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Sudden death and cardiac arrest without phenotype: the utility of genetic testing

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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 optimization 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.

Key words: Sudden death, sudden arrhythmic death syndrome, idiopathic ventricular fibrillation, genetics.

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 folds 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

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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 characterized 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 commercialized 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 germ-line 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* modeling 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 utilizing small controls have labeled 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 minimize 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.

Fig. 1 summarizes 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.

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