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

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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 (Iₒ) 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


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


### 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**.

### LQTS

**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

**Class IIa**
- 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.

**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**.
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