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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.

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].

☆ All authors take the responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation.

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Compared to other arrhythmogenic diseases, there have been relatively few studies focusing on characterizing the genetic landscape of IVF [3,10]. Genes more recently associated to IVF are *DPP6* and *SEMA3A*, identified in a Dutch family and in a Japanese cohort, respectively, and the newly-identified *CALM1* [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-monogenic 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 diagnosed with IVF carry clinically-relevant variants in genes associated to inherited arrhythmogenic diseases.

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 refrained 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-IIx platform. The alignment to the human reference genome hg19 and variant calling of

Table 1
Clinical, electrophysiological and molecular genetic findings – The Finnish cohort.

			Electrocardiogram				24 h ECG	EP study		Echocardiography			
DNA#	Age at VF	Sex	hr (min^{-1})	PQ (ms)	QRS (ms)	QTc (ms)	VES/24 h	HV (ms)	VT/VF	LVEDD (mm)	LVEDD (mm)*	EF %	Variant(s)
F1	25	M	80	144	98	416	4	39	No	45	34	55	
F2	37	M	80	130	100	393	0	NA	No	46	46	66	
F3	38	F	68	150	85	436	1646	NA	No	54	53	63	
F4	39	F	73	166	92	474	4110	56	No	51	52	65	
F5	20	M	47	198	112	372	8	NA	No	57	52	68	
F6	36	M	50	170	130	475	NA	62	No	53	49	50	
F7	18	F	50	184	86	374	24,987	50	No	47	53	64	*MYBPC3*-p.Arg1138His
F8	30	M	76	180	70	439	NA	49	No	49	56	65	
F9	12	F	94	120	76	432	0	NA	No	42	51	74	
F10	**12**	**M**	**75**	**150**	**100**	**447**	**NA**	**42**	**No**	**48**	**57**	**62**	***RYR2*-p.Leu575Phe**
F11	44	F	93	148	80	423	2981	NA	NA	55	58	68	
F12	23	F	68	144	76	383	NA	45	No	50	56	60	
F13	22	M	64	152	84	449	NA	41	No	57	57	60	
F14	44	F	60	180	150	470	NA	69	No	56	48	51	*DMD*-p.Leu1264Ser; *MYBPC3*-p.Arg1138His
F15	30	M	71	136	104	392	NA	44	No	53	55	69	
F16	15	M	73	178	96	474	10	45	Yes	51	50	66	*HCN4*-p.Arg713His; *RYR2*-p.Asn3308Ser
F17	39	M	60	150	70	400	11,321	44	Yes	53	47	60	*SGCD*-p.Ala204Ser
F18	42	F	70	164	86	464	1696	NA	NA	54	59	61	
F19	43	M	53	144	106	395	84	38	No	52	51	60	*MYH7B*-p.Arg231Trp, p.Lys108Asn; *SCN5A*-p.Ala572Asp
F20	23	F	70	140	80	432	NA	59	No	46	52	57	
F21	43	F	87	166	74	458	9797	48	No	51	50	70	*MYH7B*-p.Asp1096His; *FKRP*-p.Trp418*
F22	18	M	72	166	100	394	NA	50	No	41	43	65	
F23	22	M	90	148	94	429	NA	37	No	53	53	68	
F24	34	M	63	173	100	410	36	50	No	60	48	66	*TTN*-p.Arg24987*, p.Lys29627Thr; *SCN5A*-p.Pro2006Ala
F25	35	F	66	142	95	420	NA	36	No	56	50	54	
F26	17	F	96	120	100	430	NA	NA	NA	49	51	60	
F27	**18**	**F**	**79**	**154**	**100**	**459**	**NA**	**NA**	**NA**	**57**	**48**	**67**	***RYR2*-p.Gln3774Arg**
F28	48	M	67	176	94	391	NA	64	No	61	61	50	
F29	43	F	53	152	92	432	41	46	No	45	44	70	
F30	**13**	**F**	**65**	**150**	**80**	**448**	**0**	**32**	**No**	**35**	**43**	**60**	***CACNA1C*-p.Gly402Ser;***CRYAB-p.Pro46Leu; *DMD*-p.Leu2792Phe
F31	19	F	52	156	92	410	NA	NA	NA	48	49	65	*RYR2*-p.Gly2367Arg; *SCN5A*-p.Ala572Asp
F32	47	F	90	150	108	416	NA	NA	NA	58	58	63	
F33	38	F	53	156	90	385	NA	NA	No	53	48	71	
F34	31	M	61	170	80	403	3676	39	No	56	57	51	
F35	44	F	50	152	90	374	13	50	No	46	44	58	
F36	39	F	84	154	100	473	40	49	No	46	41	57	*SCN5A*-p.Ala572Asp
Mean	30.6		69.5	155.9	93.6	424.2		47.4		50.9	50.7	62.2	

Clinical, electrophysiological and molecular genetic findings of IVF patients. The carriers of pathogenic and likely pathogenic variants are indicated in bold. 24 h ECG = 24 h ambulatory electrocardiogram, EP study = electrophysiological study, VF = ventricular fibrillation, HR = heart rate, VES = ventricular extrasystole, VT = ventricular tachycardia, LVEDD = left ventricular end diastolic diameter, EF = ejection fraction, NA = not available. * = LVEDD and EF at the end of the follow-up.

Table 2
Clinical, electrophysiological and molecular genetic findings – The Italian cohort.

			Electrocardiogram				24 h ECG	EP study		Echocardiography				
DNA#	Age at VF	Sex	hr (min^{-1})	PQ (ms)	QRS (ms)	QTc (ms)	VES/24 h	HV (ms)	VT/VF	LVEDD (mm)	LVEDD (mm)*	EF %	EF %*	Variant(s)
I1	26	M	60	146	98	405	0	NA	NA	49	47	45	52	*MYBPC3*-p.Asp610His
I2	29	F	69	168	102	427	0	NA	Yes	49	46	55	63	*KCNE1*-p.Arg32His
I3	33	M	52	140	106	392	2	NA	Yes	51	48	52	57	
I4	63	F	100	200	80	439	NA	NA	NA	NA	NA	50	NA	
I5	0	M	110	120	70	367	0	NA	NA	30	36	59	60	
I6	41	M	47	170	90	416	129	NA	NA	48	53	60	74	
I7	51	M	77	140	100	410	1792	NA	NA	50	45	72	58	
I8	50	F	57	150	70	397	NA	NA	NA	NA	NA	45	60	*KCNE1*-p.Gln96Arg
I9	37	F	56	158	104	388	19,567	NA	NA	55	54	56	55	
I10	27	F	71	160	70	452	14,888	NA	NA	56	56	65	60	*RYR2*-p.Ala2354Thr
I11	16	F	62	128	88	430	110	NA	NA	43	49	65	58	
I12	**21**	**M**	**60**	**160**	**100**	**390**	**1595**	**NA**	**No**	**49**	**NA**	**48**	**NA**	***DSP*-p-Gln620***
I13	60	M	46	236	82	388	NA	NA	NA	NA	NA	55	NA	
I14	43	M	68	160	80	416	83	Normal	No	50	49	58	55	
I15	32	M	62	140	80	394	NA	NA	NA	NA	NA	60	NA	
I16	37	F	65	110	80	470	62	Normal	No	NA	42	60	62	*LDB3*-p.Ala340Pro
I17	47	F	64	152	102	427	530	NA	NA	51	NA	56	NA	
I18	43	M	44	225	100	387	33	NA	NA	NA	57	60	57	
I19	20	F	73	130	70	415	1	48	No	44	49	47	58	*DSP*-p.Lys1094Asn
I20	30	F	55	140	90	387	31,704	NA	NA	51	48	56	55	
I21	22	F	53	170	80	456	16,999	NA	NA	NA	NA	20	50	*CASQ2*-p.Asp351Gly; *LDB3*-p.Pro446Ser, p.Ser189Leu
I22	17	F	70	180	80	430	37	NA	NA	NA	NA	55	59	
I23	14	M	78	160	80	356	NA	NA	NA	NA	NA	35	55	*DSP*-p.Glu1898Gln
I24	29	F	52	140	80	430	869	NA	NA	56	50	62	63	*DSC2*-p.Gly863Arg
I25	25	M	55	170	84	417	3180	NA	NA	54	49	53	60	
I26	16	M	90	120	70	406	343	NA	NA	51	46	60	62	
I27	18	F	90	170	80	381	2	NA	NA	NA	48	62	60	
I28	**2**	**M**	**90**	**110**	**80**	**376**	**2**	**NA**	**NA**	**38**	**NA**	**60**	NA	***RYR2*-p.Met4002Ile**
I29	32	F	64	144	92	409	10,000	NA	NA	48	50	55	50	*MYBPC3*-p.Ser217Gly
I30	56	F	56	160	130	394	3	NA	NA	45	31	70	65	
I31	31	F	52	128	88	420	8	Normal	No	51	52	42	65	
I32	40	F	90	130	80	413	613	NA	NA	43	48	66	60	
I33	45	F	51	158	82	418	40	NA	NA	NA	45	60	72	
I34	**11**	**M**	**81**	**100**	**70**	**470**	**0**	**NA**	**NA**	**50**	**47**	**22**	**58**	***RYR2*-p.Met399Leu;** *DSP*-p.Val2518Ile; *SCN5A*-p.Thr1069Arg; *KCNE2*-p.Ile57Thr
I35	16	M	48	160	90	362	2	NA	NA	47	48	60	60	
I36	47	M	51	140	94	386	0	NA	NA	42	43	70	60	
I37	22	M	75	156	90	430	0	NA	NA	48	51	60	62	
I38	20	M	70	130	96	392	1	normal	No	48	NA	52	NA	
I39	40	M	80	160	80	476	NA	Normal	No	56	NA	58	NA	
I40	**62**	**F**	**100**	**NA**	**70**	**437**	**NA**	**NA**	**NA**	**NA**	**NA**	**58**	NA	***RYR2*-p.Glu1724Lys;** *MYH7*-p.Met113Leu
Mean	31.8		67.4	148.0	86.5	411.4				48.3	47.7	55.1	59.5	

Clinical, electrophysiological and molecular genetic findings of IVF patients. The carriers of pathogenic and likely pathogenic variants are indicated in bold. 24 h ECG = 24 h ambulatory electrocardiogram, EP study = electrophysiological study, VF = ventricular fibrillation, HR = heart rate, VES = ventricular extrasystole, VT = ventricular tachycardia, LVEDD = left ventricular end diastolic diameter, EF = ejection fraction, NA = not available. * = LVEDD and EF at the end of the follow-up.

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 provided in Supplemental Table A.4. For annotation of the variants we utilized the ANNOVAR software [15]. To ensure complete sequencing coverage of major cardiac channellopathy genes in the Finnish dataset, we subjected suboptimally covered exons in *KCNQ1*, *KCNH2*, *SCN5A*, *CACNA1C* and *RYR2* genes (Supplemental Table A.5) to Sanger sequencing.

The Italian cohort was genetically evaluated with next-generation sequencing (NGS) and a custom gene panel of 21 genes (TruSeq Custom Amplicon, MiSeq platform, Illumina) endorsing the International Expert Consensus Statement guidelines for genetic testing in patients with channelopathies and cardiomyopathies, as previously described [16–17]. All exonic regions with low coverage (<20×) were subjected to Sanger sequencing. Therefore, final sequencing coverage in the Italian cohort was 100% (Supplemental Table A.5).

For both cohorts, the genetic variants reported in the main text were verified by Sanger sequencing, as previously described [8,18].

2.3. Whole-exome and custom next-generation sequencing variant analysis

We focused our attention on protein-coding genetic variation. Considering both dominant and recessive disease models, we analyzed single nucleotide variants (SNVs) and small insertions and deletions predicted to result in missense, nonsense, splice site or frameshift mutations. In order to identify penetrant IVF-causing variants, we set a stringent minor allele frequency (MAF) filter criterion of <0.005%, based on the ExAC and gnomAD browsers containing sequencing data from over 60,000 and 120,000 individuals, respectively. Simultaneously, these databases, including Finnish and Italian samples, served as population controls for our study [19].

For the Finnish cohort that underwent WES, additional criteria included MAF < 0.05% in the Finnish SISu database (http://sisu.fimm.fi) and a PolyPhen-2 prediction suggesting a damaging or possibly damaging effect. The less stringent MAF criterion for the SISu dataset is based on the smaller size of the database and the potential enrichment of variants in the Finnish population [20]. For the recessive model, we utilized a MAF cutoff < 0.5%, to screen for potential homozygous, hemizygous and compound heterozygous mutations. Additionally, for the Finnish cohort we performed exome-wide copy number variation (CNV) analysis to screen for larger deletions and insertions involving the arrhythmia-associated genes using the XHMM pipeline [21].

Finally, data from both cohorts were analyzed for potentially clinically-significant variants falling within the 0.005–0.5% MAF range in the 21 established channelopathy and cardiomyopathy-associated genes [16,17].

2.4. Variant classification criteria

Our classification criteria are based on the current recommendations of the American College of Medical Genetics and Genomics [22] with details provided in the Supplemental Methods. Genetic variants identified in both populations were classified into five classes: 1) Pathogenic 2) Likely pathogenic, 3) Variants of Unknown Significance (VUS), 4) Rare variants with demonstrated functional effects, and 5) Likely benign.

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 missense mutation in the LQTS-associated gene *CACNA1C* (F30:p.Gly402Ser) [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. A.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*, *HCN4*, *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 *RYR2* 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.

Gene	Exon	Variant	ExAC (ALL)	ExAC (EUR-nF)	ExAC (EUR-F)	gnomAD	SNP ID	PP2	SIFT	SIFT HP	Mutation Taster	M-CAP	Conservation	Literature
A. Pathogenic and likely pathogenic variants (ExAC MAF<0.005%)														
DSP	14	c.1858C>T:p.Gln620*	–	–	–	–	–	NA	NA	NA	D	NA	NA	–
RYR2	14	c.1195A>T:p.Met399Leu	–	–	–	–	–	pD	D	T	D	P	Full	–
RYR2	18	c.1725A>C: p.Leu575Phe	–	–	–	–	–	D	D	D	D	P	High	–
RYR2	82	c.11321A>G: p.Gln3774Arg	–	–	–	–	–	D	D	D	D	P	Full	–
RYR2	37	c.5170G>A:p.Glu1724Lys	–	–	–	–	–	D	D	D	D	P	Full	[27]
RYR2	90	c.12006G>T:p.Met4002Ile	–	–	–	–	–	pD	D	T	D	P	High	[28]
CACNA1C	8 (iso 14)	c.1204G>A: p.Gly402Ser	–	–	–	–	rs80315385	D	D	D	D	P	Full	[29]
B. Variants of unknown significance (ExAC MAF < 0.005%)														
MYH7	4	c.337A>C:p.Met113Leu	–	–	–	–	–	B	T	T	D	P	Full	–
SCN5A	17	c.3206C>G:p.Thr1069Arg	–	–	–	–	–	pD	D	T	D	P	Medium	–
TTN	276	c.74959C>T:p.Arg24987*	–	–	–	4.0e-6/1	–	NA	NA	NA	D	NA	NA	–
KCNE1	4	c.287A>G:p.Gln96Arg	8.3e-06/1	–	–	7.1e-6/2	rs767972997	pD	T	D	D	P	Medium	–
HCN4	7	c.2138G>A:p.Arg713His	8.4e-06/1	–	–	8.0e-6/2	–	D	D	D	D	P	High	–
DSP	23	c.3282G>C:p.Lys1094Asn	1.7e-05/2	3.0e-05/2	–	1.1e-5/3	rs2491080	D	D	D	D	P	High	–
DSP	24	c.5692G>C:p.Glu1898Gln	–	–	–	–	–	D	D	T	D	P	High	–
DSP	24	c.7552G>A:p.Val2518Ile	1.6e-05/1	1.5e-05/1	–	3.2e-5/9	rs757394666	B	T	T	D	B	Medium	–
LDB3	10	c.1336C>T:p.Pro446Ser	2.0e-05/1	4.0e-05/1	–	–	rs757728320	B	D	T	D	P	High	–
LDB3	7 (iso 1)	c.1018G>C:p.Ala340Pro	3.4e-05/4	4.6e-05/3	0.00016/1	2.5e-5/7	rs755329877	pD	D	T	P	B	Medium	–
C. Variants of Unknown Significance (ExAC MAF 0.005%–0.5%)														
MYBPC3	19	c.1828G>C:p.Asp610His	5.8e-05/3	7.0e-05/2	0.00044/1	3.9e-5/10	rs371564200	D	D	D	D	P	Full	[30]
CASQ2	11	c.1052A>G:p.Asp351Gly	8.3e-05/10	9.0e-05/6	–	4.8e-5/12	rs200899037	D	D	T	D	P	High	–
RYR2	46	c.7060G>A:p.Ala2354Thr	6.7e-05/8	3.0e-05/2	–	4.0e-5/10	rs773070579	B	D	T	D	P	Low	–
RYR2	46	c.7099G>A:p.Gly2367Arg	0.00017/20	0.00011/7	0.0017/11	0.00014/40	rs369152386	pD	T	D	D	P	Medium	[31]
RYR2	69	c.9923A>G:p.Asn3308Ser	0.00036/35	0.00036/19	0.0028/16	0.00039/109	rs201081663	B	T	T	P	P	Low	–
DSC2	16	c.2587G>A:p.Gly863Arg	0.00027/33	0.00027/18	–	0.00027/77	rs147109895	D	D	D	D	P	High	[32]
MYBPC3	31	c.3413G>A:p.Arg1138His	0.0013/105	0.00066/30	0.017/72	0.0012/320	rs187705120	NA	D	D	D	NA	High	[33]
D. Variants with demonstrated functional effects (ExAC MAF<0.5%)														
LDB3	5	c.566C>T:p.Ser189Leu	0.00057/68	0.00072/47	0.00015/1	0.00064/179	rs45487699	B	T	T	D	B	Low	[34]
KCNE1	4	c.95G>A:p.Arg32His	5.0e-05/6	6.0e-05/4	–	5.2e-5/13	rs17857111	B	T	T	P	P	Medium	[35]
KCNE2	2	c.170T>C:p.Ile57Thr	0.00088/107	0.0011/73	0.00015/1	0.00095/269	rs74315448	D	D	D	D	P	High	[36]
MYBPC3	5	c.649A>G:p.Ser217Gly	0.0023/248	0.0010/64	0.00017/1	0.0017/462	rs138753870	pD	D	D	D	NA	Low	[37]
SCN5A	12	c.1715C>A:p.Ala572Asp	0.0043/519	0.0037/245	0.039/257	0.0053/1498	rs36210423	B	D	T	P	NA	Low	[38]
SCN5A	28	c.6016C>G:p.Pro2006Ala	0.0013/146	0.0020/124	0.0023/15	0.0011/257	rs45489199	B	T	T	P	P	Low	[39]

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) [19]. *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.

Table 4
Statistical features of patients carrying pathogenic/likely pathogenic variants.

	Age at VF	hr (min^{-1})	PQ (ms)	QRS (ms)	QTc(ms)	LVEDD (mm)	EF %
Mean[*SD*] (C)	19.9[19.5]	78.6[13.8]	137.3[25.5]	85.7[14.0]	432.4[35.5]	45.7[8.0]	53.9[15.2]
Mean[*SD*] (NC)	32.4[12.7]	67.9[15.3]	155.2[22.8]	90.3[14.8]	416.0[30.3]	50.2[5.4]	58.9[8.9]
p (NC *vs.* C)	**0.01**	NS	NS	NS	NS	NS	NS

For electrogardiogram and echocardiography findings, statistical analysis comparing patients carrying pathogenic/likely pathogenic mutations (C, carriers, $N = 7$) and rest of the patients (NC, Non-carriers, $N = 69$) was performed using non parametric Mann-Whitney *U* test. $P < 0.05$ was considered significant. NS = Not significant.

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 [9,44]. 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.ijcard.2017.10.016.

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