence of segregation in large families to strengthen association; and a reliance on *in vitro* basic electrophysiological data that may be open to interpretation. Increasing knowledge of background ethnic specific genetic variation has meant that rare variants previously thought to be pathogenic mutations are now thought to be more common and unlikely to be truly disease causing. For example, in one study involving just over 700 unaffected individuals, the *KCNJ8* variant associated with IVF and early repolarisation syndrome was found to

have a prevalence of 4% amongst Ashkenazi Jews, compared to <0.025% prevalence among most other populations (38).

Convincing evidence of a diagnostic role in genetic testing in IVF is limited to a founder haplotype at the *DPP6* gene locus on chromosome 7 that was identified in a genome-wide haplotype-sharing analysis of Dutch families with IVF (39). In the same study, *DPP6* expression was found to be 20-fold higher in the myocardium of carriers compared to controls. More recently, functional studies have pointed to a previously unreported role of this gene in the transient outward current (I_{to}) in Purkinje fibres, with *DPP6* gain-of-function being shown to selectively enhance this current (40). It has therefore been proposed that a *DPP6*-mediated syndrome may be a novel molecular mechanism for some forms of IVF. *DPP6* testing is therefore recommended in IVF survivors of Dutch ancestry (19).

Overall the combined yield from genetic screening of IVF survivors and unselected family members of SCD victims has been found to be as low as 9% (41). It is unclear if the classification of IVF survivors and unselected family members of SCD victims (as opposed to SCD victims themselves) into one group could have diluted the yield of genetic testing in IVF survivors but the findings are consistent with our clinical experience. The effectiveness of screening large numbers of genes should therefore be taken into careful consideration before embarking on genetic testing in IVF survivors without any other overt phenotype. Recommendations for genetic testing in IVF are cautious as the *a priori* likelihood of genetic disease appears low and the signal to noise ratio highly unfavourable. Thus the 2013 HRS/EHRA guidelines recommend that genetic testing *can be useful* when there is a suspicion of a specific genetic disease on clinical evaluation of the proband and/or family members (class IIa recommendation). Factors to take into account before considering genetic testing include age, gender, circumstances of cardiac arrest and whether there is a family history of sudden death. For example, in catecholaminergic polymorphic ventricular tachycardia (CPVT), sudden cardiac arrest can be the first clinical presentation in up to 30% of cases. Mutations of the gene encoding the

cardiac ryanodine receptor, *RYR2*, can therefore be regarded as a potential cause of adrenergically-mediated IVF, justifying genetic testing for this if circumstances are suggestive (19). Genetic screening of large panels of genes in IVF patients where there is no suspicion of an inherited arrhythmogenic disease after comprehensive clinical evaluation is discouraged (class III recommendation) (9).

Incompletely penetrant disease may be more evident in family members of IVF survivors than in the proband, especially if comprehensive clinical evaluation of the IVF survivor is limited by poor neurological outcome following the cardiac arrest (11). Relatives of IVF survivors who are either obligate carriers, or who have suspicious symptoms, such as cardiac syncope, should be prioritised for comprehensive clinical evaluation. The optimal management of first-degree relatives of IVF survivors with unexplained syncope but no identifiable phenotype is, however, unclear at present due to scarce data on this group. Unless a pathogenic mutation is found and cascade screening is negative, periodic clinical re-evaluation is recommended for children and young family members due to the possibility of age-related penetrance of several inherited cardiac conditions. Re-evaluation is also recommended for family members of IVF survivors who subsequently become symptomatic, or if a family history of sudden death emerges (9).

Genetic testing in SADS

Retention of tissue in SADS victims that is suitable for DNA extraction can permit post-mortem genetic testing of arrhythmia syndrome linked genes: the 'molecular autopsy'. This can lead to a genetic diagnosis of an inherited arrhythmia syndrome in 9-20% of cases (17,42). The yield is lower in an unselected cohort of SADS victims compared to phenotype-directed molecular autopsy (43,44) and also tends to vary with age and the number of genes included. Figure 2 illustrates the reported

diagnostic yields of molecular autopsy predominantly undertaken using Sanger sequencing for the major arrhythmia syndrome genes *KCNQ1*, *KCNH2*, *SCN5A* and *RYR2*, with two studies utilising exome sequencing and wider targeted panels. These studies suggested at least a doubling in yield of possible pathogenic rare variants. Bagnall et al identified 3/28 (11%) cases with 3 rare variants in the major LQTS genes and 21% (6/28) with six rare variants in 22 other common ion channel genes as well as cardiomyopathy genes despite the absence of structural disease at autopsy. Two thirds of these variants were novel and VUSs. Nunn et al identified a yield of 29% by analysing 135 genes: seven (12%) cases carrying rare or novel possible pathogenic variants and ten (12%) carrying previously published disease causing mutations (45). Both studies illustrate that the increased yield from including greater numbers of genes is accompanied by a greater uncertainty over true pathogenicity of variants, especially as our understanding of the less common channelopathy and cardiomyopathy genes is less complete than the major channelopathy genes.

[FIGURE 2]

Figure 2: Diagnostic yield of molecular autopsy (17,18,43,45–50). The horizontal lines indicate the means.

An inherited cardiac condition may be identified in up to half of families on clinical screening of first-degree relatives following a SADS death (51–53). Similar to IVF, re-evaluation is necessary for family members who subsequently become symptomatic, or if a family history of sudden death emerges (9). If a clinical diagnosis of an inherited cardiac condition is made on evaluation of a family member, they should be managed according to the diagnosis and family cascade clinical and/or phenotype-directed genetic screening should be offered. Less penetrant disease that fails to cause symptoms can still cause sudden death. Genetic disease may therefore be present in some undiagnosed families whose members remain potentially at risk. At present identifying such genetic risk is challenging unless there is a clear pathogenic mutation in the family. Thankfully, however, first-

degree relatives in whom initial clinical and genetic evaluation does not lead to a diagnosis, rarely manifest inherited cardiac disease or cardiac events during follow-up (54).

In light of these data the guidelines recommend two different diagnostic approaches be undertaken concurrently in a SADS family: 1) familial clinical evaluation followed by genetic testing targeted to phenotype, and 2) post-mortem genetic testing (molecular autopsy) in the proband. The aim is to identify clinical or subclinical inherited cardiac disease in the family in a timely manner to prevent adverse cardiac events in surviving family members (17). It is recommended that a tissue sample (whole blood, blood spot card, frozen sample of the heart, liver or spleen) is collected from all SADS cases for DNA extraction and subsequent genetic testing. Comprehensive testing for ion channelopathies *can be* useful, but targeted testing (*RYR2*, *KCNQ1*, *KCNH2*, and *SCN5A*) is recommended if the circumstances of the event suggest a clinical diagnosis of LQTS or CPVT (eg. drowning, physical exertion, emotional stress, or an acoustic trigger) (19). When a true pathogenic mutation is identified on molecular autopsy, the next step is cascade genetic screening in the parents and other family members. If the mutation is absent in both parents, it is likely to have been sporadic in the proband, in which case the family can be reassured from a genetic perspective. There is a small chance of germ-line mosaicism, meaning that there may still be a risk of transmission of a mutated allele to another child. It is therefore reasonable to offer testing to other children as a precaution. If a phenotype is identified on clinical evaluation of the first-degree relatives of the proband, then phenotype-directed genetic screening is recommended.

Other aspects of genetic risk in SCD

Attempts have been made to identify genetic markers of SCD risk in the general population. For example, the common polymorphism *SCN5A* S1103Y, present in up to 13% of African Americans, was

associated with arrhythmia (syncope, aborted sudden death, medication- or bradycardia-associated QTc prolongation or documented ventricular tachyarrhythmias) with an odds ratio of 8.7 (95% CI 3.2-23.9) when compared to the general population (55). A subsequent series of sudden deaths with morphologically normal hearts of African American ethnicity were compared to African American non-cardiac deaths. The age and sex-adjusted relative risk of *SCN5A* S1103Y for sudden death was 8.4 (95% CI 2.1-28.6) (56). Amongst 173 consecutive SADS cases referred for molecular autopsy, Tester *et al* identified a common nonsynonymous polymorphism with known functional effect in either *KCNH2*, *KCNE1*, *SCN5A* or *RYR2* in 28/128 molecular autopsies where no other putative pathogenic mutation was found (48). Whilst larger studies able to compare case and control frequencies are needed, these data support the role of common variants in the risk of SCD in SADS. There is evidence emerging of oligogenic risk in BrS. Given the lack of success in identifying monogenic causes of IVF, it is also possible that oligogenic risk may underlie IVF.

Conclusions and Future Perspectives

The role of genetic testing should always be to complement, rather than replace or supersede, clinical evaluation. It is best suited to supporting diagnosis in the setting of a clear phenotype. Genetic yields are never 100%, so a negative test does not exclude disease. The exception is cascade genetic screening where a clear phenotype and pathogenic mutation has been identified in a family, in which case predictive testing is possible. In the absence of an overt disease phenotype as in SADS and IVF, the ability to use this approach is limited and so are yields of pathogenic, clinically actionable variants. Larger NGS panels increase the background genetic noise and reduce the signal-to-noise ratio with many rare variants often being missense and/or private to a family and pathogenicity unknown: the VUS. In SADS, there is a greater probability of finding an underlying genetic condition compared to IVF, albeit at a lower frequency than in phenotype guided testing.

Large gene panels can be used to complement clinical screening. In IVF the lower prevalence of monogenic disease means that the signal-to-noise ratio is less favourable and, pending any new research, testing of large gene panels is not currently recommended. Incorrectly calling a variant as pathogenic can have major clinical, psychological and financial consequences for the affected family members. Where a VUS is found, pathogenicity should be clarified through segregation analysis in the family after appropriate counselling (the variant could be 'private' within the family), along with regular review of the scientific literature in accordance with ACMG guidelines.

High throughput functional studies may help in the future, including the use of patient-specific human induced pluripotent stem cell derived cardiomyocyte (hiPSC-CM) models. Another avenue of investigation is whole exome or genome sequencing in the proband and his/her parents (a trio) if they appear unaffected. If an unshared and therefore a *de novo* rare genetic variant in a known or novel gene is identified in the child, this could represent a likely causative variant if the gene involved is a suitable candidate. This approach can also be used to evaluate recessive inheritance, disease, although this may be more difficult to prove. Given that there is some evidence supporting a role for common genetic variation and oligogenic risk in SADS and IVF, further work in large collections of cases will be needed to explore this paradigm.

References

1. Bowker TJ, Wood DA, Davies MJ, Sheppard MN, Cary NRB, Burton JDK, et al. Sudden, unexpected cardiac or unexplained death in England: a national survey. *QJM*. 2003 Apr;96(4):269–79.
2. Doolan A, Langlois N, Semsarian C. Causes of sudden cardiac death in young Australians. *Med J Aust*. 2004 Feb 2;180(3):110–2.
3. Puranik R, Chow CK, Duflou JA, Kilborn MJ, McGuire MA. Sudden death in the young. *Heart Rhythm*. 2005 Dec;2(12):1277–82.
4. de Noronha S V, Behr ER, Papadakis M, Ohta-Ogo K, Banya W, Wells J, et al. The importance of specialist cardiac histopathological examination in the investigation of young sudden cardiac deaths. *Europace*. 2014 Jun;16(6):899–907.
5. Morentin B, Suárez-Mier MP, Aguilera B. Sudden unexplained death among persons 1-35 years old. *Forensic Sci Int*. 2003 Aug 27;135(3):213–7.
6. Winkel BG, Holst AG, Theilade J, Kristensen IB, Thomsen JL, Ottesen GL, et al. Nationwide study of sudden cardiac death in persons aged 1-35 years. *Eur Heart J*. 2011 Apr;32(8):983–90.
7. Margey R, Browne L, Murphy E, O'Reilly M, Mahon N, Blake G, et al. The Dublin cardiac arrest registry: temporal improvement in survival from out-of-hospital cardiac arrest reflects improved pre-hospital emergency care. *Europace*. 2011 Aug;13(8):1157–65.
8. Corrado D, Basso C, Thiene G. Sudden cardiac death in young people with apparently normal heart. *Cardiovasc Res*. 2001 May;50(2):399–408.
9. Priori SG, Wilde AA, Horie M, Cho Y, Behr ER, Berul C, et al. HRS/EHRA/APHR expert

- consensus statement on the diagnosis and management of patients with inherited primary arrhythmia syndromes: document endorsed by HRS, EHRA, and APHRS in May 2013 and by ACCF, AHA, PACES, and AEPC in June 2013. *Heart Rhythm*. 2013 Dec;10(12):1932–63.
10. Behr ER, Casey A, Sheppard M, Wright M, Bowker TJ, Davies MJ, et al. Sudden arrhythmic death syndrome: a national survey of sudden unexplained cardiac death. *Heart*. 2007 May;93(5):601–5.
 11. Krahn AD, Healey JS, Chauhan V, Birnie DH, Simpson CS, Champagne J, et al. Systematic assessment of patients with unexplained cardiac arrest: Cardiac Arrest Survivors With Preserved Ejection Fraction Registry (CASPER). *Circulation*. 2009 Jul 28;120(4):278–85.
 12. Priori SG, Napolitano C, Grillo M. Concealed arrhythmogenic syndromes: the hidden substrate of idiopathic ventricular fibrillation? *Cardiovasc Res*. 2001 May;50(2):218–23.
 13. Conte G, Caputo ML, Regoli F, Marcon S, Klersy C, Adjibodou B, et al. True idiopathic ventricular fibrillation in out-of-hospital cardiac arrest survivors in the Swiss Canton Ticino: prevalence, clinical features, and long-term follow-up. *Europace*. 2016 Feb 17;
 14. Priori SG, Borggrefe M, Camm AJ, Hauer RN, Klein H, Kuck KH, et al. Unexplained cardiac arrest. The need for a prospective registry. *Eur Heart J*. 1992 Nov;13(11):1445–6.
 15. Survivors of out-of-hospital cardiac arrest with apparently normal heart. Need for definition and standardized clinical evaluation. Consensus Statement of the Joint Steering Committees of the Unexplained Cardiac Arrest Registry of Europe and of the Idiopa. *Circulation*. 1997 Jan 7;95(1):265–72.
 16. Priori SG, Blomström-Lundqvist C, Mazzanti A, Blom N, Borggrefe M, Camm J, et al. 2015 ESC Guidelines for the management of patients with ventricular arrhythmias and the prevention of sudden cardiac death: The Task Force for the Management of Patients with Ventricular Arrhythmias and the Prevention of Sudden Cardiac Death of the Europe. *Eur Heart J*. 2015 Nov 1;36(41):2793–867.
 17. Miles CJ, Behr ER. The role of genetic testing in unexplained sudden death. *Transl Res*. 2016

- Feb;168:59–73.
18. Bagnall RD, Das K J, Dufflou J, Semsarian C. Exome analysis-based molecular autopsy in cases of sudden unexplained death in the young. *Heart Rhythm*. 2014 Apr;11(4):655–62.
 19. Ackerman MJ, Priori SG, Willems S, Berul C, Brugada R, Calkins H, et al. HRS/EHRA expert consensus statement on the state of genetic testing for the channelopathies and cardiomyopathies this document was developed as a partnership between the Heart Rhythm Society (HRS) and the European Heart Rhythm Association (EHRA). *Heart Rhythm*. 2011 Aug;8(8):1308–39.
 20. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*. 2015 May;17(5):405–24.
 21. Refsgaard L, Holst AG, Sadjadieh G, Haunsø S, Nielsen JB, Olesen MS. High prevalence of genetic variants previously associated with LQT syndrome in new exome data. *Eur J Hum Genet*. 2012 Aug;20(8):905–8.
 22. Risgaard B, Jabbari R, Refsgaard L, Holst AG, Haunsø S, Sadjadieh A, et al. High prevalence of genetic variants previously associated with Brugada syndrome in new exome data. *Clin Genet*. 2013 Nov;84(5):489–95.
 23. Jabbari J, Jabbari R, Nielsen MW, Holst AG, Nielsen JB, Haunsø S, et al. New exome data question the pathogenicity of genetic variants previously associated with catecholaminergic polymorphic ventricular tachycardia. *Circ Cardiovasc Genet*. 2013 Oct;6(5):481–9.
 24. Kapa S, Tester DJ, Salisbury BA, Harris-Kerr C, Pungliya MS, Alders M, et al. Genetic testing for long-QT syndrome: distinguishing pathogenic mutations from benign variants. *Circulation*. 2009 Nov 3;120(18):1752–60.
 25. MacRae CA. Closer look at genetic testing in long-QT syndrome: will DNA diagnostics ever be enough? *Circulation*. 2009 Nov 3;120(18):1745–8.

26. Ackerman MJ, Splawski I, Makielski JC, Tester DJ, Will ML, Timothy KW, et al. Spectrum and prevalence of cardiac sodium channel variants among black, white, Asian, and Hispanic individuals: implications for arrhythmogenic susceptibility and Brugada/long QT syndrome genetic testing. *Heart Rhythm*. 2004 Nov;1(5):600–7.
27. Clinical Genome Resource. ClinGen and ClinVar Partnership [Internet]. [cited 2016 Jun 5]. Available from: <https://www.clinicalgenome.org/data-sharing/clinvar/>
28. Exome Aggregation Consortium. ExAC Browser (Beta) [Internet]. [cited 2016 Jun 18]. Available from: <http://exac.broadinstitute.org/>
29. Chen Q, Kirsch GE, Zhang D, Brugada R, Brugada J, Brugada P, et al. Genetic basis and molecular mechanism for idiopathic ventricular fibrillation. *Nature*. 1998 Mar 19;392(6673):293–6.
30. Giudicessi JR, Ye D, Kritzberger CJ, Nesterenko V V, Tester DJ, Antzelevitch C, et al. Novel mutations in the KCND3-encoded Kv4.3 K⁺ channel associated with autopsy-negative sudden unexplained death. *Hum Mutat*. 2012 Jun;33(6):989–97.
31. Haïssaguerre M, Chatel S, Sacher F, Weerasooriya R, Probst V, Lousouarn G, et al. Ventricular fibrillation with prominent early repolarization associated with a rare variant of KCNJ8/KATP channel. *J Cardiovasc Electrophysiol*. 2009 Jan;20(1):93–8.
32. Medeiros-Domingo A, Tan B-H, Crotti L, Tester DJ, Eckhardt L, Cuoretti A, et al. Gain-of-function mutation S422L in the KCNJ8-encoded cardiac K(ATP) channel Kir6.1 as a pathogenic substrate for J-wave syndromes. *Heart Rhythm*. 2010 Oct;7(10):1466–71.
33. Valdivia CR, Medeiros-Domingo A, Ye B, Shen W-K, Algiers TJ, Ackerman MJ, et al. Loss-of-function mutation of the SCN3B-encoded sodium channel β 3 subunit associated with a case of idiopathic ventricular fibrillation. *Cardiovasc Res*. 2010 Jun 1;86(3):392–400.
34. Watanabe H, Nogami A, Ohkubo K, Kawata H, Hayashi Y, Ishikawa T, et al. Electrocardiographic characteristics and SCN5A mutations in idiopathic ventricular fibrillation associated with early repolarization. *Circ Arrhythm Electrophysiol*. 2011 Dec;4(6):874–81.

35. Akai J, Makita N, Sakurada H, Shirai N, Ueda K, Kitabatake A, et al. A novel SCN5A mutation associated with idiopathic ventricular fibrillation without typical ECG findings of Brugada syndrome. *FEBS Lett.* 2000 Aug 11;479(1-2):29–34.
36. Postema PG. The quest for the identification of genetic variants in unexplained cardiac arrest and idiopathic ventricular fibrillation. *PLoS Genet.* 2013 Apr;9(4):e1003480.
37. Casado-Arroyo R, Rodriguez-Mañero M, Sarkozy A, Brugada P. Letter by Casado-Arroyo et al regarding article, “Electrocardiographic characteristics and SCN5A mutations in idiopathic ventricular fibrillation associated with early repolarization”. *Circ Arrhythm Electrophysiol.* 2012 Apr;5(2):e59; author reply e60–1.
38. Veeramah KR, Karafet TM, Wolf D, Samson RA, Hammer MF. The KCNJ8-S422L variant previously associated with J-wave syndromes is found at an increased frequency in Ashkenazi Jews. *Eur J Hum Genet.* 2014 Jan;22(1):94–8.
39. Alders M, Koopmann TT, Christiaans I, Postema PG, Beekman L, Tanck MWT, et al. Haplotype-Sharing Analysis Implicates Chromosome 7q36 Harboring DPP6 in Familial Idiopathic Ventricular Fibrillation. *Am J Hum Genet.* American Society of Human Genetics; 2009;84(4):468–76.
40. Xiao L, Koopmann TT, Ördög B, Postema PG, Verkerk AO, Iyer V, et al. Unique cardiac Purkinje fiber transient outward current β -subunit composition: a potential molecular link to idiopathic ventricular fibrillation. *Circ Res.* 2013 May 10;112(10):1310–22.
41. Bai R, Napolitano C, Bloise R, Monteforte N, Priori SG. Yield of genetic screening in inherited cardiac channelopathies: how to prioritize access to genetic testing. *Circ Arrhythm Electrophysiol.* 2009 Feb;2(1):6–15.
42. Tester DJ, Ackerman MJ. The role of molecular autopsy in unexplained sudden cardiac death. *Curr Opin Cardiol.* 2006 May;21(3):166–72.
43. Winkel BG, Larsen MK, Berge KE, Leren TP, Nissen PH, Olesen MS, et al. The prevalence of mutations in KCNQ1, KCNH2, and SCN5A in an unselected national cohort of young sudden

- unexplained death cases. *J Cardiovasc Electrophysiol*. 2012 Oct;23(10):1092–8.
44. Mazzanti A, Priori SG. Molecular autopsy for sudden unexplained death? Time to discuss pros and cons. *J Cardiovasc Electrophysiol*. 2012 Oct;23(10):1099–102.
 45. Nunn LM, Lopes LR, Syrris P, Murphy C, Plagnol V, Firman E, et al. Diagnostic yield of molecular autopsy in patients with sudden arrhythmic death syndrome using targeted exome sequencing. *Europace*. 2015 Oct 25;
 46. Larsen MK, Berge KE, Leren TP, Nissen PH, Hansen J, Kristensen IB, et al. Postmortem genetic testing of the ryanodine receptor 2 (RYR2) gene in a cohort of sudden unexplained death cases. *Int J Legal Med*. 2013 Jan;127(1):139–44.
 47. Tester DJ, Ackerman MJ. Postmortem long QT syndrome genetic testing for sudden unexplained death in the young. *J Am Coll Cardiol*. 2007 Jan;49(2):240–6.
 48. Tester DJ, Medeiros-Domingo A, Will ML, Haglund CM, Ackerman MJ. Cardiac channel molecular autopsy: insights from 173 consecutive cases of autopsy-negative sudden unexplained death referred for postmortem genetic testing. *Mayo Clin Proc*. 2012 Jun;87(6):524–39.
 49. 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 Nov;79(11):1380–4.
 50. Skinner JR, Crawford J, Smith W, Aitken A, Heaven D, Evans C-A, et al. Prospective, population-based long QT molecular autopsy study of postmortem negative sudden death in 1 to 40 year olds. *Heart Rhythm*. 2011 Mar;8(3):412–9.
 51. Behr E, Wood DA, Wright M, Syrris P, Sheppard MN, Casey A, et al. Cardiological assessment of first-degree relatives in sudden arrhythmic death syndrome. *Lancet (London, England)*. 2003 Nov 1;362(9394):1457–9.
 52. Tan HL, Hofman N, van Langen IM, van der Wal AC, Wilde AAM. Sudden unexplained death: heritability and diagnostic yield of cardiological and genetic examination in surviving relatives.

- Circulation. 2005 Jul 12;112(2):207–13.
53. Behr ER, Dalageorgou C, Christiansen M, Syrris P, Hughes S, Tome Esteban MT, et al. Sudden arrhythmic death syndrome: familial evaluation identifies inheritable heart disease in the majority of families. *Eur Heart J*. 2008 Jul;29(13):1670–80.
 54. van der Werf C, Stiekema L, Tan HL, Hofman N, Alders M, van der Wal AC, et al. Low rate of cardiac events in first-degree relatives of diagnosis-negative young sudden unexplained death syndrome victims during follow-up. *Heart Rhythm*. 2014 Oct;11(10):1728–32.
 55. Splawski I, Timothy KW, Tateyama M, Clancy CE, Malhotra A, Beggs AH, et al. Variant of SCN5A sodium channel implicated in risk of cardiac arrhythmia. *Science*. 2002 Aug 23;297(5585):1333–6.
 56. Burke A, Creighton W, Mont E, Li L, Hogan S, Kutys R, et al. Role of SCN5A Y1102 polymorphism in sudden cardiac death in blacks. *Circulation*. 2005 Aug 9;112(6):798–802.

Figure HRS/EHRA Genetic Testing Guidelines and HRS/EHRA/APHSR Guidelines for Management of Arrhythmia Syndromes

LQTS

Comprehensive or targeted LQT1-3 testing -

Class I:

- Patients with a strong clinical suspicion of LQTS by means of clinical history, family history, ECG and stress test(s);
- Asymptomatic QTc prolongation (>500ms in adults) in the absence of other conditions that may prolong QTc;
- Family mutation-specific testing after identification in an index case

Class IIb:

- Asymptomatic QTc prolongation (>480ms in adults)

BrS

Class I:

- Family mutation-specific testing after identification of a BrS-causative mutation in an index case

Class IIa:

- Comprehensive or targeted testing in patients with a strong clinical suspicion of BrS by means of clinical history, family history, ECG and stress test(s)

Class III:

- Not indicated in the setting of an isolated type 2 or type 3 BrS ECG

CPVT

Class I:

- Comprehensive or targeted testing in patients with a strong clinical suspicion of CPVT by means of clinical history, family history, ECG and stress test(s)
- Family mutation-specific testing after identification of the CPVT-causative mutation in an index case

IVF

Class IIa:

- Genetic testing *can be useful* when there is a suspicion of a specific genetic disease following clinical evaluation of the IVF patient and/or family members.

Class III:

- Genetic screening of a large panel of genes in IVF patients in whom there is no suspicion of an inherited arrhythmogenic disease after clinical evaluation *should not be performed*.

Molecular Autopsy

Class I

- Collection of a tissue sample is *recommended* in all SADS cases
- Mutation-specific genetic testing is *recommended* for family members following identification of a pathogenic mutation in the decedent (proband)
- Targeted post-mortem genetic testing is *recommended* if circumstantial evidence suggests LQTS or CPVT

Class IIa:

Comprehensive post-mortem genetic testing of an arrhythmia syndrome panel *can be useful*.

Figure

