

Genetic testing in Idiopathic Ventricular Fibrillation: Searching for a needle in a haystack?

Greg Mellor MB BChir¹, Elijah R Behr MD²

¹Dept. of Cardiology, Papworth Hospital NHS Foundation Trust, Cambridge, UK

²Cardiovascular Sciences Dept., St. George's, University of London, London, UK

Dr. Behr was supported by the Higher Education Funding Council for England

Correspondence:

Dr. Elijah R Behr

Cardiovascular Sciences Dept.

St. George's, University of London

Cranmer Terrace,

London, UK

SW17 0RE

ebehr@sgul.ac.uk

T: +442087252994, F: +442087253416

Word Count (inc. title page): 1384

Sir Thomas More, the sixteenth century philosopher and author who was beheaded (and later canonised) for his opposition to Henry VIII's reformation, first wrote that: "to seek out one line in his books would be to go look (for) a needle in a meadow." Almost 500 years later, attempts to identify a single pathogenic variant in more than 20,000 genes that make up the human genome may be considered a similarly thankless task. In usual practice, a well-established clinical phenotype will dramatically reduce the size of the 'meadow' or 'haystack' and improve the chances of identifying the 'needle'. However, idiopathic ventricular fibrillation (IVF) presents a unique challenge: a life-threatening condition without clinical signs other than the sudden and unpredictable onset of ventricular fibrillation.

IVF is diagnosed: "in a resuscitated cardiac arrest victim[...]in whom known cardiac respiratory, metabolic and toxicological etiologies have been excluded through clinical evaluation"¹. Advances in the diagnosis of inherited arrhythmia syndromes have demonstrated that phenotypes may be subtle and highly variable. Detailed clinical evaluation in patients previously labeled as IVF may reveal diagnoses including the long QT and Brugada syndromes, arrhythmogenic right ventricular cardiomyopathy and catecholaminergic polymorphic ventricular tachycardia. Furthermore, survivors of an initially unexplained cardiac arrest may develop a detectable phenotype years after the index event² and some victims of sudden unexpected death with a normal post-mortem examination (i.e. Sudden Arrhythmic Death Syndrome, SADS), which may be considered analogous to IVF, may have pathogenic variants in cardiomyopathy-associated genes identified by molecular autopsy³. Therefore

electrical instability and the risk of life-threatening arrhythmias may precede clinically or pathologically identifiable phenotypes and a proportion of IVF is attributable to primary arrhythmia syndromes or cardiomyopathies without an expressed phenotype. In other IVF cases, monogenic causes have been identified. These include: the well established Dutch founder haplotype in the arrhythmia gene *DPP6*⁴; pathogenic variants in calmodulin⁵ and the transcription factor *IRX3*⁶; and a loss of function variant in *RYR2*⁷.

Genetic testing may therefore diagnose a concealed arrhythmia syndrome or the genetic etiology of IVF. However, genetic testing in such cases is not currently recommended with high costs and frequent identification of variants of uncertain significance (VUS) cited as reasons in the 2011 HRS/EHRA expert consensus document⁸. As sequencing technology becomes increasingly rapid, affordable and widely available, reappraisal of this recommendation may be required.

In this issue of Heart Rhythm, Visser et. al.⁹ describe the findings of an extended next generation sequencing panel of 179 genes in 33 IVF survivors and report a yield of only one pathogenic or likely pathogenic variant - a truncating variant in the titin (*TTN*) gene seen in a single patient.

The authors are to be congratulated on their efforts to thoroughly evaluate their cohort. They completed and supplemented the original historical assessments in order to exclude alternative causes of VF. However, despite their attempts, clinical evaluation was not exhaustive. Although all patients had a 12-lead ECG, coronary imaging, echocardiography, exercise and ambulatory ECG, cardiac MRI was only performed in 45% and sodium channel blocker provocation in 58%. No

comment was made of the use of high right ventricular lead ECGs either at rest or during provocation testing.

Whilst at first glance their findings portray a negative message regarding the utility of genetic testing in IVF, it must be noted that these 33 patients were a sub-selection of a larger cohort of apparent IVF survivors in whom previous genetic testing had already proven negative. This had consisted of either phenotype targeted testing or a 33-gene panel of predominantly arrhythmia syndrome associated genes¹⁰. The yield of the first stage of genetic testing is reported as 15%¹⁰, with the 33-gene panel in particular identifying pathogenic or likely pathogenic variants in: *KCNQ1* (associated with LQTS type 1); *MYL2* (associated with hypertrophic cardiomyopathy (HCM)); and *DPP6* in 3 individuals.

Of the 179 genes included in second-line testing only a small number have been reliably associated with either IVF or with established phenotypes that carry a risk of ventricular fibrillation; variants in sarcomeric genes *MYBPC3*, *MYH7*, *TNNI3* and *TNNT2* are recognized causes of HCM, while truncating variants in *TTN* are seen in cases of dilated cardiomyopathy and peri-partum cardiomyopathy. For the remaining genes, evidence that variants are associated with 'sudden death' phenotypes is limited. It is therefore unsurprising that inclusion of these genes in large panels such as this offers little added yield. Furthermore, as the authors state, the 'genetic noise' inevitably increases as more genes are tested. One or more VUS was identified in 24% of patients with the initial 33-gene panel, which increased to 34% overall with the extended panel.

Genetic testing in IVF therefore offers a modest but potentially significant diagnostic yield, identifying either rare monogenic causes of IVF or concealed arrhythmia syndromes where clinical evaluation has failed. It should therefore be considered in conjunction with thorough and systematic clinical evaluation of probands, where it may guide treatment, and relatives, where it may aid the identification of other at-risk individuals. While testing should be comprehensive, in that genes associated with both arrhythmia syndromes and cardiomyopathies may be included, the temptation to increase yield through ever expanding panels should be avoided and only those genes with robust association with disease should be included. Due to the relatively high rate of VUSs, testing should be limited to experienced centres with the ability and infrastructure to assess variants and robustly follow-up those individuals until the significance of a variant can be determined.

Future research should also be focused upon identifying potential polygenic and non-genetic causes that likely underpin much of IVF. These may have wider implications for the understanding of the mechanisms of ventricular fibrillation in other more common scenarios such as ischaemic heart disease. Rather than finding the one needle, we need to understand the interaction between the blades of grass in the stack.

Bibliography

1. Priori SG, Wilde A a, Horie M, et al.: Executive summary: HRS/EHRA/APHRs expert consensus statement on the diagnosis and management of patients with inherited primary arrhythmia syndromes. *Europace* 2013; 15:1389–1406.

2. Herman ARM, Cheung C, Gerull B, et al.: Outcome of Apparently Unexplained Cardiac Arrest: Results From Investigation and Follow-Up of the Prospective Cardiac Arrest Survivors With Preserved Ejection Fraction Registry. *Circ Arrhythm Electrophysiol* 2016; 9:e003619.
3. Bagnall R, Weintraub R, Ingles J, et al.: A Prospective Study of Sudden Cardiac Death among Children and Young Adults. *N Engl J Med* 2016; 374:2441–2452.
4. Alders M, Koopmann TT, Christiaans I, et al.: Haplotype-Sharing Analysis Implicates Chromosome 7q36 Harboring DPP6 in Familial Idiopathic Ventricular Fibrillation. *Am J Hum Genet The American Society of Human Genetics*, 2009; 84:468–476.
5. Marsman RF, Barc J, Beekman L, et al.: A mutation in CALM1 encoding calmodulin in familial idiopathic ventricular fibrillation in childhood and adolescence. *J Am Coll Cardiol* 2014; 63:259–266.
6. Koizumi A, Sasano T, Kimura W, et al.: Genetic defects in a His-Purkinje system transcription factor, IRX3, cause lethal cardiac arrhythmias. *Eur Heart J* 2016; 37:1469–1475.
7. Roston TM, Sanatani S, Wayne Chen SR: Suppression-of-function mutations in cardiac ryanodine receptor RyR2: emerging evidence for a novel arrhythmia syndrome? *Heart Rhythm*, 2017; 14:108-109.
8. Ackerman MJ, Priori SG, Willems S, 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). *Europace* 2011; 13:1077–1109.

9. Visser M, Dooijes D, van der Smagt JJ, Van Der Heijden JF, Doevendans PA, Loh P, Asselbergs FW, Hassink RJ: Next Generation Sequencing of a large panel in patients initially diagnosed with idiopathic ventricular fibrillation. *Heart Rhythm* 2017; .
10. Visser M, Van Der Heijden JF, Van Der Smagt JJ, Doevendans PA, Wilde AA, Loh P, Hassink RJ: Long-Term Outcome of Patients Initially Diagnosed with Idiopathic Ventricular Fibrillation. *Circ Arrhythmia Electrophysiol* 2016; 9:e004258.