The genetics underlying idiopathic ventricular fibrillation: A special role for catecholaminergic polymorphic ventricular tachycardia?☆

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ABSTRACT

Background: Ventricular fibrillation (VF) is a major cause of sudden cardiac death. In some cases clinical investigations fail to identify the underlying cause and the event is classified as idiopathic (IVF). Since mutations in arrhythmia-associated genes frequently determine arrhythmia susceptibility, screening for disease-predisposing variants could improve IVF diagnostics.

Methods and results: The study included 76 Finnish and Italian patients with a mean age of 31.2 years at the time of the VF event, collected between the years 1996–2016 and diagnosed with idiopathic, out-of-hospital VF. Using whole-exome sequencing (WES) and next-generation sequencing (NGS) approaches, we aimed to identify genetic variants potentially contributing to the life-threatening arrhythmias of these patients. Combining the results from the two study populations, we identified pathogenic or likely pathogenic variants residing in the RYR2, CACNA1C and DSP genes in 7 patients (9%). Most of them (5, 71%) were found in the RYR2 gene, associated with catecholaminergic polymorphic ventricular tachycardia (CPVT). These genetic findings prompted clinical investigations leading to disease reclassification. Additionally, in 9 patients (11.8%) we detected 10 novel or extremely rare (MAF < 0.005%) variants that were classified as of unknown significance (VUS).

Conclusion: The results of our study suggest that a subset of patients originally diagnosed with IVF may carry clinically-relevant variants in genes associated with cardiac channelopathies and cardiomyopathies. Although misclassification of other cardiac channelopathies as IVF appears rare, our findings indicate that the possibility of CPVT as the underlying disease entity should be carefully evaluated in IVF patients.

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1. Introduction

Ventricular fibrillation (VF) is a severe form of cardiac arrhythmia, often resulting in sudden cardiac death (SCD). Typically, VF results from an underlying ischemic, electrical, infectious or structural disease of the heart. Rarely, clinical examinations fail to identify an underlying cause and VF is classified as idiopathic [1–3]. Idiopathic ventricular fibrillation (IVF) is a diagnosis by exclusion, with a likely complex etiology, although in some cases it may have a strong genetic basis. During the past decades, several genetic arrhythmia disorders, such as Brugada syndrome and long QT syndrome (LQTS), used to reside within the category of IVF [3, 4]. Despite the improvements in the diagnosis of these syndromes, concealed forms of these known genetic disorders may still explain a proportion of IVF or SCD incidents [3,5]. For instance, catecholaminergic polymorphic ventricular tachycardia (CPVT), typically caused by mutations in the RYR2 gene, may still get misclassified as IVF [6]. Moreover, mutations in other arrhythmia-associated genes such as SCN5A and KCNH2 may initially manifest as VF, although in most of these cases an underlying electrical disease is later identified [7–9].

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Compared to other arrhythmic diseases, there have been relatively few studies focusing on characterizing the genetic landscape of IVF [3,10]. Genes more recently associated with IVF include DPP6 and SEMA3A, identified in a Dutch family and in a Japanese cohort, respectively, and the newly-identified CALMI [11–13]. The reported yield of genetic testing in all IVF cohorts is however relatively low [3,10]. Overall, these observations suggest that the genetic background of IVF is likely heterogeneous and that it could also be of non-mono- genic origin [3,10].

By combining the results from two independent European IVF cohorts, we aimed to characterize the spectrum of genetic variation underlying IVF. Our study suggests that a subset of patients initially referred for clinical, electrophysiological and molecular genetic testing in all IVF cohorts is however relatively low [3,10].

2. Methods

2.1. Subjects

The Finnish cohort includes 36 unrelated patients (15 males and 21 females) drawn from the Finnish Inherited Arrhythmia Disorder Registry. These patients were resuscitated following out-of-hospital VF of unknown cause between the years 1996 and 2011, and had a mean age of 31 ± 11 years when experiencing the arrhythmic episode (range 12–48 years). The Italian cohort includes 40 patients (20 males and 20 females) referred to the investigators of the Center for Cardiac Arrhythmias of Genetic Origin from 2006 to 2016. The patients’ mean age at VF was 32 ± 16 years (ranges 0–63 years). The main clinical characteristics of the patients are summarized in Tables 1 and 2.

All patients underwent routine clinical work-up in order to identify the cause of VF, before receiving a diagnosis of IVF and an implantable cardiac defibrillator (ICD) in the referring hospital. The details of the clinical tests performed for each patient are provided in Supplemental Tables A.1 and A.2. No patient had a previous history of a cardiac condition, nor clinical findings in resting electrocardiogram (ECG), coronary artery angiography, or cardiac imaging explaining the VF. For the purpose of the current study, patients’ medical history was re-evaluated by reviewing available hospital records. The patients were contacted if abnormal findings, related to potentially significant genetic variants, emerged. In the analysis of the relatives we relied on anamnestic information and referred from a broader clinical investigation if there was no reason to suspect a particular disease entity. Informed consent was obtained from all patients. All investigations were performed in accordance with the Helsinki Declaration and approved by the local ethical review boards.

2.2. DNA sequencing

The Finnish cohort was genetically evaluated with whole exome sequencing (WES) focusing on 100 genes associated with channelopathies and cardiomyopathies selected based on the review by Wilde and Behr (Supplemental Table A.3) [14]. DNA sequencing was performed at the Technology Center at the Institute for Molecular Medicine Finland, FIMM. The exome targets were captured with the NimbleGenSeqCapEZ Human Exome Library v2.0 (www.nimblegen.com/products/seqcap/ez/index.html), followed by sequencing with the Illumina Genome Analyzer-Ibx platform. The alignment to the human reference genome hg19 and variant calling of
chromosomal regions was performed using the variant calling pipeline of the FIMM. The mean target coverage in the exome sequencing was 44.7×. Details about coverage are chromosomal regions was performed using the variant calling pipeline of the FIMM. The mean target coverage in the exome sequencing was 44.7×. Details about coverage are chromosomal regions was performed using the variant calling pipeline of the FIMM. The mean target coverage in the exome sequencing was 44.7×. Details about coverage are chromosomal regions was performed using the variant calling pipeline of the FIMM. The mean target coverage in the exome sequencing was 44.7×. Details about coverage are chromosomal regions was performed using the variant calling pipeline of the FIMM. The mean target coverage in the exome sequencing was 44.7×. Details about coverage are chromosomal regions was performed using the variant calling pipeline of the FIMM. The mean target coverage in the exome sequencing was 44.7×. Details about coverage are.
3. Results

3.1. Clinical characteristics of the patients

The study population consisted of two independent European cohorts, from Finland and Italy. The resting ECG findings, electrophysiological study (EP) data, and cardiac ultrasonography results for each patient are summarized in Tables 1 and 2. Except for two cases, in the resting ECGs, the PQ interval was ≤200 ms and QTc ≤480 ms in all patients. The mean QTc was 424 ± 30.5 ms and 411 ± 29 ms in the Finnish and Italian cohorts, respectively. Based on cardiac ultrasonography, the left ventricular ejection fraction was ≥50% in all patients either after the episode or in the follow-up. The mean follow-up time with the ICD was 9.1 ± 3.4 years in the Finnish and 3.2 ± 3.3 years in the Italian cohort.

A review of the patients' medical records showed that the number of premature ventricular complexes (PVCs) in 24 h exceeded 1000 in 8 of 52 subjects studied by ambulatory 24-hour ECG recordings. Signal-averaged ECG was performed in 14 patients (18%) and showed late potentials in three cases (F1, F19 and I17). Endomyocardial biopsy was performed in 22 patients and altogether 47 (62%) patients underwent cardiac magnetic resonance imaging (MRI), without clinically significant findings. An EP study with programmed ventricular stimulation was carried out in 39 (51%) patients. In four cases (F16, F17, I2, I3) ventricular stimulation induced sustained polymorphic ventricular tachycardia (VT) which degenerated into VF. An exercise stress test was carried out in 25 (69%) Finnish and 32 (80%) Italian patients. Altogether 15 (20%) patients received appropriate ICD shocks during the follow-up.

3.2. Genetic findings

To identify putative highly penetrant IVF-associated genetic variants, we initially filtered the datasets by variant frequency (MAF < 0.005%) corresponding to a disease incidence of <1:10,000. Adding PolyPhen-2 filtering for the WES dataset, the total yield was 25 candidate variants, 13 in the Finnish study population and 12 in the Italian. Upon subsequent scrutiny of the patients' clinical data and assessment of the segregation patterns of the variants in the respective families, when available, 7 variants in 7/76 patients (9%) were classified as pathogenic or likely pathogenic (Table 3A).

3.3. Pathogenic and likely pathogenic variants

Three of the pathogenic and likely pathogenic variants were identified in the Finnish and four in the Italian population (Table 3A). The majority of these variants affected the CPVT gene RYR2, with two Finnish patients and three Italian patients carrying 3 novel (F10:p.Leu575Phe; F27:p.Gln3774Arg; I34:p.Met399Leu) and 2 CPVT associated (I28:p.Met4002Ile; I40:p.Glu1724Lys) pathogenic variants. One Finnish patient carried a previously described nonsense mutation in the LQT5-associated gene CACNA1C (F30:p.Gly4025Ser) [23], while one Italian patient carried a novel nonsense mutation in the ARVC-related DSP gene (I12:p.Gln620*). Notably, for 4 of these patients (F10, F30, I28 and I34) the mutations have arisen de novo and were absent from the unaffected parents (Supplemental Fig. A1.1). Although the parents of the remaining cases were unavailable for genetic testing, two of these patients (F27 and I40) showed positive family history of SCD. Most importantly, the genetic findings spurred re-evaluation of the original clinical data and led to a renewed clinical assessment of the patients, causing disease reclassification. Particularly, for all five cases with RYR2 mutations, the updated clinical data was compatible with a CPVT diagnosis. Clinical descriptions of all cases carrying pathogenic and likely pathogenic variants, as well as some of the cases with VUSs and likely benign variants, are provided in the Appendix.

Patients carrying pathogenic and likely pathogenic variants were generally younger at the time of the VF incident in comparison to the remaining 69 patients (19.9 ± 19.5 SD [median 13] vs 32.4 ± 12.7 SD [median 32] years, P = 0.01). When comparing other clinical parameters (Tables 1, 2 and 4), these subjects did not significantly differ from the rest of the cohort.

In both datasets, the likely pathogenic variants and the VUSs were SNVs detected under the analysis model considering dominant mutations. In the Finnish data, the extended gene sequencing, as well as the recessive model, failed to identify additional pathogenic variants. Moreover, no large deletions/insertions were identified in the CNV analysis.

3.4. Identification of variants of unknown significance (VUSs) and variants with demonstrated functional effects

For most of the patients we did not identify variants meeting all the criteria for pathogenic variants. However, in addition to the 7 pathogenic or likely pathogenic variants, we detected 10 variants (3 novel; 13%) satisfying the frequency cut-off in 7 different genes (SCN5A, KCNE1, KCNQ4, MYH7, DSP, LDB3, TTN) in 9 subjects. Although bioinformatics tools support a functional effect for most, they did not satisfy all the criteria for assignment of a pathogenic or likely pathogenic status and were classified as VUS (Table 3B, and Appendix). Current genetic knowledge implies that beyond the classical disease-causing single-gene defects, the overall genetic background might be particularly relevant in disease manifestation and expressivity [24]. To complete the genetic characterization of our cohorts, after concentrating on potentially highly penetrant variants, we focused our attention on the 21 arrhythmia- and cardiomyopathy-associated genes interrogated in both datasets. We first chose to interrogate variants in the 0.005–0.05% MAF range, falling below the estimated prevalence of arrhythmogenic diseases such as LQTS (1:2000) and ARVC (1:5000) [25,26]. Within this MAF range we identified 7 variants in 8 IVF subjects in 4 different genes (CASQ2, RYR2, MYBPC3, DSC2) that were classified as VUSs (Table 3C).

Lastly, we chose to analyze our sequencing datasets for the presence of rare variants (MAF < 0.5%) in the aforementioned genes with a previously demonstrated functional effect in vitro. Six such variants in 5 different genes (KCNE1, KCNE2, SCN5A, MYBPC3 and LDB3) were present in 8 subjects of our IVF cohort (Table 3D). Although these variants are certainly not primarily disease-causing, they may however play a favoring role in disease manifestation and arrhythmia susceptibility.

4. Discussion

The present study provides the first evidence of a clearly quantifiable (9%) contribution by identifiable genetic cardiac disorders to the occurrence of IVF. Importantly, for the patients with a pathogenic or likely pathogenic variant, the molecular genetic findings prompted re-evaluation of the clinical data, revealing incomplete clinical investigation in some cases and ultimately leading to disease reclassification. As 71% of these variants were identified in the CPVT gene, our data point to CPVT as the most commonly missed diagnosis. Therefore, the present findings suggest that after excluding the obvious causes, the possibility that CPVT might have caused the IVF event should be considered with high priority [41].

Interestingly, the results from two independent European IVF cohorts, representing the northern and southern borders of the continent, depict a rather uniform picture of the genetic architecture of IVF. Whereas both cohorts included a significant proportion of cases with RYR2 variants, the majority of patients in both study populations were left without a genetic diagnosis. Yet, some of the novel or extremely rare (MAF < 0.005%) genetic variants that we stringently classified as VUSs (n = 10) may in the end prove to be disease-associated variants, thereby further increasing the yield of genetic testing.
### Table 3
Main genetic findings in the Finnish and Italian IVF patients.

<table>
<thead>
<tr>
<th>ExAC (ALL)</th>
<th>ExAC (EUR-nF)</th>
<th>gnomAD</th>
<th>SNP ID</th>
<th>PP2</th>
<th>SIFT</th>
<th>SIFT HP</th>
<th>Mutation Taster</th>
<th>M-CAP</th>
<th>Conservation</th>
<th>Literature</th>
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<tbody>
<tr>
<td><strong>A. Pathogenic and likely pathogenic variants (ExAC MAF &lt; 0.005%)</strong></td>
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<tr>
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<td>pD</td>
<td>D T D</td>
<td>P Full</td>
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<tr>
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<tr>
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<td>D D D D</td>
<td>P Full</td>
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<tr>
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<td>P Full</td>
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<tr>
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<tr>
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<td>B T T D</td>
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<tr>
<td><strong>SCN5A</strong> 55</td>
<td>c.2877A&gt;G;p.Glu96Arg</td>
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<td>B T T D</td>
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<td>B T T D</td>
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<tr>
<td><strong>RYR2</strong> 37</td>
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<td>D D D D</td>
<td>P Full</td>
<td>–</td>
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<tr>
<td><strong>RYR2</strong> 90</td>
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<td>–</td>
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<td>–</td>
<td>pD</td>
<td>D T D</td>
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<tr>
<td><strong>MYBPC3</strong> 31</td>
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<td>B T T D</td>
<td>P Full</td>
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**B. Variants of unknown significance (ExAC MAF < 0.005%)**

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<th>Conservation</th>
<th>Literature</th>
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<td><strong>MYH7</strong> 4</td>
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<td><strong>SCN5A</strong> 17</td>
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**C. Variants of Unknown Significance (ExAC MAF 0.005–0.5%)**

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<td><strong>D. Variants with demonstrated functional effects (ExAC MAF &gt; 0.5%)</strong></td>
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<tr>
<td><strong>MYBPC3</strong> 31</td>
<td>c.3413G&gt;A;p.Arg1138His</td>
<td>0.0013/105</td>
<td>0.00066/30</td>
<td>0.017/72</td>
<td>0.0012/320</td>
<td>rs371564200 D</td>
<td>D D D D</td>
<td>P High</td>
<td>–</td>
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**Genetic variants identified in the Finnish and Italian IVF cohorts**

Mutation nomenclature conforming to the latest Human Genome Variation Society guidelines has been assigned according to the isoforms specified in Supplemental Table 3, unless otherwise stated [40]. MAF/Allele count are reported separately for each variant in the European Finnish (EUR-F), European non-Finnish (EUR-nF) and the total population (ALL) of the Exome Aggregation Consortium browser (ExAC) and the Genome Aggregation database (gnomAD). In silico variant effect predictions (see Supplemental References in the Appendix) are presented by Polyphen-2 (PP2; D-Probably Damaging, pD-Possibly Damaging, B-Benign), SIFT (D-Damaging, T-Tolerated), SIFT Human Protein (SIFT HP; D-Damaging, T-Tolerated), Mutation Taster (D-Disease-causing, P-Polymorphism), M-CAP (P-Possibly Pathogenic, B-Likely Benign). NA denotes Not available/Not applicable. Conservation has been interrogated in the UCSC genome browser (https://genome.ucsc.edu) by multiple alignment of 100 vertebrates (Full- fully conserved; High- at least 95% conserved; Medium- at least 70% conserved; Low< 70% conserved, in all species available). Literature references for each variant refer either to the first published report or the most relevant functional study.
4.1. Identification of pathogenic and likely pathogenic variants predisposing to IVF

Idiopathic VF is a diagnosis by exclusion, established in the absence of clinical findings explaining the arrhythmia. However, despite comprehensive clinical examinations it may be challenging to distinguish IVF from established cardiac channelopathies. For example, while a positive exercise stress test is highly suggestive of CPVT, a negative test does not rule out the diagnosis [42]. Furthermore, after a cardiac arrest, an exercise stress test off therapy - the gold standard for the diagnosis of CPVT - is not always feasible. Thus, screening of established arrhythmia-associated genes may favor a correct clinical diagnosis. Indeed, here we show that molecular screening supported the identification of five previously undiagnosed CPVT cases. These patients carry either novel likely pathogenic RYR2 variants (n = 3), most of which have arisen de novo (n = 2), or previously-described CPVT-associated pathogenic variants in the RYR2 gene (n = 2), generally located within or in the proximity of CPVT-associated RYR2 mutation hotspots [43]. An exercise stress test off beta-blocker therapy was not originally performed in four cases. For I28 and I40 severe post-anoxic sequelae occurred, for I34 beta-blocker therapy was immediately started to stabilize the clinical condition, and F27 initially refused to undergo the test. In the case of F10, findings suggestive of CPVT were originally ignored. Altogether, in our cohort we thus identified a CPVT-related mutation in 6.6% of IVF patients. This percentage is not surprising considering a reported 13% prevalence of CPVT in patients with cardiac arrest without overt heart disease [4,5]. Nonetheless, our results underscore the fact that CPVT could be a commonly missed diagnosis in IVF, stressing the importance of including an exercise stress test before starting beta-blocker therapy, whenever possible.

While the current study enabled the diagnosis of CPVT in five patients, the only pathogenic mutation detected in an LQTS-associated gene in our IVF cohort was a mutation in CACNA1C. This result implies that undiagnosed LQTS is not a major contributor to IVF cases assessed in expert referral centers, unlikely to miss a prolongation of the QT interval. Indeed, in the Italian group’s experience some cases initially referred as IVF were soon attributed to LQTS or Brugada Syndrome after a comprehensive clinical and molecular investigation [4,44]. Unfortunately, sequencing data were not available for CACNA1C in the Italian cohort. However, one Italian patient (I12) carried a pathogenic nonsense mutation in DSP with clear changes in cardiac electrophysiology, but without definite clinical manifestations typical for DSP-associated cardiomyopathies. Noteworthy, the Italian cohort contained three other carriers of rare variants in DSP (patients I19, I23, I34), classified as VUS, whereas rare DSP variants were absent in the Finnish population. A potential explanation for this discrepancy might be the higher estimated prevalence of ARVC in Italy.

4.2. Challenges in IVF genetic testing

The recent understanding of human genetic variation indicates that healthy individuals in fact may carry dozens of variants disrupting gene function, most of which have little or no effect on health [45]. This questions the pathogenicity of some of the variants previously associated with disease and highlights current limitations in evaluating variant pathogenicity, also for syndromes like CPVT [46–48]. Obviously, stringent standards are warranted when interpreting the consequences of sequence variants, as we feel we have applied in our study, albeit without functional validation. Partly due to the lack of high-throughput methods to functionally study the NGS findings, it is imperative that any uncertainties or ambiguities are clearly conveyed and revisited over time for variants which are considered clinically-actionable [49]. Our results also support a recent study showing that large gene panels in IVF do not considerably increase the yield of positive results compared to targeted sequencing [50]. This may reflect limitations in the current genetic knowledge, or the fact that for many patients IVF is not primarily monogenic in origin.

The concept of IVF being not exclusively a monogenic disease is not new in the field [3]. As our understanding of the complexity of the genome constantly increases with newly-acquired genetic methodologies, so does the appreciation that in several instances single gene defects constitute only a small portion of the etiology behind genetic diseases, let alone the complex traits [24]. While single genetic variants with large effects may explain almost a tenth of IVF cases, as in the current study, genetic variants with smaller effects acting in synergy may prove to form part of particular genetic disease signatures. In the latter category, non-coding variation is naturally expected to come into play. And then the plot is expected to further thicken.

4.3. Study limitations

The limitations of the study include different screening methods for our two cohorts, despite similar results for the 21 genes under the primary focus of the study. Moreover, our studies rely on publicly available reference sequence data as a control dataset, which has been generated independently from our study. We also lack functional studies for the identified variants.

5. Conclusions

Our data suggest that WES and NGS strategies, focusing on variants in established arrhythmia-associated genes, may lead to a more accurate diagnosis for a subset of patients resuscitated from VF without an identifiable cause. Particularly, our results emphasize the importance of excluding CPVT as the cause for the unexplained arrhythmia, both clinically and molecularly. For the majority of the patients, however, pathogenic mutations could not be identified, likely reflecting the complex etiology underlying IVF.

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Conflict of interest disclosures
None.

Appendix A. Supplementary data
Supplementary data to this article can be found online at https://doi.org/10.1016/j.jjcard.2017.10.016.

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