American Heart Association.

Circulation: Arrhythmia and Electrophysiology

Manuscript Submission and Peer Review System

URL: https://circep-submit.aha-journals.org

Title: Familial Evaluation in Idiopathic Ventricular Fibrillation: Diagnostic Yield and Significance of J-Wave Syndromes

Manuscript number: CIRCAE/2020/009089-T2

Author(s): Greg Mellor, Royal Papworth Hospital NHS Foundation Trust, Cambridge, UK
Lennart Blom, University Medical Centre Utrecht
Sanne Groeneveld, University Medical Center Utrecht
Bo Winkel, Copenhagen University Hospital, Rigshospitalet
Bode Ensam, St George's University of London
Johannes Bargehr, University of Cambridge
Bianca van Rees, Maastricht University Medical Centre
Chiara Scrocco, St. George's, University of London, St George's University Hospitals NHS Foundation Trust

Ingrid Krapels, Maastricht University Medical Center

Paul Volders, Maastricht University Medical Centre

Jacob Tfelt-Hansen, The Heart Centre, Copenhagen University Hospital, Rigshospitalet, and Department of Medicine and Surgery, University of Copenhagen, Copenhagen

Andrew Krahn, University of British Columbia

Rutger Hassink, University Medical Center Utrecht

Elijah Behr, St George's University of London

Familial Evaluation in Idiopathic Ventricular Fibrillation: Diagnostic Yield and Significance of J-Wave Syndromes

Mellor & Blom; IVF Family Screening

Greg J Mellor MD[1]*, Lennart J Blom MD[3]*, Sanne A Groeneveld MD[3], Bo G Winkel PhD[4], Bode Ensam MBBS[2], Johannes Bargehr PhD[1,5], Bianca van Rees MD[6], Chiara Scrocco MD[2], Ingrid PC Krapels MD PhD[7], Paul GA Volders[6] MD PhD, Jacob Tfelt-Hansen DMSc[4], Andrew D Krahn MD[8], Rutger J Hassink[3] MD PhD, Elijah R Behr MD[2]

1 Cardiology Department, Royal Papworth Hospital, Cambridge, UK

2 Cardiovascular Clinical Academic Group, Molecular and Clinical Sciences Research Institute, St. George's, University of London and St. George's University Hospitals NHS Foundation Trust, London UK

3 Department of Cardiology, University Medical Centre, Utrecht, The Netherlands

4 Department of Cardiology, Rigshospitalet, Copenhagen, Denmark

5 Division of Cardiovascular Medicine, University of Cambridge, Cambridge, UK

6 Department of Cardiology, Cardiovascular Research Institute Maastricht (CARIM), Maastricht University Medical Center, Maastricht, The Netherlands

7 Department of Clinical Genetics, Maastricht University Medical Center, Maastricht, The Netherlands

8 Division of Cardiology, University of British Columbia, Vancouver, BC, Canada

* These authors contributed equally to this work

Word Counts:

Abstract	298
Manuscript (inc. tables/figure legends)	4200
Bibliography	1775

Journal Subject Terms:

Sudden Cardiac Death / Genetics / Electrocardiology (ECG) / Clinical Studies

Corresponding Authors:

1) Prof. Elijah Behr

Cardiology Clinical Academic Group

St. George's, University of London

Cranmer Terrace

London

SW17 0RE

ebehr@sgul.ac.uk

+44208 7252994

2) Dr. Greg Mellor

Cardiology Dept.

Royal Papworth Hospital

Papworth Road

Cambridge

CB2 0QT

gregmellor@nhs.net

+441223 639791

Abstract

Background

Familial cascade screening is well established in patients with heritable cardiac disease and in cases of Sudden Arrhythmic Death Syndrome (SADS). The clinical benefit of family screening in idiopathic ventricular fibrillation (IVF) is unknown.

Methods

Patients with IVF were identified from national and institutional registries. All underwent systematic and comprehensive clinical evaluation to exclude identifiable causes of cardiac arrest with a minimum requirement of ECG, cardiac (echocardiogram and/or MRI) and coronary imaging, exercise ECG and sodium channel blocker (SCB) provocation. Additional investigations including genetic testing were performed at the physician's discretion. First-degree relatives who were assessed with at least a 12-lead ECG were included in the final cohort. Results of additional investigations, performed at the physician's discretion, were also recorded. Results were coded as normal, abnormal or minor findings.

Results

We identified 201 first-degree relatives of 96 IVF patients. In addition to a 12 lead ECG, echocardiography was performed in 159 (79%) and ≥ 1 additional investigation in 162 (80%) relatives. An inherited arrhythmia syndrome was diagnosed in 5 (3%) individuals from 4 (4%) families. Two relatives hosted the DPP6 risk haplotype identified in a single proband, one of whom received a primary prevention ICD. In three separate families an asymptomatic parent of the IVF proband developed a type 1 Brugada ECG pattern during SCB provocation. All were managed with lifestyle measures only. The Early Repolarisation ECG pattern (ER) was present in

16% probands and was more common in relatives in those families than those where the proband did not have ER (25% vs. 8%, p=0.04).

Conclusions

The yield of family screening in relatives of IVF probands is low when the proband is comprehensively investigated. The significance of J wave syndromes in relatives and the role for systematic SCB provocation are, however, uncertain and require further research.

Keywords: Idiopathic VF; family screening; sudden cardiac death; J waves; Brugada syndrome; early repolarisation

Non-standard Abbreviations and Acronyms

BrS	Brugada Syndrome
ER	Early Repolarisation
ERS	Early Repolarisation Syndrome
IVF	Idiopathic Ventricular Fibrillation
PVC	Premature Ventricular Contraction
SADS	Sudden Arrhythmic Death Syndrome
SCB	Sodium Channel Blocker
UCA	Unexplained Cardiac Arrest
VUS	Variant of Uncertain Significance

Introduction

Cardiac arrest due to ventricular fibrillation is a common presentation and important cause of death[1]. The majority of cases are due to ischaemic heart disease. Alternatively, manifest cardiomyopathy or ion channel disease may be evident. In a significant minority, initial investigation with ECG, transthoracic echocardiogram and coronary imaging will be non-diagnostic[2]. Systematic clinical investigation of such 'unexplained cardiac arrest' (UCA)[3] may reveal a diagnosis in approximately one third of cases[4]. Frequently, the diagnosis made will be an inherited arrhythmia syndrome or cardiomyopathy which will lead to a recommendation to perform cascade screening of immediate family members[5,6].

If no diagnosis is made, a label of idiopathic ventricular fibrillation (IVF) is used although there is currently no consensus on which investigations are required before IVF is diagnosed[7]. Therefore, those patients labelled with IVF may represent a variety of underlying pathologies including concealed forms of established arrhythmia syndromes or cardiomyopathies, short-coupled VF without documentation of the initiating premature ventricular contraction (PVC); or unidentified environmental triggers in otherwise normal hearts.

Heritability in sudden death[8] is recognised and familial evaluation in sudden unexplained death is well-established with evidence of inherited heart disease identified in around one third of families[9]. However, the benefit of family cascade screening in idiopathic VF is not known. Long-term follow-up has revealed a heritable diagnosis in a fifth of idiopathic VF patients[10] and incomplete penetrance and variable expressivity are well recognised in the inherited arrhythmia syndromes[11,12]. We hypothesized that familial screening may reveal heritable diagnoses in first-degree relatives that were not apparent in the IVF proband.

Methods

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Cohort Identification

Eligible patients were identified through a 2-step process. Initially, cardiac arrest patients meeting the criteria for IVF, as outlined below, were identified. Next, first-degree relatives of these patients who had undergone assessment with at least a 12-lead ECG were identified, with further evaluation of relatives being performed at the discretion of the treating physician. Families with an IVF proband and ≥1 relative assessed were included in the final cohort (figure 1).

Cases were identified from national and institutional registries in Canada, Denmark, the Netherlands and the United Kingdom. The breakdown of the cohort by registry is described in the Supplemental Material. Individuals were diagnosed with IVF if they had been resuscitated from cardiac arrest with documentation of VF and no diagnosis had been made after comprehensive clinical assessment comprising a minimum of: resting 12-lead ECG, cardiac imaging with echocardiogram and/or MRI, coronary artery imaging, exercise ECG and sodium channel blocker provocation (SCB). Further investigations were performed at the treating physician's discretion. Cases with positive toxicology were excluded. Genetic testing was not required for inclusion although results were recorded where performed. In addition, minor or non-diagnostic findings from clinical investigations were recorded. Presence of the early repolarization (ER) ECG pattern and documented short-coupled PVC initiating VF were considered part of the spectrum of IVF and therefore individuals with these diagnoses were included. ER pattern was defined as ≥0.1 mV J-point elevation in 2 contiguous inferior and/or

lateral leads[7,13]. The study was approved by the relevant institutional review boards and subjects gave informed consent as required. A summary of the minimum required clinical assessment is shown in figure 1.

Data Collection

Clinical characteristics of cardiac arrest patients and relatives were collected. Results of investigations performed were recorded and coded by study investigators at each site as: normal, abnormal (meeting recognised disease-specific diagnostic criteria) or minor findings (outside of recognised normal limits but not diagnostic). Investigations with positive results were noted and associations with proband's and relatives' characteristics were sought. A familial diagnosis was recorded when ≥1 first-degree relatives had investigation findings compatible with a diagnosis of a recognised inherited cardiac condition as per guidelines[5,14,15].

Statistical Methods

Continuous data are presented as median (Inter Quartile Range) and were compared using the Mann-Whitney U test. Categorical variables are presented as n (%) and were compared with Fisher's exact test. A $p<0.05$ was considered significant. Statistical analysis was performed with SPSS 24 (SPSS, Inc., Chicago, IL).

Results

We included 96 families of IVF probands where cascade screening with at least a 12-lead ECG was performed. The final cohort comprised 201 first-degree relatives of these 96 idiopathic VF probands.

Proband characteristics

The clinical characteristics of probands are summarised in table 1. The mean age was 39±12 years, 51 (53%) were male. Self-reported ethnicities were: Caucasian (91%), South East Asian (n=3), African-Caribbean (n=3), Chinese (n=1) and Middle Eastern (n=1). One individual self-reported as 'other ethnicity'. The circumstance of cardiac arrest was recorded in 81 (84%). The majority occurred at rest (45, 56%). Prior suspected arrhythmic syncope was reported in 17 (18%). A prior familial history of sudden death was reported in 12 (13%). Additional investigations performed above those required for inclusion are listed in table 1.

Minor investigation findings were common (specific findings are listed in the supplemental material). The inferolateral ER pattern was seen in 17% of probands. A type 2 Brugada ECG pattern which did not convert to a type 1 pattern with SCB provocation (including high RV leads) was seen in two probands. Other minor ECG findings including minor conduction delay, repolarisation changes and PVCs were seen in 20%. Minor imaging findings were present in 18% on echo and 16% on MRI with significant overlap of findings between modality. Minor changes in right ventricular dimension or contractility (Echo 59%, MRI 80%) were most common. Minor findings, most commonly isolated PVCs (65%), were present in 18% of exercise tests.

Relative Characteristics

The clinical characteristics of relatives are displayed in table 2. The mean age was 39±20 years and 114 (57%) were male. Sixty (30%) were parents, 69 (34%) were siblings and 72 (36%) were offspring of the proband. Prior syncope was reported in 12 (6%). A prior familial history of sudden death (other than the proband) was reported in 7 (4%).

As per the protocol, all relatives had a resting 12-lead ECG, with additional high right ventricular leads performed in 52 (26%). Further investigations included: an echocardiogram in 159 (79%), echocardiogram and cardiac MRI in 22 (11%), exercise ECG in 124 (62%), SAECG in 59 (29%), SCB provocation in 20 (10%) and adrenaline provocation in 6 (3%) relatives.

Diagnostic Yield in Relatives

Five (3%) relatives from 4 (4%) families had a positive test result in keeping with a heritable condition (See Figure 2). There were no significant differences between the clinical characteristics of probands where a familial diagnosis was made in first-degree relatives compared to those where no familial diagnosis was made, although the proband age tended to be lower (28±17 years vs 39±13 years, p=0.08). High RV lead ECG and SCB provocation were both performed more often in relatives where a heritable diagnosis was made (p=0.02, 0.01 respectively). The finding of a pathogenic/likely pathogenic variant on genetic testing was associated with a familial diagnosis (p=0.01). The families with positive findings are summarized below:

Family 1

A 54-year-old male of Dutch heritage suffered VF during sleep. Clinical assessment revealed only dyskinesia of the right ventricular lateral wall and a RV ejection fraction of 44%. There was no ER pattern. Genetic testing with a 34 gene panel identified the *DPP6* risk-haplotype. Six relatives (4 siblings and 2 children) underwent cascade genetic testing. The patient's brother and daughter were found to carry the haplotype. A primary prevention subcutaneous ICD was implanted in the daughter.

Family 2

A 21-year-old male suffered VF during sleep. He had a history of prior syncope. There was an inferior ER pattern on the resting ECG and further clinical assessment revealed no positive findings. Three relatives underwent clinical assessment. His 62-year-old father was found to have a type 2 Brugada ECG pattern at rest which converted to a type 1 pattern with ajmaline provocation. Genetic testing in both proband and father was negative. The father was asymptomatic and received lifestyle advice only. Ajmaline provocation in the patient's sister was negative and was not performed in his mother (See Figure 3).

Family 3

An 18-year-old male suffered VF during exercise. Clinical assessment revealed only basal septal hypokinesia on echocardiogram and MRI. There was no ER pattern. Five relatives underwent clinical assessment. The patient's 49-year-old father was found to have a type 2 Brugada ECG pattern which converted to a type 1 pattern with ajmaline provocation. During exercise testing changes in RV conduction approaching a type 1 Brugada ECG pattern were also noted. Genetic testing in both proband and father was negative. The father was asymptomatic and received lifestyle advice only. The ECG of the patient's 16-year-old brother showed partial RBBB. Ajmaline provocation was negative. Ajmaline provocation was not performed in the patient's mother or two other siblings with normal resting ECGs.

Family 4

A 19-year-old female suffered VF at rest. Clinical assessment revealed no positive findings. There was no ER pattern. Genetic testing with a 212 gene panel revealed a variant of uncertain significance (VUS) in *CACNA1C* (c.6637G>A, p.Asp2213Asn). Her 61-year-old mother received intravenous flecainide for treatment of atrial fibrillation. During the infusion she developed a

type 1 Brugada ECG pattern. She was found to carry the same variant in *CACNA1C*. She was asymptomatic and received lifestyle advice only.

Other Findings in Relatives

ECG abnormalities were present in 22% of relatives. The ER pattern was seen in 11 (5%) relatives from 10 families. Where the proband had ER (n=16), ≥1 relative also had ER in 4 families (25%). ER was less common in families where the proband did not have ER (6/80 8%, p=0.04). Minor findings were reported on echocardiogram (9%) and exercise ECG (11%). Two relatives were diagnosed with left ventricular hypertrophy secondary to hypertension and a single relative was found to have a significant atrial septal defect which was subsequently closed.

Genetic Testing

Genetic testing was performed in 77 (77%) probands. Testing strategies varied from single phenotype panels to broad multi-phenotype panels of up to 212 genes. A pathogenic/likely pathogenic variant was identified in 2 (2%). A male proband of Dutch ancestry was found to carry the *DPP6* risk haplotype. A truncating *PKP2* (Arg79Ter) variant was identified in a single proband who experienced VF at rest. His ECG and cardiac imaging were unremarkable both at the time of arrest and when repeated several years later, although a small number of PVCs were seen during exercise ECG at repeat assessment. Predictive testing was carried out in families of the probands with the *DPP6* risk haplotype (two relatives positive, four negative) and the pathogenic *PKP2* variant (one relative negative). In relatives where Brugada Syndrome (BrS) was diagnosed, two had negative panel testing and one was found to carry a VUS in *CACNA1C* previously identified in the familial proband.

Discussion

This study provides a unique insight into the heritability and genetic risk in idiopathic VF. We describe a large multi-centre international cohort of IVF probands fulfilling stringent and strict negative investigative criteria, designed to ensure that acquired disease and expressed genetic disorders have been excluded as fully as possible. We then assessed the yield of genetic disease following evaluation of relatives with at least an ECG performed. We identified a 3% yield of families with a drug-induced type 1 Brugada pattern in first degree relatives, a higher prevalence of the ER pattern in relatives of probands with pre-existing ER pattern and a role for targeted *DPP6* haplotype testing in Dutch families.

Stringency of IVF patient investigation

A recent study has found that 6.9% of all cardiac arrest patients were described as IVF, although there was significant variability in clinical assessment carried out before arriving at the diagnosis[2]. Indeed, whilst IVF is a diagnosis of exclusion, there remains no consensus on the minimum clinical evaluation required before a diagnosis can be made. Previous studies of IVF have contained heterogeneous cohorts of patients with investigations carried out in varying depth[16]. The inclusion criteria for this study were therefore strict in order to arrive at as homogenous a population as possible.

Diagnostic Yield of Familial Evaluation

The yield of clinical familial evaluation was low with only 5 (3%) relatives from 4 (4%) families with a heritable diagnosis. This is in contrast to previous studies of familial evaluation in autopsy-negative and toxicology-negative sudden unexplained death (also known as Sudden Arrhythmic Death Syndrome [SADS][17]) which have reported diagnoses of an inherited cardiac

condition in 18-53% of families[17–23]. Furthermore, post-mortem genetic testing (molecular autopsy) series in SADS have reported potentially disease-causing variants in genes associated with ion channel disease and cardiomyopathy in 13-30% cases[24–26].

Clinical assessment of SADS probands is very limited by default as most deaths are unheralded and therefore very few would have had ante-mortem cardiac assessment[27,28]. Comparison between sudden death and cardiac arrest survivors is not straightforward and fundamental differences between cardiac arrest survivors and those who die suddenly cannot be excluded [20,29]. However, it is probable that, if SADS cases survived to clinical evaluation, a proportion would have an identifiable heritable phenotype and that testing in these families would account for much of the expected positive diagnostic yield. The remainder would presumably therefore overlap phenotypically with the IVF cohort described here and be expected to have a lower yield from family evaluation.

Studies of familial evaluation in cardiac arrest survivors are fewer with varying yields. Kumar *et. al.*[22] reported a 62% yield of familial evaluation in UCA although relatives were only assessed if a heritable diagnosis was made in the proband. Familial evaluation in the Cardiac Arrest Survivors with Preserved Ejection Fraction Registry (CASPER) included relatives of UCA patients with and without a diagnosis after clinical evaluation. This led to a definite diagnosis in 5.4% of first-degree relatives, and a probable diagnosis in a further 11.8%[30]. A single study of idiopathic VF probands with similar inclusion criteria to our study found no familial diagnosis in 72 relatives of 33 probands[31].

The low yield in this current cohort therefore reflects that probands with identifiable phenotypes where cardiac screening would be expected to have the highest yield have been actively excluded through extensive evaluation of the VF patient. Therefore, the original hypothesis that

a significant proportion of idiopathic VF may be due to concealed forms of inherited heart disease is only weakly supported by our data. Since relatives were investigated at a single time point, we are unable to comment on whether further serial evaluation would have revealed additional diagnoses over time.

Diagnostic Yield of Genetic Testing

Genetic testing of the proband was not required for inclusion in the study and was performed at the discretion of the treating physician. One proband of Dutch heritage was identified with the *DPP6* risk haplotype[32]. Four family members were tested with 2 positive and one receiving a primary prevention ICD. A second proband had a pathogenic truncating *PKP2* variant identified through whole genome sequencing. A single family member was negative for the variant thus providing welcome reassurance.

The *DPP6* risk haplotype is currently the only well-established genetic culprit associated with familial IVF[32]. However, identified cases have been limited to a recognised founder effect in Dutch families[33]. The yield of pathogenic and likely pathogenic variants in other studies of IVF and UCA is higher than ours at 9-17%[16,34,35]. Established genes associated with both ion channel disease and cardiomyopathy are frequently implicated. However, clinical investigation in these studies was not as systematic and comprehensive in excluding these disorders as in our cohort.

The low yield of genetic testing in this and previous studies may be seen as evidence of significant non-genetic risk factors in idiopathic VF. More comprehensive genetic testing in probands may have led to an increased number of diagnoses in relatives. However, this study was not designed to be able to dissect what is likely to be a highly complex interaction between many potential non-genetic as well as genetic contributing factors.

Brugada Syndrome and Early Repolarisation

The sole familial condition identified through clinical assessment (n=3) was BrS following positive SCB provocation testing i.e. >2mm J point elevation (or J wave) in the right precordial leads with coved ST elevation and T wave inversion. Overall, SCB provocation was performed in 20 relatives from 12 families giving a positive rate of 15% and 25% at the family level. Use of SCB provocation and high lead ECGs was, however, highly selected and the absolute numbers of positive tests may have been higher if they were undertaken more systematically. Nonetheless, this yield is similar to reported yields of 14-20% in recent large studies of ajmaline use in families with a history SADS, sudden unexpected death and UCA[36,37].

The diagnosis of BrS by SCB provocation in asymptomatic patients has been a matter of debate due to low rates of arrhythmic events[38] and positive test results in patients with apparently unrelated conditions[39]. Accordingly, recently adopted diagnostic criteria for BrS classifies the combination of positive SCB provocation and history of UCA in a first-degree relative only as 'possible' BrS unless the cardiac arrest event itself was suspicious for BrS[14].

However, the complex nature of heritability of BrS must also be considered. While traditionally considered a Mendelian autosomal dominant trait, common genetic variation has also been shown to influence disease susceptibility with cumulative effects on overall disease expression[40] and the type 1 ECG pattern response to SCB[41]. It is therefore possible that some shared genetic predisposition is responsible, at least in part, for idiopathic VF in one family member and a positive SCB provocation test in another.

Another form of J wave, the inferior ER pattern is over-represented in IVF probands[42] which has led to the proposed diagnosis of Early Repolarisation Syndrome (ERS)[7] as a further J wave syndrome alongside BrS[14]. Previous reports have demonstrated heritability of the ER pattern

and it is more common in relatives of SADS cases than control groups[43,44]. ER was present in a significant number of probands in this study and was more common in their relatives than relatives of probands without ER, supporting previous findings in a similar small cohort[31]. As in BrS, recent data support that common genetic variation associates with the ER pattern[45] and there is little evidence of autosomal dominant disorders underlying ERS[14].

Our data therefore suggest a genetic predisposition to ER and/or ERS in some IVF patients and their families, as well as a genetic predisposition to the drug-induced type 1 pattern and/or BrS in others. Future systematic ECG, electrophysiological and genomic research of ER and Brugada ECG patterns in IVF probands and their relatives, coupled with long-term follow-up, will be required to determine their true significance as predisposition for unexpected cardiac arrest in this setting.

Clinical Implications

The comprehensive evaluation of the cardiac arrest patient should be a priority to ensure exclusion of well-established genetic causes. We advocate a similar approach to our inclusion criteria (figure 1). While the current study was not designed to assess the utility of genetic testing in the proband, the impact of positive findings on familial cascade testing can support its inclusion albeit with a focus on established genes.

However, given the low yield of family screening in our study, the justification of routine comprehensive testing of relatives of idiopathic VF cases is weak. And yet, ER pattern and drug-induced type 1 Brugada ECG patterns suggest that J wave syndromes form an important sub-group of heritable risk in IVF families. Based upon current understanding routine SCB provocation in asymptomatic relatives of IVF probands is not indicated unless there is a strong prior suspicion of BrS such as the type 2 Brugada patterns seen in our families. The long-term

management will usually be with lifestyle measures to avoid arrhythmic triggers together with monitoring for any evolution of risk[36]. No clinical action can be taken in relatives with ER given the low absolute risk of arrhythmia in asymptomatic individuals with ER. Cardiac syncope and/or a strong family history for premature SCD would otherwise be indications for intervention[7].

Limitations

The study was a retrospective analysis; evaluation of family members and genetic testing in probands was not standardized. Therefore, bias cannot be excluded. While the cohort is large in terms of a rare disease, its size limits the analysis of clinical predictors for familial diagnosis. The majority of individuals were of white European ethnicity and so application of the results to other groups may not be possible. Furthermore, as these findings were generated from tertiary services with variable catchment areas, they may not be generalisable to a non-tertiary population and should be investigated prospectively.

Future Studies

A prospective study with systematic and standardised genetic testing in probands and routine use of SCB provocation in relatives would be important to further develop the conclusions drawn from this study.

Conclusion

The yield of family screening in relatives of IVF probands is low. Comprehensive clinical evaluation of the UCA proband is the cornerstone of management in these families. The

significance of J wave Syndromes in relatives of IVF cases, and the role for SCB provocation testing, require further systematic research.

Sources of Funding

None

Disclosures

None

Bibliography

1. Zipes DP, Wellens H. Sudden Cardiac Death. *Circulation*. 1998;98:2334–2351.

2. Waldmann V, Bougouin W, Karam N, Dumas F, Sharifzadehgan A, Gandjbakhch E, Algalarrondo V, Narayanan K, Zhao A, Amet D, et. al. Characteristics and clinical assessment of unexplained sudden cardiac arrest in the real-world setting: Focus on idiopathic ventricular fibrillation. *Eur Heart J*. 2018;39:1981–1987.

3. Krahn AD, Healey JS, Chauhan V, Birnie DH, Simpson CS, Champagne J, Gardner M, Sanatani S, Exner D V., Klein GJ, et. al. Systematic assessment of patients with unexplained cardiac arrest: Cardiac arrest survivors with preserved ejection fraction registry (CASPER). *Circulation*. 2009;120:278–285.

4. Herman ARM, Cheung CC, Gerull B, Simpson CS, Birnie DH, Klein GJ, Champagne J, Healey JS, Gibbs K, Talajic M, 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

5. Priori SG, Blomstrom-Lundqvist C, Mazzanti A, Bloma N, Borggrefe M, Camm J, Elliott PM, Fitzsimons D, Hatala R, Hindricks G, 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 Europea. *Eur Heart J*. 2015;36:2793-2867l.

6. Stiles MK, Wilde AAM, Abrams DJ, Ackerman MJ, Albert CM, Behr ER, Chugh SS, Cornel MC, Gardner K, Ingles J, et. al. 2020 APHRS/HRS expert consensus statement on the investigation of decedents with sudden unexplained death and patients with sudden

cardiac arrest, and of their families. *Hear Rhythm*. 2021;18:E1-50.

7. Priori SG, Wilde AAM, Horie M, Cho Y, Behr ER, Berul C, Blom N, Brugada J, Chiang C-E, Huikuri H V., et. al. Executive summary: HRS/EHRA/APHRS expert consensus statement on the diagnosis and management of patients with inherited primary arrhythmia syndromes. *Europace*. 2013;15:1389–406.

8. Jouven X, Desnos M, Guerot C, Ducimetière P. Predicting sudden death in the population. The Paris prospective study I. *Circulation*. 1999;99:1978–1983.

9. Raju H, Behr ER. Unexplained sudden death, focussing on genetics and family phenotyping. *Curr Opin Cardiol*. 2013;28:19–25.

10. Visser M, Van Der Heijden JF, Van Der Smagt JJ, Doevendans PA, Wilde AAM, Loh P, Hassink RJ. Long-Term Outcome of Patients Initially Diagnosed with Idiopathic Ventricular Fibrillation. *Circ Arrhythmia Electrophysiol*. 2016;9:e004258.

11. Probst V, Wilde AAM, Barc J, Sacher F, Babuty D, Mabo P, Mansourati J, Le Scouarnec S, Kyndt F, Le Caignec C, et. al. SCN5A Mutations and the role of genetic background in the pathophysiology of brugada syndrome. *Circ Cardiovasc Genet*. 2009;2:552–557.

12. Mazzanti A, Maragna R, Vacanti G, Monteforte N, Bloise R, Marino M, Braghieri L, Gambelli P, Memmi M, Pagan E, et. al. Interplay Between Genetic Substrate, QTc Duration, and Arrhythmia Risk in Patients With Long QT Syndrome. *J Am Coll Cardiol*. 2018;71:1663–1671.

13. Macfarlane PW, Antzelevitch C, Haissaguerre M, Huikuri H V., Potse M, Rosso R, Sacher F, Tikkanen JT, Wellens H, Yan G-X. The Early Repolarization Pattern: A Consenus Paper. *J Am Coll Cardiol*. 2015;66:470–477.

14. Antzelevitch C, Yan GX, Ackerman MJ, Borggrefe M, Corrado D, Guo J, Gussak I, Hasdemir C, Horie M, Huikuri H, et. al. J-Wave syndromes expert consensus conference report: Emerging concepts and gaps in knowledge. Hear. Rhythm. 2016;13:e295–e324.

15. Marcus FI, McKenna WJ, Sherrill D, Basso C, Bauce B, Bluemke D a, Calkins H, Corrado D, Cox MGPJ, Daubert JP, et. al. Diagnosis of arrhythmogenic right ventricular cardiomyopathy/dysplasia: proposed modification of the task force criteria. *Circulation*. 2010;121:1533–41.

16. Leinonen JT, Crotti L, Djupsjöbacka A, Castelletti S, Junna N, Ghidoni A, Tuiskula AM, Spazzolini C, Dagradi F, Viitasalo M, et. al. The genetics underlying idiopathic ventricular fibrillation: A special role for catecholaminergic polymorphic ventricular tachycardia? *Int J Cardiol*. 2018;250:139–145.

17. Behr ER, Wood D a, 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;362:1457–9.

18. 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;112:207–13.

19. Behr ER, Dalageorgou C, Christiansen M, Syrris P, Hughes S, Tome Esteban MT, Rowland E, Jeffery S, McKenna WJ. Sudden arrhythmic death syndrome: familial evaluation identifies inheritable heart disease in the majority of families. *Eur Heart J*. 2008;29:1670–80.

20. van der Werf C, Hofman N, Tan HL, van Dessel PF, Alders M, van der Wal AC, van

Langen IM, Wilde AAM. Diagnostic yield in sudden unexplained death and aborted cardiac arrest in the young: the experience of a tertiary referral center in The Netherlands. *Heart Rhythm*. 2010;7:1383–9.

21. McGorrian C, Constant O, Harper N, O'Donnell C, Codd M, Keelan E, Green A, O'Neill J, Galvin J, Mahon NG. Family-based cardiac screening in relatives of victims of sudden arrhythmic death syndrome. *Europace*. 2013;15:1050–8.

22. Kumar S, Peters S, Thompson T, Morgan N, Maccicoca I, Trainer A, Zentner D, Kalman JM, Winship I, Vohra JK. Familial cardiological and targeted genetic evaluation: Low yield in sudden unexplained death and high yield in unexplained cardiac arrest syndromes. *Hear Rhythm*. 2013;10:1653–1660.

23. Hansen BL, Jacobsen EM, Kjerrumgaard A, Tfelt-hansen J, Winkel BG, Bundgaard H, Christensen AH. Diagnostic yield in victims of sudden cardiac death and their relatives. *Europace*. 2020;1–8.

24. Bagnall RD, Weintraub R, Ingles J, Duflou J, Yeates L, Lam L, Davis A, Thompson T, Connell V, Wallace J, et. al. A Prospective Study of Sudden Cardiac Death among Children and Young Adults. *N Engl J Med*. 2016;374:2441–2452.

25. Lahrouchi N, Raju H, Lodder EM, Papatheodorou E, Ware JS, Papadakis M, Tadros R, Cole D, Skinner JR, Crawford J, et. al. Utility of Post-Mortem Genetic Testing in Cases of Sudden Arrhythmic Death Syndrome. *J Am Coll Cardiol*. 2017;69:2134–2145.

26. Tester DJ, Ackerman MJ. Postmortem long QT syndrome genetic testing for sudden unexplained death in the young. *J Am Coll Cardiol*. 2007;49:240–6.

27. Mellor G, Raju H, de Noronha S V, Papadakis M, Sharma S, Behr ER, Sheppard MN.

Clinical Characteristics and Circumstances of Death in the Sudden Arrhythmic Death Syndrome. *Circ Arrhythm Electrophysiol*. 2014;7:1078–1083.

28. Raju H, Papadakis M, Govindan M, Bastiaenen R, Chandra N, O'Sullivan A, Baines G, Sharma S, Behr ER. Low prevalence of risk markers in cases of sudden death due to Brugada syndrome relevance to risk stratification in Brugada syndrome. *J Am Coll Cardiol*. 2011;57:2340–5.

29. Semsarian C, Wilde AAM. Genetic Causes in Cardiac Arrest Survivors: Fake News or the Real Deal? *Circ Cardiovasc Genet*. 2017;10:8–10.

30. Steinberg C, Padfield GJ, Champagne J, Sanatani S, Angaran P, Andrade JG, Roberts JD, Healey JS, Chauhan VS, Birnie DH, J et. al. Cardiac Abnormalities in First-Degree Relatives of Unexplained Cardiac Arrest Victims. *Circ Arrhythmia Electrophysiol*. 2016;9:e004274.

31. Honarbakhsh S, Srinivasan N, Kirkby C, Firman E, Tobin L, Finlay M, Hunter RJ, Murphy C, Lowe MD, Schilling RJ, Lambiase PD. Medium-term outcomes of idiopathic ventricular fibrillation survivors and family screening: A multicentre experience. *Europace*. 2017;19:1874–1880.

32. Alders M, Koopmann TT, Christiaans I, Postema PG, Beekman L, Tanck MWT, Zeppenfeld K, Loh P, Koch KT, Demolombe S, et. al. Haplotype-Sharing Analysis Implicates Chromosome 7q36 Harboring DPP6 in Familial Idiopathic Ventricular Fibrillation. *Am J Hum Genet*. 2009;84:468–476.

33. Postema PG, Christiaans I, Hofman N, Alders M, Koopmann TT, Bezzina CR, Loh P, Zeppenfeld K, Volders PGA, Wilde AAM. Founder mutations in the Netherlands: Familial

idiopathic ventricular fibrillation and DPP6. *Netherlands Hear J*. 2011;19:290–296.

34. 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. *Hear Rhythm*. 2017;14:1035-1040

35. Mellor G, Laksman ZWM, Tadros R, Roberts JD, Gerull B, Simpson CS, Klein GJ, Champagne J, Talajic M, Gardner M, et. al. Genetic Testing in the Evaluation of Unexplained Cardiac Arrest: From the CASPER (Cardiac Arrest Survivors with Preserved Ejection Fraction Registry). *Circ Cardiovasc Genet*. 2017;10:1–8.

36. Papadakis M, Papatheodorou E, Mellor G, Raju H, Bastiaenen R, Wijeyeratne YD, Wasim S, Ensam B, Finocchiaro G, Gray B, et. al. The Diagnostic Yield of Brugada Syndrome After Sudden Death With Normal Autopsy. *J Am Coll Cardiol*. 2018;71:1204–1214.

37. Tadros R, Nannenberg EA, Lieve K V., Škorić-Milosavljević D, Lahrouchi N, Lekanne Deprez RH, Vendrik J, Reckman YJ, Postema PG, et. al. Yield and Pitfalls of Ajmaline Testing in the Evaluation of Unexplained Cardiac Arrest and Sudden Unexplained Death: Single-Center Experience With 482 Families. *JACC Clin Electrophysiol*. 2017;3:1400–1408.

38. Probst V, Veltmann C, Eckardt L, Meregalli PG, Gaita F, Tan HL, Babuty D, Sacher F, Giustetto C, Schulze-Bahr E, Borggrefe M, et. al. Long-term prognosis of patients diagnosed with Brugada syndrome: Results from the FINGER Brugada Syndrome Registry. *Circulation*. 2010;121:635–43.

39. Hasdemir C, Payzin S, Kocabas U, Sahin H, Yildirim N, Alp A, Aydin M, Pfeiffer R. High prevalence of concealed Brugada syndrome in patients with atrioventricular nodal

reentrant tachycardia. *Hear Rhythm*. 2015;12:1584–1594.

40. Bezzina CR, Barc J, Mizusawa Y, Remme CA, Gourraud J-B, Simonet F, Verkerk AO, Schwartz PJ, Crotti L, Dagradi F, et. al. Common variants at SCN5A-SCN10A and HEY2 are associated with Brugada syndrome, a rare disease with high risk of sudden cardiac death. *Nat Genet*. 2013;45:1044–9.

41. Tadros R, Tan HL, Mathari S el, Kors JA, Postema PG, Lahrouchi N, Beekman L, Radivojkov-Blagojevic M, Amin AS, Meitinger T, et. al. Predicting cardiac electrical response to sodium channel blockade and Brugada syndrome using polygenic risk scores. *Eur Heart J*. 2019;1–12.

42. Haïssaguerre M, Derval N, Sacher F, Jesel L, Deisenhofer I, de Roy L, Pasquié J-L, Nogami A, Babuty D, Yli-Mayry S,. Sudden cardiac arrest associated with early repolarization. *N Engl J Med*. 2008;358:2016–23.

43. Nunn LM, Bhar-Amato J, Lowe MD, Macfarlane PW, Rogers P, McKenna WJ, Elliott PM, Lambiase PD. Prevalence of J-point elevation in sudden arrhythmic death syndrome families. *J Am Coll Cardiol*. 2011;58:286–90.

44. Mellor G, Nelson CP, Robb C, Raju H, Wijeyeratne YD, Hengstenberg C, Reinhard W, Papadakis M, Sharma S, Samani NJ, Behr ER. The Prevalence and Significance of the Early Repolarization Pattern in Sudden Arrhythmic Death Syndrome Families. *Circ Arrhythmia Electrophysiol*. 2016;9:e003960

45. Teumer A, Trenkwalder T, Kessler T, Jamshidi Y, van den Berg ME, Kaess B, Nelson CP, Bastiaenen R, de Bortoli M, Rossini A, et. al. KCND3 potassium channel gene variant confers susceptibility to electrocardiographic early repolarization pattern. *JCI Insight*.

2019;4:e131156.

Tables and Figures

Characteristic, n (%)	All probands (*n*=96)	Negative family screening (*n*=92)	Positive family screening (n=4)	*p*-value
Age, mean±SD	39±12	39±12	28±17	0.08
Male Sex (%)	51 (53)	47 (51)	3 (75)	0.35
Caucasian (%)*	87 (91)	83 (90)	4 (100)	0.33
Circumstance of cardiac arrest				
Sleep (%)	12 (15)	10 (13)	2 (50)	0.12
Rest (%)	45 (56)	44 (57)	1 (25)	
Exercise (%)	24 (30)	23 (30)	1 (25)	
Prior syncope (%)	17 (18)	16 (17)	1 (25)	0.70
Other FH SD (%)	12 (13)	12 (13)	0 (0)	0.44
Investigations				
ECG (*n*=96, 100%)				
Normal	61 (64)	58 (63)	3 (75)	0.63
Early Repolarisation	16 (17)	15 (16)	1 (25)	
Other Minor findings	19 (20)	19 (21)	0 (0)	
High lead ECG (*n*=84, 85%)				
Normal	82 (98)	78 (98)	4 (100)	0.75
Type 2 Brugada pattern	2 (2)	2 (2)	0 (0)	
SAECG (*n*=48, 52%)				
Normal (0/3)	36 (75)	35 (74)	1 (100)	1.0
Abnormal (≥2/3)	7 (15)	7 (15)	0 (0)	
Equivocal (1/3)	5 (10)	5 (11)	0 (0)	
Echo (*n*=96, 100%)				

Normal Minor finding	79 (82) 17 (18)	76 (83) 16 (17)	3 (75) 1 (25)	0.70
CMR (*n*=81, 85%)				
Normal Minor finding	68 (84) 13 (16)	66 (86) 11 (14)	2 (50) 2 (50)	0.06
Exercise ECG (*n*=96, 100%)				
Normal Minor finding	79 (82) 17 (18)	75 (82%) 17 (18%)	4 (100) 0 (0)	0.34
Adrenaline provocation (*n*=33, 34%)				
Negative QT prolongation >30ms QT prolongation 1-30ms	23 (70) 1 (3) 9 (26)	21 (68) 1 (3) 9 (28)	2 (100) 0 (0) 0 (0)	0.35
Sodium channel blocker provocation (*n*=96, 100%)				
Negative Type 1 Brugada ECG	96 (100) 0 (0)	92 (100) 0 (0)	4 (100) 0 (0)	-
Genetics (*n*=73, 77%)				
Negative inc. VUS P/LP variant	71 (97) 2 (3)	68 (99) 1 (1)	3 (75) 1 (25)	0.01

Table 1. Clinical characteristics and summary of investigation findings in idiopathic VF probands. Stratified by presence of a positive finding during evaluation in a first-degree relative. *The self-reported ethnicities of the remaining 9% were South East Asian (n=3), African-Caribbean (n=3), Chinese (n=1) and Middle Eastern (n=1). One individual self-reported as 'other ethnicity'. *ECG: Electrocardiogram; FH SD: Family history of Sudden Cardiac Death; P/LP: Pathogenic or likely pathogenic; SAECG: Signal-averaged electrocardiogram; SD: Standard deviation; VUS: variant of uncertain significance.*

	All relatives (n=201)	No familial diagnosis (*n*=185)	Familial diagnosis (n=16)	*p*-value
Age, mean±SD	37 (23-57)	36 (23-56)	48 (23-61)	0.80
Male (%)	114 (57)	107 (58)	7 (44)	0.28
Relationship to proband (%)				
Child	72 (36)	70 (38)	2 (13)	0.08
Sibling	69 (34)	60 (32)	9 (56)	
Parent	60 (30)	55 (30)	5 (31)	
Syncope (%)	12 (6)	12 (7)	0 (0)	0.29
Other FH SD (%)	7 (4)	6 (4)	1 (6)	0.53
Investigations performed				
ECG (%)	201 (100)	185 (100)	16 (100)	-
High lead ECG (%)	52 (26)	44 (24)	8 (50)	0.02
SAECG (%)	59 (29)	53 (29)	6 (38)	0.46
Echocardiogram (%)	159 (79)	149 (81)	10 (63)	0.09
MRI (%)	22 (11)	21 (11)	1 (6)	0.53
Exercise ECG (%)	124 (62)	117 (63)	7 (44)	0.13
Adrenaline provocation (%)	6 (3)	6 (3)	0 (0)	0.46
Na channel blocker provocation (%)	20 (10)	15 (8)	5 (31)	0.01

Table 2. Clinical characteristics and investigations performed in relatives. Stratified by presence of positive findings during familial evaluation. *ECG: electrocardiogram; CMR: cardiac magnetic resonance imaging; SAECG: signal-averaged electrocardiogram; FH SD: Family history of sudden cardiac death.*

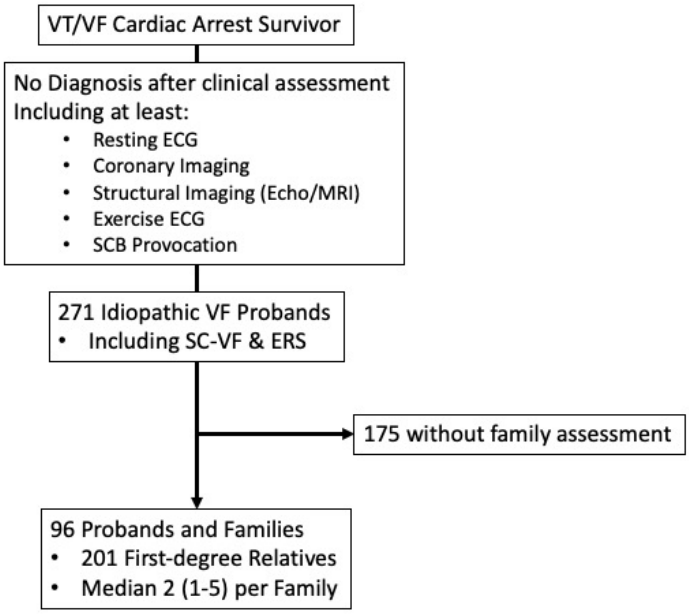


Figure 1. Cohort Selection. Idiopathic VF probands were identified after comprehensive clinical evaluation. Families where ≥1 first degree relative were assessed with at least a resting ECG were included. *SCB=Sodium Channel Blocker*

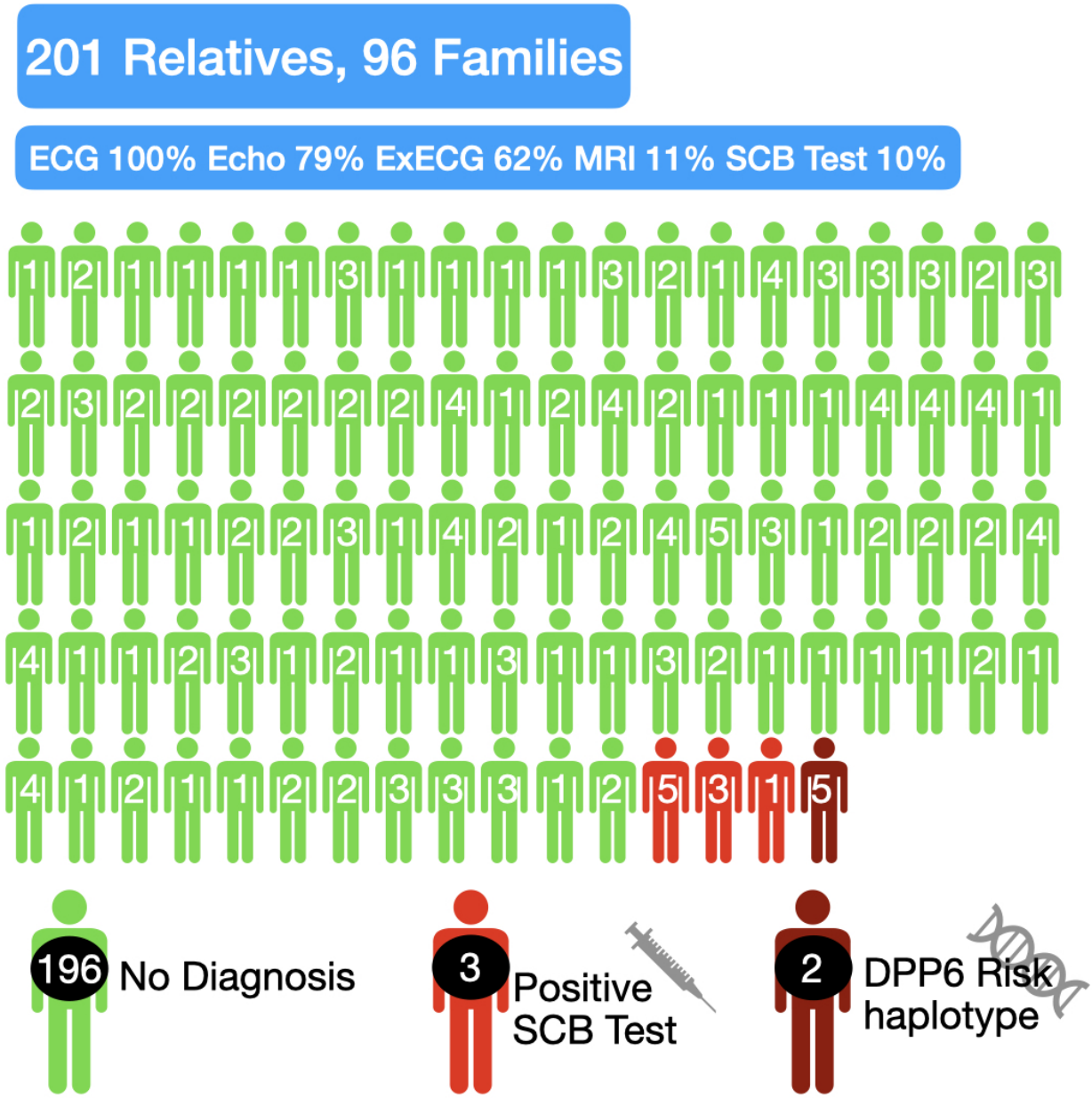


Figure 2. Outcomes of family assessment in Idiopathic VF. Each person icon represents one family with the number of relatives assessed noted inside the icon. *SCB=Sodium Channel Blocker*

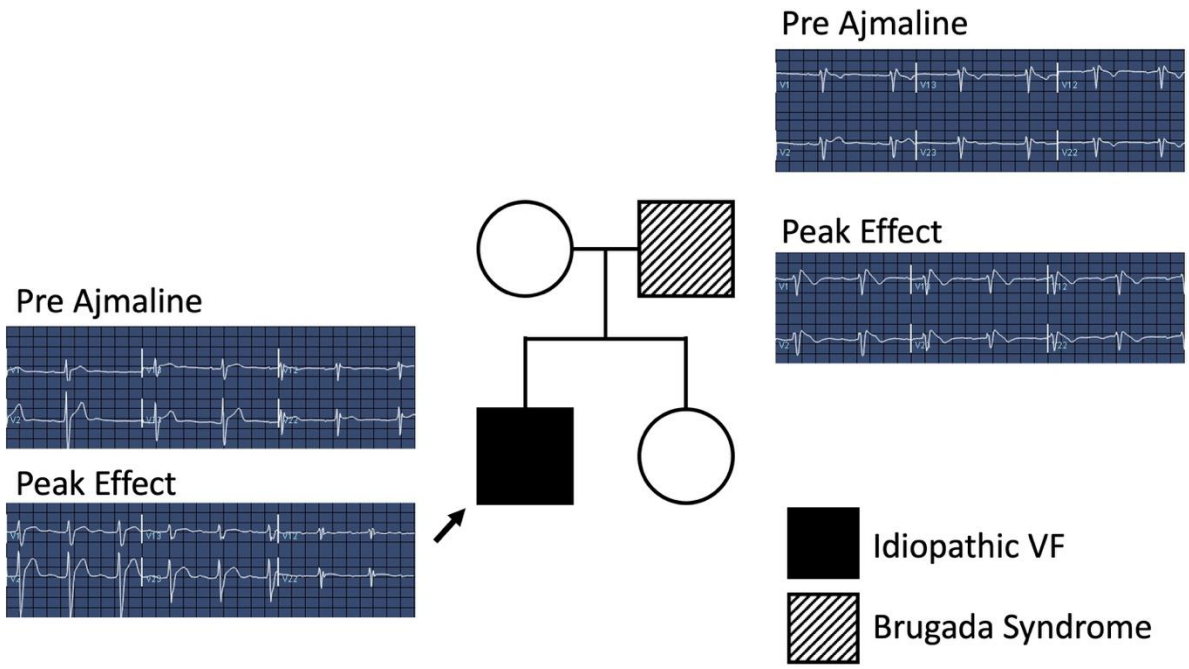
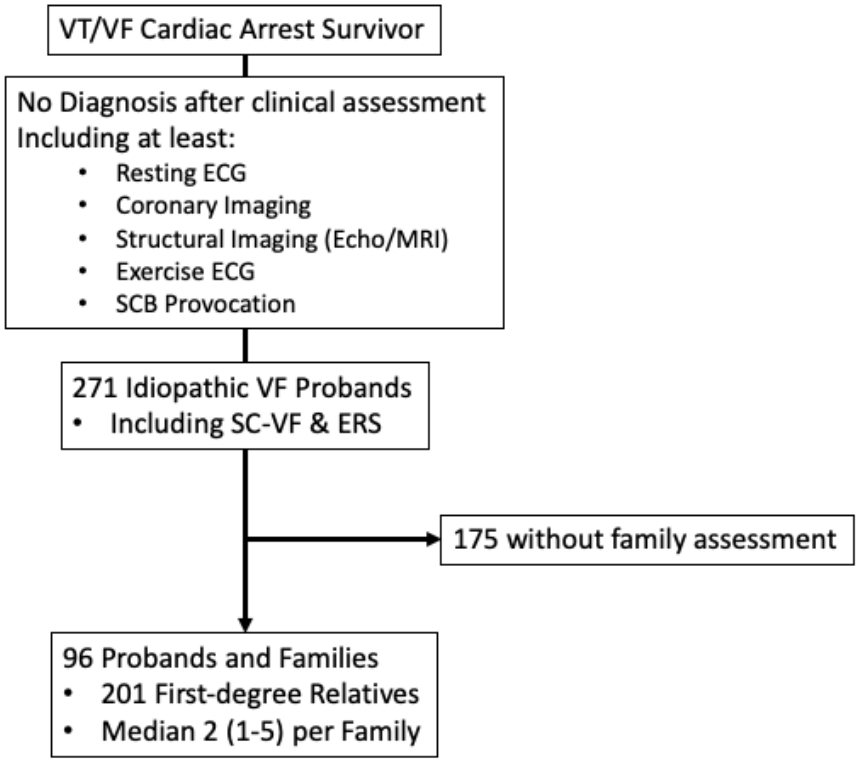
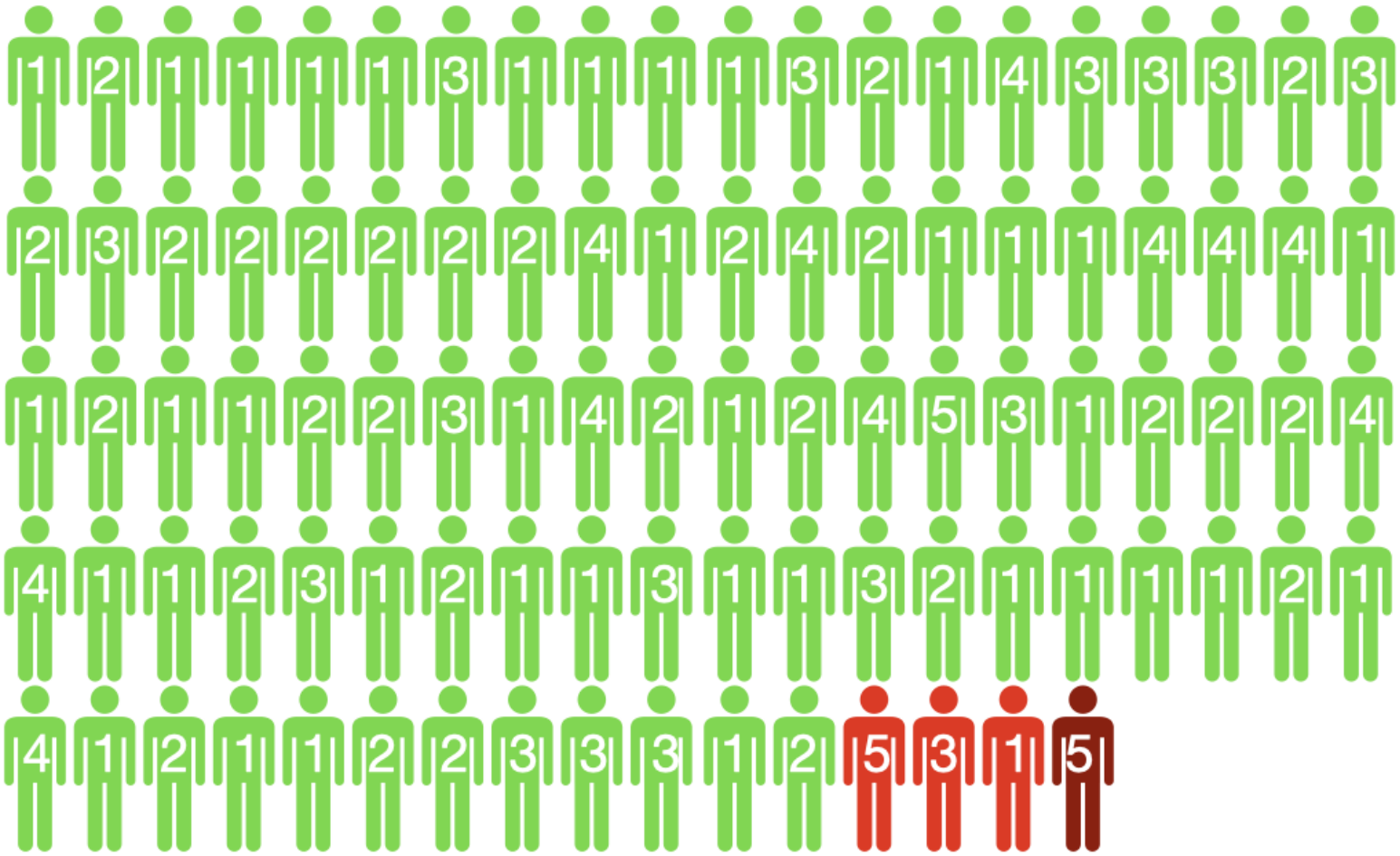


Figure 3. Sodium Channel Blocker Provocation Results in Family 2. An 18-year-old male idiopathic VF proband had a negative ajmaline provocation. The patient's father had a positive test with a type 1 Brugada ECG pattern seen. 1mg/kg Ajmaline was infused over 5 mins. Continuous digital ECGs were recorded with V_1 and V_2 in the standard and high lead positions (labelled V_{12}/V_{22} for second intercostal space, V_{13}/V_{23} for third intercostal space.


VT/VF Cardiac Arrest Survivor
No Diagnosis after clinical assessment
Including at least:
• Resting ECG
• Coronary Imaging
• Structural Imaging (Echo/MRI)
• Exercise ECG
• SCB Provocation
271 Idiopathic VF Probands
• Including SC-VF & ERS
175 without family assessment
96 Probands and Families
• 201 First-degree Relatives
• Median 2 (1-5) per Family

201 Relatives, 96 Families

ECG 100% Echo 79% ExECG 62% MRI 11% SCB Test 10%

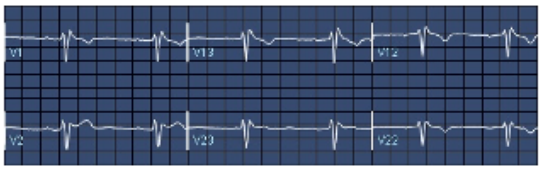


196 No Diagnosis

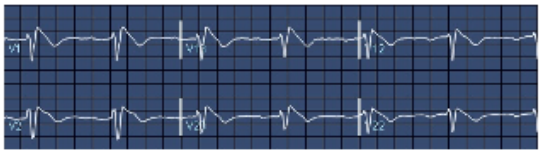
3 Positive SCB Test

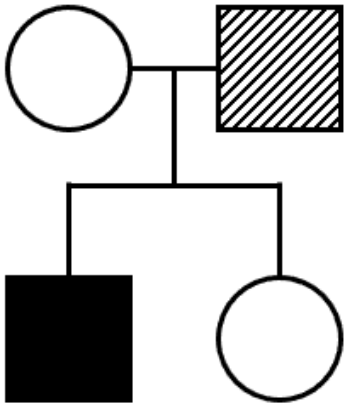
2 DPP6 Risk haplotype

Pre Ajmaline

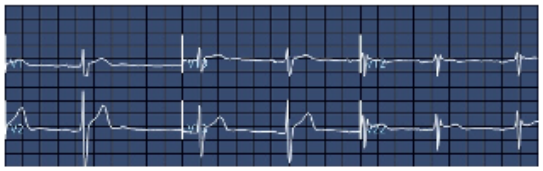


Peak Effect

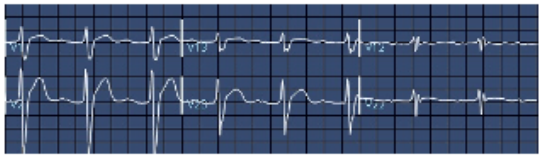




Pre Ajmaline



Peak Effect



Idiopathic VF

Brugada Syndrome