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**CLINICAL RESEARCH**

# The genetic basis of apparently idiopathic ventricular fibrillation: a retrospective overview

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**Aims** During the diagnostic work-up of patients with idiopathic ventricular fibrillation (VF), next-generation sequencing panels can be considered to identify genotypes associated with arrhythmias. However, consensus for gene panel testing is still lacking, and variants of uncertain significance (VUS) are often identified. The aim of this study was to evaluate genetic testing and its results in idiopathic VF patients.

**Methods and results** We investigated 419 patients with available medical records from the Dutch Idiopathic VF Registry. Genetic testing was performed in 379 (91%) patients [median age at event 39 years (27–51), 60% male]. Single-gene testing was performed in 87 patients (23%) and was initiated more often in patients with idiopathic VF before 2010. Panel testing was performed in 292 patients (77%). The majority of causal (likely) pathogenic variants (LP/P, $n = 56$, 15%) entailed the *DPP6* risk haplotype ($n = 39$, 70%). Moreover, 10 LP/P variants were found in cardiomyopathy genes (*FLNC*, *MYL2*, *MYH7*, *PLN* (two), *TTN* (four), *RBM20*), and 7 LP/P variants were identified in genes associated with cardiac arrhythmias (*KCNQ1*, *SCN5A* (2), *RYR2* (four)). For eight patients (2%), identification of an LP/P variant resulted in a change of diagnosis. In 113 patients (30%), a VUS was identified. Broad panel testing resulted in a higher incidence of VUS in comparison to single-gene testing (38% vs. 3%, $P < 0.001$).

**Conclusion** Almost all patients from the registry underwent, albeit not broad, genetic testing. The genetic yield of causal LP/P variants in idiopathic VF patients is 5%, increasing to 15% when including *DPP6*. In specific cases, the LP/P variant is the underlying diagnosis. A gene panel specifically for idiopathic VF patients is proposed.



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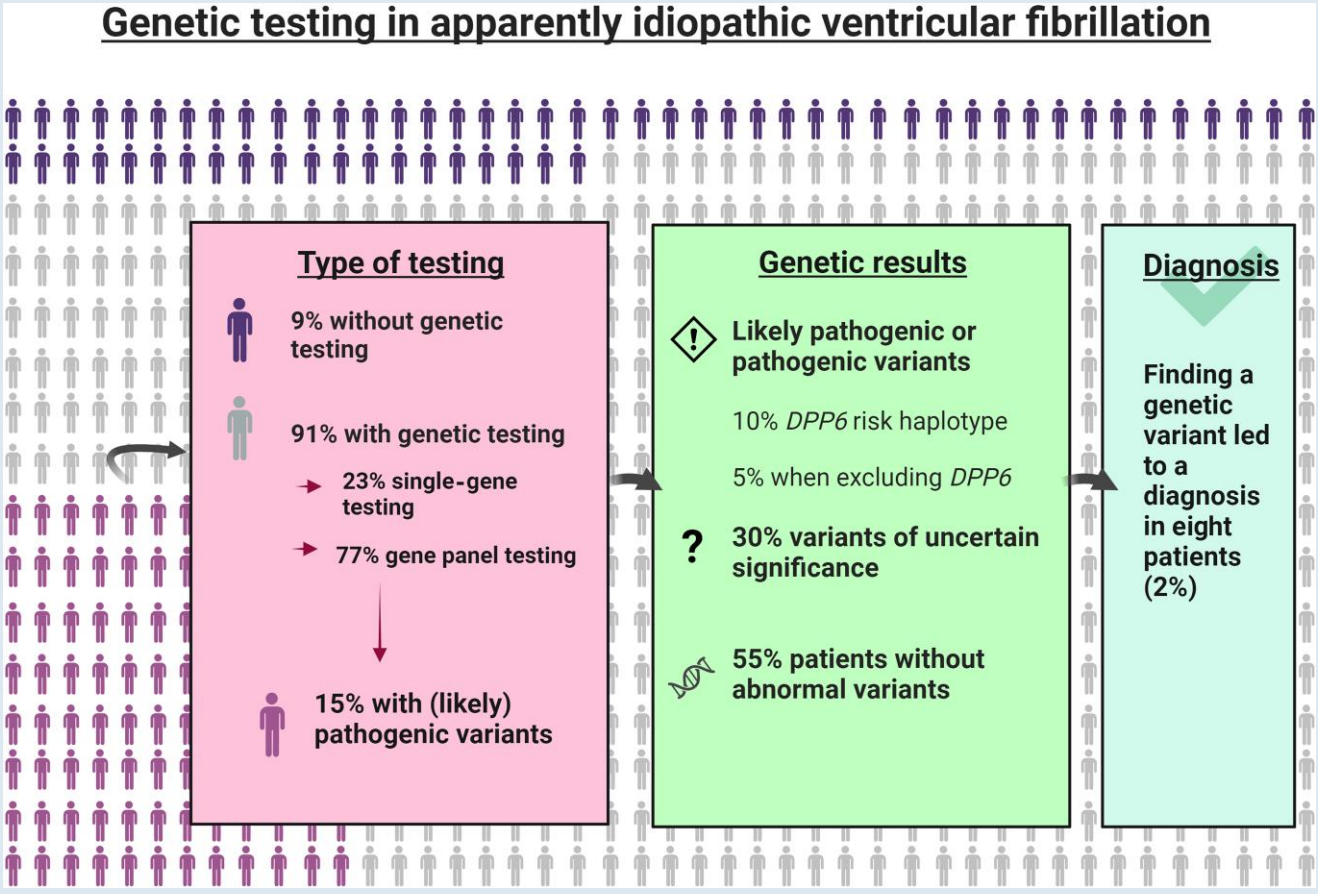
**Graphical Abstract**




**Keywords** Idiopathic ventricular fibrillation • Sudden cardiac arrest • Genetic testing

## What's new?

- Over the years, the type of genetic testing has changed for patients with idiopathic ventricular fibrillation (VF), but genetic testing in general is regularly performed.
- For a subset of idiopathic VF patients, finding a likely pathogenic or pathogenic variant contributes to recognizing a previously concealed disease phenotype.
- A gene panel for idiopathic VF patients is proposed.

# Introduction

Idiopathic ventricular fibrillation (VF) is a diagnosis per exclusion that should only be made after a complete diagnostic work-up.[1] Only a small proportion of patients presenting with sudden cardiac arrest (SCA) remains undiagnosed, i.e. without a myocardial disease phenotype after work-up.[2] Discovering the underlying cause of SCA will guide treatment, including lifestyle advises, and can prompt family screening. Most patients presenting with SCA have VF in the setting of myocardial ischaemia owing to coronary artery disease. In younger patients, channelopathies or non-ischaemic cardiomyopathies precipitating lethal arrhythmias are important underlying causes.[3] It is known that several of these diseases have an inheritable origin.[4] Several studies, and more recent a consensus statement, have focused on a specific diagnostic work-up to be performed in SCA survivors.[3,5] The current recommendation is to perform genetic testing in SCA survivors based on phenotypic abnormalities revealed with standard diagnostic testing [including (extended family) history taking]. Uncertainty remains about expanding genetic testing with next-generation sequencing panels when no specific diagnosis for SCA is revealed. Primarily the costs, the uncertain value of a negative result and the finding of variants of uncertain significance (VUS), are important factors to consider.[5] Studies focusing on the value of genetic testing in unexplained cardiac arrest (UCA) survivors have shown genetic yields up to 48%.[6–13] A significant number of these variants were found in genes associated with cardiomyopathies. This indicates the importance of genetic testing to reveal the so-called concealed cardiomyopathies as a cause for a SCA without apparent structural abnormalities.[7,9] Recently, Pannone *et al.*[14] showed that the yield of genetic testing in a small group of 45 probands with idiopathic VF was 7%. Post-mortem genetic testing, and especially the combination of clinical and genetic evaluation, is also of importance in sudden arrhythmic deaths.[15] In these patients, a genetic yield of 13% has been reported, which increased when combined with clinical evaluation of family members.[13]

To further assess the value of genetic testing in idiopathic VF, the aim of this study was two-fold. First, we investigated a large cohort of unrelated idiopathic VF patients, evaluating the routine use of genetic testing and genetic results in the Netherlands. Secondly, we focused on the impact of genetic test results on ultimate diagnosis and the implications for family screening.

# Methods

## Study population

The study population was derived from the Dutch Idiopathic VF Registry. This is a national, multicentre, observational cohort including patients initially diagnosed with idiopathic VF. The diagnosis idiopathic VF is based on the latest guideline criteria.[3] We included all patients from the registry with available patient records to assess their genetic and cardiac evaluation. The Medical Ethics Committee of the University Medical Centre Utrecht exempted the study from Medical Research Involving Human Subjects (14-254/C). The study adheres to the Declaration of Helsinki.

## Clinical evaluation

Demographic characteristics, medical history, and circumstances before SCA were collected. Results from laboratory testing (including toxicological screening), electrocardiographic testing [12-lead electrocardiogram (ECG), Holter monitoring, exercise treadmill testing, signal averaged ECG], cardiac imaging [echocardiography, cardiac magnetic resonance (CMR), computed tomography/coronary angiography, positron emission tomography], provocation testing (sodium channel blocker provocation to exclude Brugada syndrome, ergonovine to exclude coronary artery spasm), and endomyocardial biopsy were collected. Non-diagnostic imaging abnormalities were defined as any non-diagnostic finding determined with either echocardiography or CMR. Non-diagnostic electrical abnormalities were the presence of either premature ventricular complexes (PVC) or (non-sustained) ventricular tachycardia on Holter or exercise treadmill test.

## Genetic evaluation

Performed genetic tests and their results were collected. Genetic testing was initiated at the discretion of the treating physician together with the clinical geneticist using the best available method at that specific time point. Testing methods ranged from single-gene testing by Sanger sequencing, to gene panel testing with next-generation sequencing, and whole-exome sequencing. In single-gene testing, only single candidate genes were investigated. Next-generation sequencing panels included arrhythmia, cardiomyopathy, or sudden cardiac death (SCD) panels, as indicated by the local institution. For further analysis, we grouped arrhythmia and SCD panels as 'arrhythmia panels'. When both single-gene testing and panel testing was initiated, a patient was considered as panel testing when no abnormalities were found with single-gene testing. When single-gene testing revealed a likely pathogenic (LP) or pathogenic (P) variant, the patient was eventually considered as single-gene testing. Broad panel testing included panels with genes associated with cardiomyopathies, channelopathies, congenital heart disease, and/or other cardiac diseases. When both arrhythmia and cardiomyopathy panels were tested, this was also considered as broad panel testing. Supplementary material online, *Table S1* provides a detailed overview of each panel. Besides routine genetic testing, we also performed in-depth genetic testing for research purposes. In such cases, baseline genetic testing with an arrhythmia panel was expanded with broad panel testing, as previously published by Visser *et al.*[16] To determine the yield of genetic testing, we included the results of expanded broad testing. Cascade screening was initiated when an LP/P variant was found. Family members at risk were identified by the treating physicians, and testing of the specific family variant was performed. When available, information regarding cascade screening was collected.

## Variant classification

Variant classification was first derived from patient records or genetic testing records, depending on availability. Secondly, all variants were reclassified according to the American College of Medical Genetics and Genomics Guidelines[17,18] by the investigators. Variants were classified with standard terminology including: benign (Class 1), likely benign (Class 2), VUS (Class 3), LP (Class 4), and P (Class 5). We defined three groups: (i) patients with benign, likely benign, or no variants, (ii) patients with one or multiple VUS, and (iii) patients with an LP or P variant, either with or without an additional VUS.

## Follow-up and outcomes

Follow-up was collected from the index event (SCA due to VF) until the last available clinical evaluation. Diagnostic evaluation during follow-up was initiated by the treating physician. Primary outcome was the establishment of an ultimate diagnosis (i.e. a previously concealed disease phenotype related to VF). Accepted diagnostic criteria for known underlying causes were used, as described previously.[19] Secondary outcomes were the recurrence of ventricular arrhythmias, defined as ventricular tachycardia, VF, appropriate implantable cardioverter defibrillator (ICD) intervention, resuscitated SCA, or SCD in the absence of an ICD.

## Statistical analysis

Data were analysed with SPSS version 26.0.0.1. Categorical variables are presented as numbers (percentages) and analysed using $\chi^2$ or Fisher's exact tests, as appropriate. Continuous variables are presented as mean ± standard deviation (SD) or median [interquartile range (IQR)] and analysed using Student's *t*-test or Mann–Whitney *U* test, as appropriate. $P$ values $< 0.05$ were considered significant.

# Results

## Study population

Among 453 patients included in the Dutch Idiopathic VF Registry, we enrolled 419 patients with available patient records. In 379 patients (91%), genetic testing was performed, at baseline or during follow-up (see Supplementary material online, *Figure S1*).

## Clinical characteristics and diagnostic work-up

Patients who underwent genetic testing had their event at a median age of 39 [IQR 27–51] years, and 60% were male. Compared with patients without genetic testing, patients with genetic testing were younger at first event and had their event in a more recent year (*Table 1*). Overall, patients without genetic testing received a more limited diagnostic approach in terms of high-yield diagnostic tests, with CMR imaging and sodium channel blocker provocation testing significantly less often performed. A complete overview of the diagnostic work-up is shown in Supplementary material online, *Table S2*. Non-diagnostic imaging abnormalities and electrical abnormalities were similar between groups. Supplementary material online, *Figures S2 and S3* further specify these abnormalities. Reasons for abstaining from genetic testing varied. Most patients experienced their event between 1986 and 2004, before availability of genetic testing, and/or were lost to follow-up ($n = 20$). Other reasons were patient refusal ($n = 8$), genetic testing was considered as not indicated based on phenotype ($n = 1$), only genes for aortopathies were tested ($n = 1$), or the reason was unknown ($n = 10$). Patients with a family history of SCD who did not receive genetic testing ($n = 5$) experienced their event between 1986 and 2009.

## Genetic evaluation

Single-gene testing was initiated in 87 patients (23%). Gene panel testing was performed in 292 (77%) patients. Most patients underwent gene panel testing with an arrhythmia panel ($n = 152$; 52%), broad panel testing was performed in 112 patients (38%), and in the minority, gene panel testing included only a cardiomyopathy panel ($n = 25$; 9%). For three patients, the specifics about gene panel testing could not be retrieved (1%). In total, 77 patients received gene panel testing besides specific single-gene testing. For three patients with additional single-gene testing besides gene panel testing, single-gene testing revealed an LP/P variant. *Figure 1* shows the yield of various genetic strategies. Over the years, the use of gene panel testing increased (62% in patients with idiopathic VF before 2010 vs. 87% in patients with an event after 2010, $P < 0.01$). Of patients with broad panel testing, 33 patients

**Table 1** Clinical characteristics of idiopathic VF patients stratified by genetic evaluation

| | All ($n = 419$) | Genetic testing performed ($n = 379$) | No genetic testing performed ($n = 40$) | *P* value |
|---|---|---|---|---|
| Baseline characteristics | | | | |
| Median age at event[a] | 40 (28–52) | 39 (27–51) | 47 (34–63) | <0.01 |
| Year event[a] | 2012 (2005–2017) | 2012 (2007–2017) | 2002 (1991–2013) | <0.01 |
| Male sex | 253 (60%) | 226 (60%) | 27 (68%) | 0.43 |
| Asymptomatic before event | 248 (62%) | 228 (63%) | 20 (51%) | 0.28 |
| Family history of SCD[b] | 70/410 (17%) | 65/371 (18%) | 5/39 (13%) | 0.60 |
| Performed diagnostic work-up | | | | |
| Cardiac magnetic resonance | 318/416 (76%) | 303/376 (81%) | 15/40 (38%) | <0.01 |
| Exercise treadmill test | 297/401 (74%) | 264/361 (73%) | 33/40 (83%) | 0.28 |
| Sodium channel blocker provocation | 269/408 (66%) | 256/368 (70%) | 13/40 (33%) | <0.01 |
| Phenotype | | | | |
| Non-diagnostic imaging abnormalities | 159/419 (38%) | 143/379 (38%) | 15/40 (38%) | 1.00 |
| Non-diagnostic electrical abnormalities[c] | 170/355 (48%) | 157/317 (50%) | 13/38 (34%) | 0.11 |

SCD, sudden cardiac death.
[a]Data are provided as median (IQR).
[b]Family history of SCD is defined as a first-degree family member with SCD < 50 years or multiple second-degree family members with SCD.
[c]Non-diagnostic electrical abnormalities are defined as the presence of either premature ventricular contractions (PVC) or (non-sustained) ventricular tachycardia on Holter or exercise treadmill test.

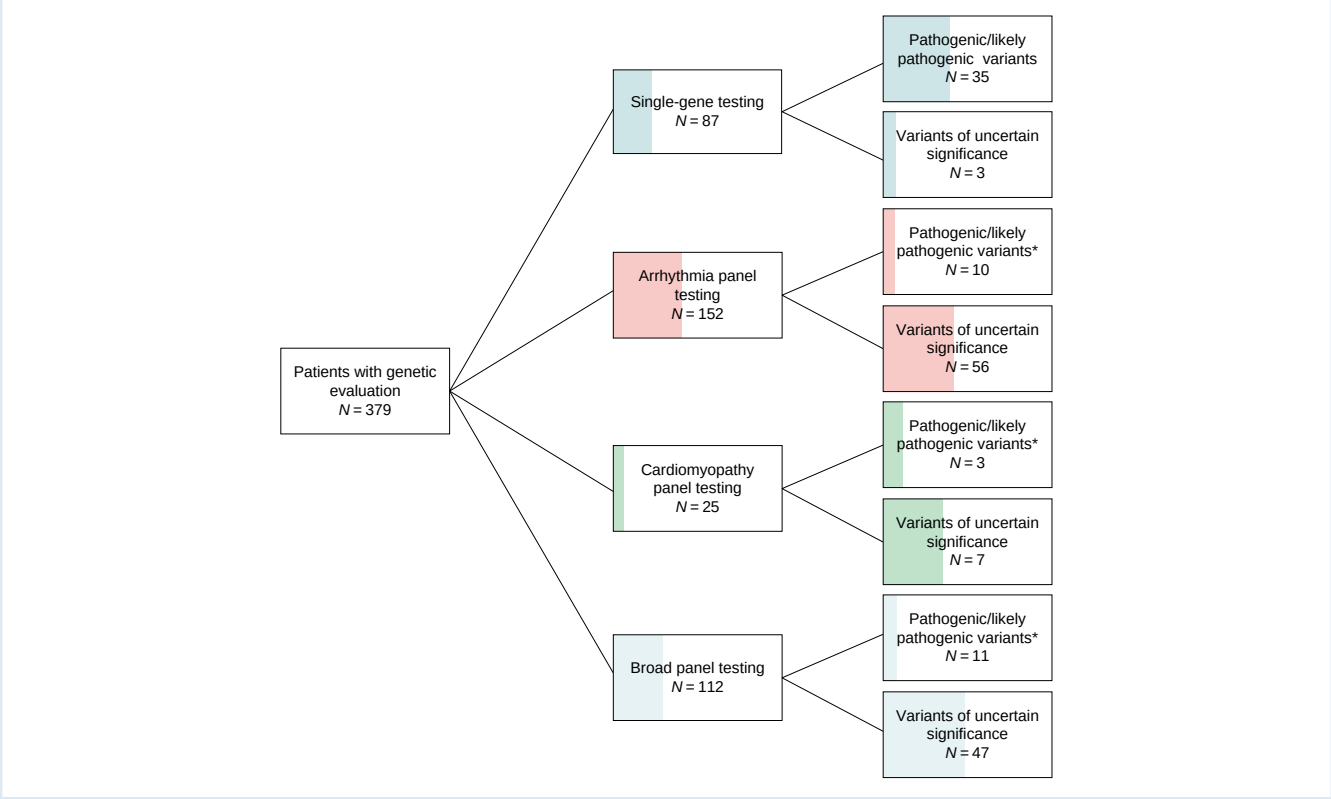



**Figure 1** Flowchart presenting genetic evaluation with results from all patients with genetic testing. Patients with LP/P variants and VUS are shown; colours represent percentages. No abnormalities, benign, or likely benign variants were found in the remaining patients. Three patients had additional single-gene testing besides panel testing which revealed an LP/P variant, highlighted in the figure with *. Three patients with unknown type of panel testing are not shown in this figure.

underwent this in a research setting. Phenotypic characteristics in terms of non-diagnostic imaging abnormalities did significantly differ between patients who underwent genetic testing including cardiomyopathy genes (see Supplementary material online, *Table S3*).

## Genetic results

In total, 59 patients carried a total of 60 LP/P variants. The *DPP6* risk haplotype was most often identified ($n = 39$, 65%), for 33 patients with single-gene testing and in 6 cases through gene panel analysis (*Table 2*). Ten LP/P variants were found in genes associated with a cardiomyopathy (*FLNC*, *MYL2*, *MYH7*, *PLN* (two), *TTN* (four), *RBM20*) and seven in genes associated with cardiac arrhythmias (*KCNQ1*, *SCN5A* (two), *RYR2* (four)). Four heterozygous variants were found in genes that were considered not causal to VF or only considered disease-causing in case of biallelic pathogenic variants (*NEB*, *CPT2*, *TRDN*, *PPA2*). The total yield of causal LP/P variants in patients excluding the *DPP6* risk haplotype was 5% (*Table 3*). *Table 4* presents each specific LP/P variant. The yield was not influenced by the presence of non-diagnostic abnormalities (see Supplementary material online, *Table S4*). After re-evaluation of all variants, one VUS was re-classified as LP, and three LP/P variants were downgraded to a VUS or benign variation. Thirty-two variants were considered a VUS before re-evaluation but are currently classified as benign/likely benign. A total of 168 VUS remained, which were identified in 124 patients, among whom 113 (30%) only carried 1 or multiple VUS. Eleven patients also carried an LP/P variant. A detailed list of all variants can be found in Supplementary material online, *Table S5*. To provide a complete overview of all found variants, benign and likely benign variants are also included in this table.

**Table 2** Genes with LP/P variants stratified by type of genetic testing that revealed the LP/P variant

| Single-gene testing | Arrhythmia panel testing | Cardiomyopathy panel testing | Broad panel testing |
|---|---|---|---|
| *DPP6* (33) | *DPP6* (5) | *MYH7* (1) | *DPP6* (1) |
| *PLN* (1) | *PLN* (1) | *TTN* (1) | *CPT2* (1) |
| *RYR* (2) | *RYR2* (1) | | *FLNC* (1) |
| *RBM20* (1) | *KCNQ1* (1) | | *MYL2* (1) |
| *TTN* (1) | *SCN5A* (1) | | *NEB* (1) |
| | | | *RYR2* (1) |
| | | | *TTN* (2) |
| | | | *SCN5A* (1) |
| | | | *PPA2* (1) |
| | | | *TRDN* (1) |

Genes (number of patients with a variant) are listed.

## Clinical course

The clinical disease course of patients with an LP/P variant in a gene associated with a cardiomyopathy or cardiac arrhythmia is presented in *Figure 2*. Among patients with an LP/P variant ($n = 17$, excluding *DPP6* risk haplotype), nine received a clinical diagnosis during follow-up. For eight cases, genetic testing influenced the recognition of this disease (8/17, 47%). Variants found in genes associated with cardiac arrhythmia resulted in a diagnosis among five of seven (71%) patients [catecholaminergic polymorphic ventricular tachycardia (three), long-QT syndrome (one), and arrhythmogenic cardiomyopathy (ACM) (one)]. Variants in genes associated with cardiomyopathies less often resulted in a diagnosis (3/10, 30%). Four out of 17 patients had ventricular arrhythmia recurrences during follow-up. Information regarding ventricular arrhythmia recurrences during follow-up for all patients who underwent genetic testing can be found in Supplementary material online, *Table S6*. For one patient, genetic testing was performed post-mortem in research setting. This revealed a pathogenic variant in the *PLN* gene, associated with the diagnosis ACM in this patient.

## Cascade screening of cardiomyopathy variants

Cascade screening results in relatives of patients carrying a variant in cardiomyopathy genes were available for five patients (*MYL2*, *TTN* (three), and *RBM20*). Fourteen family members who underwent genetic testing for the evaluation of one of the three *TTN* variants did either not carry the variant (seven family members) or not yet developed a phenotype (seven family members) fitting dilated cardiomyopathy (DCM). Genetic testing for *RBM20* was initiated after genetic testing in a second-degree family member with DCM that revealed this familial variant. Cascade screening in family members of the patient carrying the *MYL2* variant identified 6 carriers among 12 family members who underwent genetic testing. In this family, both SCD (paternal aunt, age 46 years, SCD in bed, no autopsy) and SCA (paternal cousin, age 27 years) was present; both family members were (obligate) carriers of the familial variant. One asymptomatic carrier was diagnosed with hypertrophic cardiomyopathy (HCM) during follow-up.

# Discussion

This study is, to our knowledge, the largest retrospective study assessing genetic testing in idiopathic VF patients and enhancing our

**Table 3** Yield of LP/P variants and VUS after re-classification, stratified by type of testing that revealed the pathogenic variant

| | All $n = 379$ | Panel testing $n = 289$ | Single-gene testing $n = 90$ | *P* value |
|---|---|---|---|---|
| Patients with LP/P variants | 59 (16%) | 21 (7%) | 38 (42%) | <0.01 |
| Without *DPP6* haplotype patients | 20/340 (6%) | 15/283 (5%) | 5/57 (9%) | 0.35 |
| Causal LP/P variants | 17/340 (5%) | 12/283 (4%) | 5/57 (9%) | 0.18 |
| Patients with ≥ VUS | 113 (30%) | 110 (38%) | 3 (3%) | <0.01 |

Number (%) of patients carrying a variant is presented. Patients with additional single-gene testing besides gene panel testing in which single-gene testing revealed the LP/P variant are listed under single-gene testing.
LP, likely pathogenic; P, pathogenic; VUS, variant of uncertain significance.

**Table 4** LP/P variants in genes associated with a cardiomyopathy or arrhythmia

| Pt. | Gene | Transcript | Nucleotide | Peptide | Classification |
|---|---|---|---|---|---|
| 1 | *SCN5A* | NM_198056.3 | c.392 + 3del | NMD | Class 4: LP |
| 2 | *RYR2* | NM_001035.3 | c.7009G > A | p.(Gly2337Arg) | Class 4: LP |
| 3 | *RYR2* | NM_001035.3 | c.14173T > C | p.(Tyr4725His) | Class 4: LP |
| 4 | *RYR2* | NM_001035.3 | c.11368T > C | p.(Phe3790Leu) | Class 5: P |
| 5 | *RYR2* | NM_001035.3 | c.1244C > G | p.(Thr415Arg) | Class 4: LP |
| 6 | *KCNQ1* | NM_000218.3 | c.1066C > T | p.(Gln356*) | Class 5: P |
| 7 | *SCN5A* | NM_198056.3 | c.2184_2186del | p.(Leu729del) | Class 5: P |
| 8 | *PLN* | NM_002667.5 | c.40_42del | p.(Arg14del) | Class 5: P |
| 9 | *MYL2* | NM_000432.4 | c.64G > A | p.(Glu22Lys) | Class 5: P |
| 10 | *TTN* | NM_001267550.2 | c.52198G > T | p.(Glu17400*) | Class 5: P |
| 11 | *TTN* | NM_001267550.2 | c.76352dup | p.(Pro25452fs) | Class 5: P |
| 12 | *PLN* | NM_002667.5 | c.40_42del | p.(Arg14del) | Class 5: P |
| 13 | *TTN* | NM_001267550.2 | c.8560C > T | p.(Gln2854*) | Class 4: LP |
| 14 | *RBM20* | NM_001134363.3 | c.846_853del | p.(Tyr283fs) | Class 4: LP |
| 15 | *TTN* | NM_001267550.2 | c.4583G > A | p.(Trp1528*) | Class 4: LP |
| 16 | *FLNC* | NM_001458.5 | c.3180del | p.(Asp1061fs) | Class 5: P |
| 17 | *MYH7* | NM_000257.4 | c.5754C > G | p.(Asn1918Lys) | Class 5: P |

LP, likely pathogenic; NMD, non-sense-mediated decay (RNA from blood); P, pathogenic.

knowledge about its role in the diagnostic work-up of these patients. Our study has several interesting findings. First, we show that most idiopathic VF patients received a form of genetic testing. Secondly, the yield for causal LP/P variants is 5%, increasing to 15% due to a large proportion (10%) of variants formed by the *DPP6* risk haplotype. Thirdly, in specific cases, finding an LP/P variant led to recognizing a disease phenotype. Bearing in mind that when the arrest itself does not serve a sufficient phenotype for a diagnosis, a genetic finding alone does not automatically unveil an underlying diagnosis.

## Results of previous studies focusing on genetic testing

Over the years, several studies have been conducted to investigate the yield of genetic testing in SCA survivors without a clear phenotype. Although limited by small sample sizes and depending on the specific population, the studies show similar results indicating the importance of genetic testing (*Table 5*).[6–14,16,20,21] The latest study focusing on idiopathic VF patients showed a similar yield when compared with our study.[14]

## Genetic testing in current clinical practice

It is currently recommended to perform genetic testing in SCA survivors with a suspected genetic cause, and testing should only include genes with an evident gene disease association (Class I).[5] Arrhythmia and cardiomyopathy gene panels may be considered [defined as 'may do this' according to the 2022 European Heart Rhythm Association (EHRA)/Heart Rhythm Society (HRS)/Asia Pacific Heart Rhythm Society (APHRS)/Latin American Heart Rhythm Society (LAHRS) consensus statement on the stage of genetic testing for cardiac diseases and a Class IIb recommendation according to the 2022 ESC guidelines for the management of patients with ventricular arrhythmias and the prevention of SCD] in UCA/idiopathic VF survivors.[3,22] We show that genetic testing is frequently performed in idiopathic VF patients (91%) in the Netherlands. Genetic testing in our study occurred more frequently compared with a previous retrospective overview of genetic testing in UCA from the CASPER study, in which 175 of the 375 UCA survivors (47%) had received genetic testing.[6] In a large population of idiopathic VF patients described by Conte *et al.*,[23] 18% underwent genetic testing. Indeed, these studies date from 2017 and 2019, which is in line with our findings, suggesting that genetic testing is increasingly performed during recent years. Panel testing in our study was abundant (77%), indicating that genetic testing with next-generation sequencing panels in idiopathic VF patients is now increasingly common in clinical practice.

## The genetic yield in idiopathic ventricular fibrillation

Different yields for genetic testing in UCA survivors and idiopathic VF patients have been reported. The difference between these groups is mainly based on the thoroughness of the diagnostic work-up; idiopathic VF patients generally receive a broader diagnostic work-up. In this light, our yield is consistent with that of Grondin *et al.*,[8] who recently showed that the yield of systematic genetic testing is 6% in UCA patients with a complete diagnostic work-up. As expected, higher genetic yields have been reported when a phenotype was present and ranged between 25 and 48%.[6,8,11] In our study, the yield for the *DPP6* risk haplotype, a Dutch founder variant known to be associated with idiopathic VF, was double as high compared with the yield for cardiomyopathy and other arrhythmia variants (39/379, 10%).[24] Since this risk haplotype is associated with idiopathic VF, although apparently mainly limited to the Netherlands, it justifies genetic testing in these patients. After the discovery of the *DPP6* risk haplotype, single-gene testing was initiated in idiopathic VF patients suspected for carrying this variant. This explains why the yield of single-gene testing was abundant in our study. Moreover, while the *PLN* R14del mutation is not limited to the Netherlands, its presence may be higher in our cohort compared

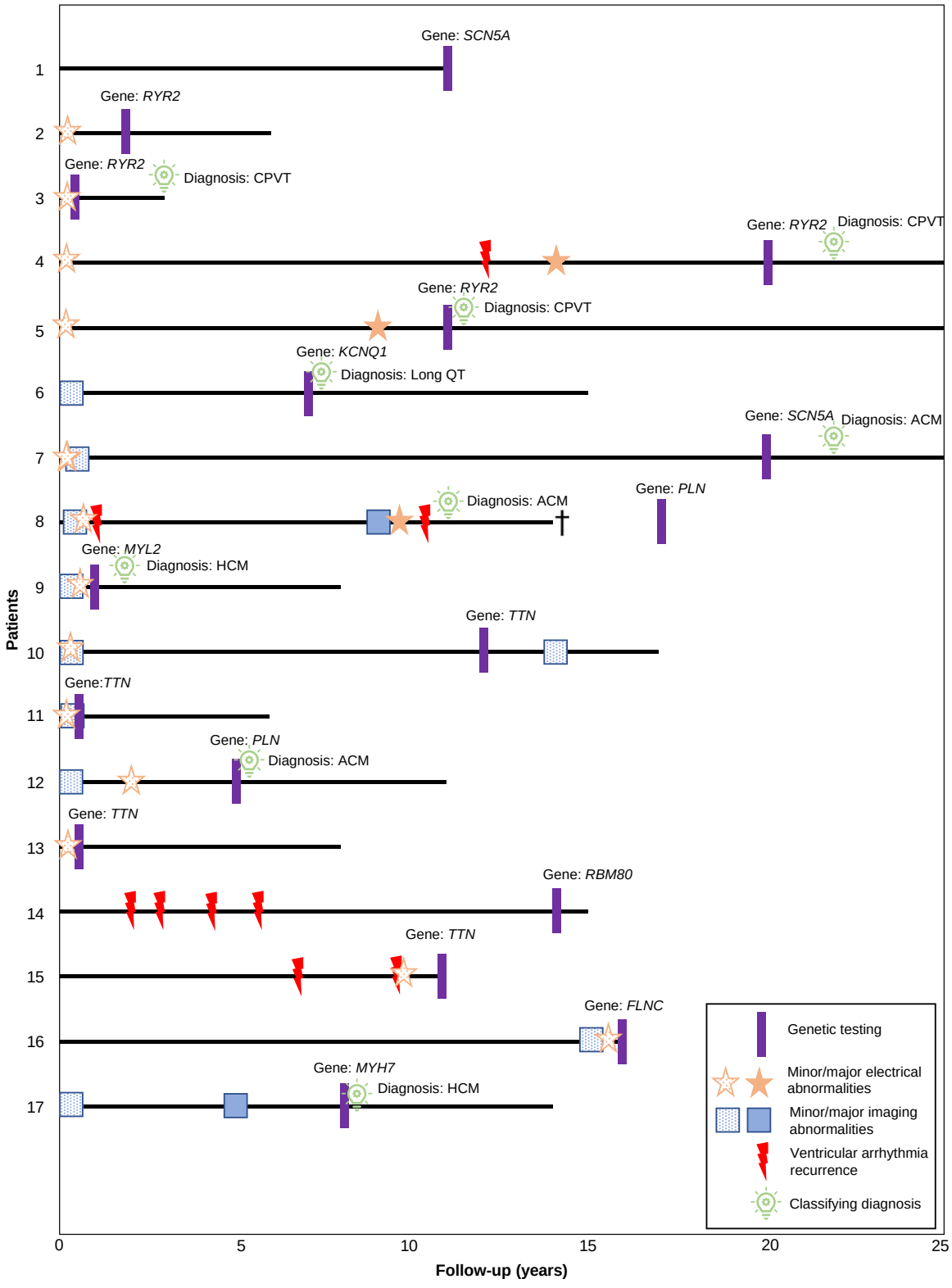



**Figure 2** Clinical course of patients with variants in genes associated with a cardiomyopathy or cardiac arrhythmia. The clinical course of each patient with an LP/P variant is shown. Each patient is represented by a line; the line starts at the moment of VF. The development of phenotypic characteristics (imaging abnormalities or electrical abnormalities), the initiation of genetic testing, ventricular arrhythmia recurrence, and the establishment of a diagnosis are plotted over follow-up years. Electrical abnormalities are premature ventricular contractions or (non-sustained) arrhythmias on Holter or exercise treadmill test. Imaging modalities are structural abnormalities determined by echocardiography or CMR imaging.

**Table 5** Overview of previous studies focusing on genetic testing in patients with UCA or idiopathic VF

| Study | Population | *n* | Gene panel | Yield[a] | LP/P genes |
|---|---|---|---|---|---|
| Asatryan *et al.*[10] | SCA patients without phenotype | 36 | 185 genes associated with hereditary cardiovascular diseases | 10/36 (28%) LP/P<br>18/36 (50%) VUS | *ABCC9*, *DSP*, *KCNE2*, *RANGRF*, *RYR2*, *SCN5A*, *TRPM4*, *TTN* |
| Giudicessi *et al.*[20] | UCA survivors | 49 | No standardized panel of genes | 1/49 (2.0%) LP/P<br>11/49 (22%) VUS | *RYR2* |
| Grondin *et al.*[8] | UCA survivors (CASPER registry)<br>Unexplained after initial testing<br>Idiopathic VF | 228<br>209<br>120 | 53 151 184 or 45 genes associated with sudden cardiac death, cardiomyopathies, or channelopathies | 23/228 (10%) LP/P<br>92/228 (40%) VUS<br>17/207 (8%) LP/P<br>7/120 (6%) LP/P | *CACNA1C*, *COA6*, *DSG2*, *FLNC*, *KCNQ1*, *LMNA*, *MYBPC3*, *MYH7*, *PLN*, *PTPN11*, *RBM20*, *RYR2*, *SCN5A*, *TNNI3K* |
| Isbister *et al.*[7] | Clinical idiopathic SCA survivors | 36 | 174 or 184 genes associated with channelopathies and cardiomyopathies | 8/36 (22%) LP/P<br>25/31 (77%) VUS | *ACTN2*, *DES*, *DSP*, *MYH7*, *MYPBC3*, *PKP2*, *SCN5A* |
| Jiménez-Jáimez *et al.*[21] | UCA survivors | 24 | 126 genes associated with cardiomyopathies and channelopathies | 5/24 (21%) diagnosed after genetic testing | *KCNH2*, *RYR2* |
| Kumar *et al.*[11] | UCA survivors | 52 | Molecular testing when a phenotype was suspected or proven | 12/25 (48%) LP/P | *KCNH2*, *KCNQ1*, *MYBPC3*, *PKP2*, *SCN5A*, *RYR2* |
| Leinonen *et al.*[13] | Unexplained OHCA | 76 | 100 or 21 genes associated with cardiomyopathies and channelopathies | 7/76 (9%) LP/P<br>9/76 (12%) VUS | *CACNA1C*, *DSP*, *RYR2* |
| Mellor *et al.*[6] | UCA survivors (CASPER registry)<br>Phenotype negative | 174<br>102 | No standardized panel of genes | 29/174 (17%) LP/P<br>32/174 (18%) VUS<br>13/102 (13%) LP/P<br>22/102 (22%) VUS | *CACNA1C*, *DSC2*, *DSG2*, *DSP*, *KCNE1*, *KCNE2*, *KCNH2*, *KCNQ1*, *LMNA*, *MYBPC3*, *PKP2*, *PLN*, *RYR2*, *SCN5A*, *TTN* |
| Neves *et al.*[9] | UCA survivors, after arrhythmia panel testing | 38 | 24 cardiomyopathy genes | 3/38 (8%) LP/P<br>4/38 (11%) VUS | *FLNC*, *TTN* |
| Pannone *et al.*[14] | Idiopathic VF patients | 45 | Gene panel for both channelopathies and cardiomyopathies | 3/45 (7%) LP/P<br>9/45 (20%) VUS | *FKTN*, *RYR2* |
| Stepien-Wojno *et al.*[12] | UCA survivors | 31 | 53 arrhythmia-associated genes and 28 HCM-associated genes | 2/31 (7%) LP/P<br>16/31 (52%) VUS | *FLNC* |
| Visser *et al.*[16] | Idiopathic VF patients with normal arrhythmia panel | 33 | 179 genes associated with cardiomyopathies, arrhythmias, or congenital heart disease | 1/33 (3%) LP/P<br>5/33 (15%) VUS | *TTN* |

HCM, hypertrophic cardiomyopathy; LP, likely pathogenic; OHCA, out of hospital cardiac arrest; P, pathogenic; SCA, sudden cardiac arrest; UCA, unexplained cardiac arrest; VUS, variant of uncertain significance; VF, ventricular fibrillation.
[a]Patients carrying a variant or receiving a diagnosis.

with other cohorts.[25] Even though our absolute yield is low (5%), for eight patients (2%) of our cohort, finding an LP/P variant contributed to obtaining a diagnosis and identifying family members at risk. With the impact of VF during life and the implications for family screening, unravelling a diagnosis, even in a small group of idiopathic VF patients, is extremely important. Apart from the yield for LP/P variants, VUS were frequently identified. In 30% of patients, one or more VUS were found. Finding ≥1 VUS with gene panel testing occurs frequently. In smaller cohorts, a prevalence ≥50% has been reported.[7,10,12] The number of VUS increases when larger genetic panels are analysed, which does not necessarily result in a significant rise in yield.[8,16] In a patient group with uncertainties due to the missing diagnosis, increasing uncertainty with VUS is particularly unfavourable. Therefore, appropriate pre- and post-test counselling by specialized genetic counsellors is mandatory.

## Cascade screening and non-diagnostic findings

In addition to an evident phenotype increasing diagnostic yield, for sudden unexplained death, recent studies have shown that the presence of non-diagnostic structural abnormalities (including non-specific late enhancement) increases the yield of pathogenic cardiomyopathy variants in sudden unexplained deaths.[9,26] Due to our relatively low yield in absolute numbers, we were not able to statistically support this finding. However, with our cascade screening and by evaluating the influence

of these variants on diagnosing a patient, our results corroborate that non-diagnostic abnormalities might influence the yield of LP/P variants. Cascade screening in family members of an index patient with an *MYL2* variant shows the importance of combining non-diagnostic findings and genetic results. *MYL2* variants are associated with HCM.[27] In the presented case, CMR revealed septal wall thickness of 12–14 mm, a non-diagnostic finding without a pathogenic genetic variant associated with HCM.[28] Screening of cardiomyopathy genes revealed a pathogenic *MYL2* variant, which eventually resulted in identifying HCM as the cause for the arrest. Without testing cardiomyopathy genes, family members at risk would not have been identified. A similar case with non-diagnostic septal wall thickness is presented in the study from Grondin *et al.*[8] Truly 'concealed' cardiomyopathies were not (yet) identified in our population. Without any phenotype, an LP/P variant cannot result in a diagnosis but can guide further clinical management and cascade screening. The question remains if findings in patients with *TTN*, *FLNC*, and *RBM20* LP/P variants are just bystanders or rather the (contributing) cause of the arrest. We found four LP/P *TTN* variants (4/379; 1.1%), a low prevalence but in accordance with the prevalence of LP/P *TTN* variants in the general population, ranging between 0.14 and 3%.[29–31] However, Bourfiss *et al.*[31] showed, in the UK Biobank, carriers of a DCM-associated variant without a phenotype had a significantly higher prevalence of ventricular arrhythmias.[31] This suggests that even without a phenotype, an LP/P cardiomyopathy variant can contribute to inducing arrhythmias.[7]

**Table 6** Proposed gene panel for idiopathic VF patients

| | | | |
|---|---|---|---|
| *ACTC1* | *ACTN2* | *BAG3* | *CACNA1C* |
| *CALM1* | *CALM2* | *CALM3* | *CASQ2* |
| *DES* | *DMD* | *DPP6* | *DSC2* |
| *DSG2* | *DSP* | *FLNC* | *JUP* |
| *KCNE1* | *KCNE2* | *KCNH2* | *KCNJ2* |
| *KCNQ1* | *LAMP2* | *LMNA* | *MYBPC3* |
| *MYH7* | *MYL2* | *MYL3* | *PKP2* |
| *PLN* | *PPA2* | *PRKAG2* | *RBM20* |
| *RYR2* | *SCN5A* | *SLC4A3* | *TECRL* |
| *TMEM43* | *TNNC1* | *TNNI3* | *TNNT2* |
| *TPM1* | *TRDN* | *TTN* | |

Table 5 and Supplementary material online, Table S8 show specific cardiogenetic disease associations of each gene and current literature.

## Recommendations for the use of genetic testing

Our results are in accordance with previous studies showing that single-gene testing resulted in a high signal-to-noise ratio and broad panel testing in a smaller signal-to-noise ratio.[6,8] We suggest including at least the genes found in our study next to cardiomyopathy genes with a high risk for arrhythmias (*RBM20*, *LMNA*, *FLNC*, *PLN*) in arrhythmia panels.[3,22] Testing additional cardiomyopathy genes may be indicated, in line with suggestions made in previous studies.[7–9]

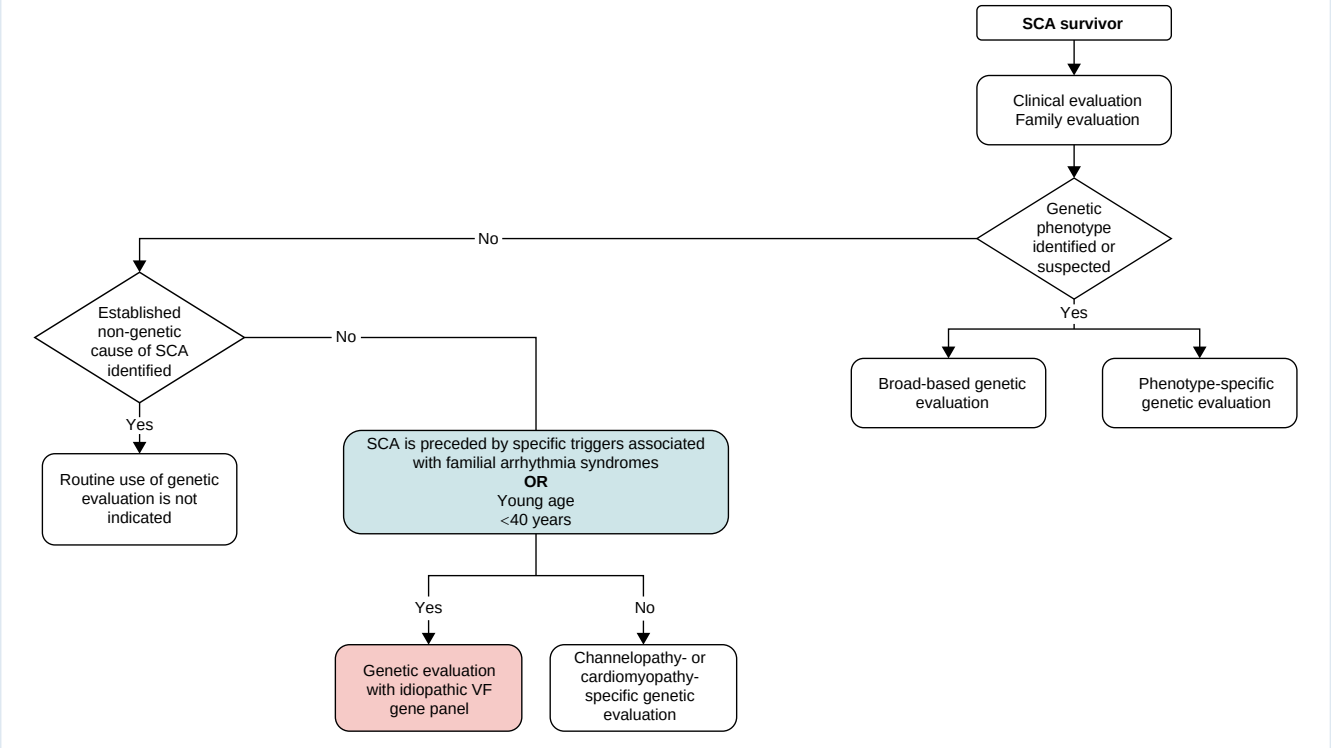



**Figure 3** Proposed flowchart for the genetic evaluation of SCA survivors. Triggers associated with familial arrhythmia syndromes and young age are added to guide indication for genetic testing. When applicable, our proposed idiopathic VF panel can be performed. Modified from Stiles *et al.*, 2020 APHRS/HRS expert consensus statement on the investigation of decedents with sudden unexplained death and patients with sudden cardiac arrest, and of their families, 2021, with permission from Elsevier.[5] SCA, sudden cardiac arrest; VF, ventricular fibrillation.

We propose a specific idiopathic VF gene panel with genes that should be included at minimum for the diagnostic work-up of idiopathic VF patients. The panel includes both arrhythmia and cardiomyopathy genes with definite/good evidence of disease based on the latest consensus statement, our results and that of others (*Table 6*).[6–14,16,20–22,32,33] Broader panel testing with panels including multiple cardiac disease related genes should only be performed in a research setting. Our results show that broad panel testing can result in finding LP/P variants for which a causal link to VF cannot be established (in our study *NEB* and *CPT2*). These variants are therefore not included in our idiopathic VF panel. Other LP/P variants without definite/good evidence of disease found in other studies are not yet included in our panel (*RANGRF*, *ABCC9*, *TRPM4*, *PTPN11*, *TNNI3K*, *COA6*, *FKTN*) but deserve re-evaluating when appropriate.[8,10,14] In addition, by performing a gene panel with only specific indicated genes, the number of VUS will decrease. When evaluating patients who received our proposed panel (patients with either broad panel testing or both an arrhythmia panel and cardiomyopathy panel), we found a yield of 6% (7 causal LP/P genes among 111 patients). In 42%, a VUS was identified. When excluding genes not included in our proposed panel, this number decreases to 27%.

## Implantation in current guidelines

The consensus statement by Stiles *et al.*[5] provides detailed flowcharts on how to initiate genetic testing in SCA survivors (Figure 15 from the expert consensus statement). When we currently follow this flowchart, a Class 2b recommendation for channelopathy- or cardiomyopathy-specific genetic evaluation is presented for idiopathic VF patients. We incorporated our idiopathic VF gene panel into the flowchart and added a step with indicators for genetic testing in idiopathic VF patients to offer guidance for future clinical management (*Figure 3*). Specific triggers preceding familial arrhythmia syndromes are defined as in the consensus statement: competitive athlete, emotional or physical stress, swimming, drug use, acoustic triggers, and seizure.[5] When following the flowchart, the majority of causal LP/P variants besides the *DPP6* risk haplotype will be identified by genetic testing ($n = 14$). The yield for causal LP/P variants in patients with an indication based on any trigger preceding SCA or a young age is 6% (14/257) and 2% (3/162) for patients without an indication. Overall, the yield for causal LP/P variants will slightly decrease to 4%. Supplementary material online, *Table S7* provides an overview of the yield and found variants when stratified by these guidelines. Since the *DPP6* risk haplotype is associated with idiopathic VF in the Netherlands, this will result in a Class 1 recommendation for phenotype-specific genetic evaluation of *DPP6* in the Netherlands. When no triggers are present or patient age is ≥40, consideration of specific genetic evaluation is as previously recommended. Importantly, genetic results should always be combined with a complete diagnostic clinical work-up and its results should be discussed in specialized cardiogenetic centres.[5,19,22,34,35] When no genetic abnormality is revealed, it is currently advised to perform clinical evaluation of only first-degree family members of UCA survivors once.[22] In addition to initiating genetic testing with a uniform approach, reassessing genetic testing and its results are noteworthy. The value of variant re-interpretation has been shown previously.[36] Indeed, our results also indicate the importance of evaluating the performed genetic testing. Since 23% idiopathic VF patients only received single-gene testing, an LP/P may be missed.

## Limitations

Our study has several limitations. First, with the retrospective aspect of the study, genetic testing was not systematically initiated. Confounding by indication, e.g. performing only a cardiomyopathy panel when a cardiomyopathy is suspected, is therefore present and limits our conclusions. Our retrospective design also limited our ability to determine if an indication for genetic testing was present at baseline. Second, referral bias from centres without genetic testing to academic centres cannot be excluded, as referral might only be initiated after the suspicion of a genetic underlying disease. Last, this study is mainly observational with, even though it is currently the largest, only a small number of patients.

# Conclusion

This study provides a detailed overview of genetic testing in idiopathic VF patients according to current practice in the Netherlands. Most idiopathic VF patients received genetic testing. Our yield of causal LP/P variants is 15% and includes a common cause present in the Netherlands (*DPP6* risk haplotype). Finding an LP/P variant can contribute to the recognition of disease, especially when non-diagnostic findings are present. An extension of the current guideline and an idiopathic VF gene panel is proposed.

# Supplementary material

Supplementary material is available at *Europace* online.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## Data availability

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

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