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Modeling Idiopathic Ventricular Fibrillation Using iPSC Cardiomyocytes and Computational Approaches:

A Proof-of-Concept Study

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Abstract

Idiopathic ventricular fibrillation (IVF) is an unrefined diagnosis representing a heterogeneous patient group without a structural or genetic definition. IVF treatment is not mechanistic-based due to the lack of experimental patient-models. We sought to create a methodology to assess cellular arrhythmia mechanisms for IVF as a proof-of-concept study. Using IVF patient-specific induced pluripotent stem cell–derived cardiomyocytes, we integrate electrophysiological optical mapping with computational modeling to characterize the cellular phenotype. This approach flips the traditional paradigm using a biophysically detailed computational model to solve the problem inversely. Insight into the cellular mechanisms of this patient's IVF phenotype could also serve as a therapeutic testbed.

Keywords

cardiac arrhythmia; idiopathic ventricular fibrillation; patient-specific stem cells; ventricular fibrillation

Idiopathic ventricular fibrillation (IVF) represents an unmet clinical challenge. Despite comprehensive attempts at clinical phenotype-genotype relationships,[1] ~50% of patients with primary electrical sudden cardiac death (SCD)/sudden cardiac arrest do not meet diagnostic criteria for other arrhythmic SCD syndromes and are labeled with a catch-all diagnosis of IVF.[1,2] Genetic linkage analysis for IVF patients has exposed a nonmonogenic disorder[2] and, often, multiple genetic variants are identified without a link to ventricular

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fibrillation arrhythmogenesis.[3,4] Without morphologic, genetic, or electrocardiogram biomarkers from which to assess, IVF patients are left vulnerable to management without evidence- or mechanistic-based approaches, causing low efficiency but high morbidity and mortality.[2,4–6]

We sought to create a methodology to assess cellular arrhythmia mechanisms for an IVF patient as a proof-of-concept study. Using IVF patient-specific induced pluripotent stem cell–derived cardiomyocytes (iPSC-CMs), we combine electrophysiological optical mapping with computational modeling to characterize the cellular phenotype and uncover the arrhythmogenic mechanism(s).

METHODS

A 38-year-old man suffered a ventricular fibrillation arrest and was defibrillated in the field. Extensive laboratory, toxicology, electrocardiogram, cardiac magnetic resonance, echocardiogram, and coronary angiogram failed to identify an arrhythmia syndrome phenotype. There was no family history of cardiac arrhythmia or SCD. Genetic testing revealed no known pathological variants, but 3 variants of uncertain significance were identified. Written informed consent was obtained from the patient in accordance with the Declaration of Helsinki and with the University of Wisconsin–Madison Institutional Review Board. Patient iPSC clones were generated, karyotyped, characterized, and cryopreserved. The human iPSC control/nondiseased line iPS-DF19–9-11T (male, WiCell; from here referenced as control) was used for control. Control iPSC or IVF iPSC lines were differentiated into iPSC-CMs using a modified GSK3β inhibitor/Wnt inhibitor protocol (GiWi), as previously described.[7] CM purities of >95% were demonstrated by flow cytometry (not shown).

ELECTROPHYSIOLOGICAL OPTICAL MAPPING.

Intracellular Ca^{2+} transients were performed at physiological temperature (37 °C ± 2 °C) using fluorescent single-cell optical mapping. The iPSC-CMs were loaded with 10 μg Fluo-4 AM Ca^{2+} sensitive dye in 10 mL RPMI+ medium in the following bath solution (mM): NaCl 148, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 1, HEPES 15, $NaHPO_4$ 0.4, D-glucose 5.5, and pH adjusted to 7.4 (NaOH). Field stimulation pacing at 0.5 Hz to 1 Hz was performed. Imaging was conducted on an inverted Nikon microscope (Eclipse Ti) equipped with a MiCAM Ultima-L CMOS (SciMedia, USA Ltd) camera and sampled at 1,000 to 1,500 frames/s. Fluorescent signals were amplified, digitized, and visualized during the experiment using specialized software (SciMedia, USA Ltd). Data were analyzed using a MATLAB custom program. Statistical comparisons were performed using one-way analysis of variance with post hoc Tukey correction for multiple comparisons using Prism 10 (GraphPad). Differences were significant at $P < 0.05$.

QUANTIFICATION OF TRIGGERED ACTIVITY.

To quantify proarrhythmic behavior, we used a custom semisupervised MATLAB program to detect different proarrhythmic behaviors in our sample traces. Thresholds for all forms of proarrhythmic behavior were determined using both healthy and arrhythmic transients and

confirmed manually. Sample traces which contained 2 or fewer Ca^{2+} events were excluded from proarrhythmic analyses.

MODEL INVERSION USING A GENETIC ALGORITHM.

We combined a genetic algorithm and the Kernik et al[8] model of iPSC-CM action potentials and Ca^{2+} handling to identify iPSC-CM differential IVF vs control model parameters by fitting the simulated Ca^{2+} transients (CaTs) of the computational model to match experimental CaT traces. Raw CaT traces of iPSC-CMs (paced at 0.5 and 1 Hz, ~6 seconds in length) were denoised through median and finite impulse response filters, aligned, and normalized. The Kernik iPSC-CM model[8] was paced at 0.5 Hz and 1 Hz for 100 seconds before simulated CaTs were recorded. Model fitness error was computed as the sum of absolute differences between the normalized experimental and simulated CaTs at 0.5 and 1 Hz. A genetic algorithm was applied to minimize the model fitness error (using function '*ga*' from MATLAB R2021, MathWorks; population size = 300; maximal generations = 50) to systematically perturb 16 model parameters describing maximum channel conductance, rates for pumps and exchangers, and sarcoplasmic reticulum fluxes.

RESULTS

IVF iPSC-CMs HAVE ABNORMAL Ca^{2+} HANDLING.

iPSC-CMs from IVF and control lines were paced using field stimulation at 0.5 Hz and 1Hz. Compared to control at 0.5 Hz, IVF iPSC-CMs have longer CaT duration ($P < 0.0001$), time to peak ($P < 0.0001$), time to 90% decay ($P < 0.0001$), and rate of decay of CaT (Tau; $P < 0.0001$) (Figure 1A). At 1-Hz pacing, CaT was faster in IVF compared to control cells ($P < 0.0001$), as was time to peak ($P < 0.0001$) and rate of decay (Tau; $P < 0.01$). However, time to 90% decay was comparable between control and IVF ($P = 0.23$) (Figure 1B).

IVF iPSC-CMs SHOW INCREASED PROARRHYTHMIC ACTIVITY.

Proarrhythmic behaviors were quantified using custom semisupervised MATLAB software in our sample traces. These include: 1) Alternans, in which the amplitude of Ca^{2+} alternates between paced activity (Figure 1C); 2) spontaneous or arrhythmic electrical activity, in which the peak-to-peak duration varies significantly (Figure 1C); and 3) impaired Ca^{2+} decay, in which the Ca^{2+} transient signal fails to reach 70% normalized decay between paced activity (Figure 1C). We found that IVF samples display an increased rate of proarrhythmic behavior at both 0.5 Hz (61 of 189 [32.3%] vs 18 of 118 [15.3%]) and 1 Hz (59 of 149 [39.6%] vs 6 of 61 [9.8%]) compared to control. Our future work will include further development and automation of this software for unbiased analysis of proarrhythmic behavior in iPSC-CMs.

GENETIC ALGORITHM COMPUTATIONAL MODELING.

To elucidate the cellular mechanisms (ie, differential model parameters) responsible for the observed differences from optical mapping, we used a genetic algorithm to optimize a biophysically detailed iPSC-CM model[8] recapitulating IVF and control CaTs. For each group, we obtained the best performing variants from 50 optimization trials with a random parameter initialization. Experimental CaTs shown are reported as a calcium signal (Norm.

Ca^{2+}), as plotted in Figure 1D. The average CaT is plotted from experimental data. Simulated data (grey traces) are superimposed with experimental data (blue trace = control, red trace = IVF). Comparison of model parameter uncovered cellular compositions that underlie the observed CaT differences in IVF vs control iPSC-CMs revealing significant differences in several model parameters (Figure 1D, bottom). Specifically, the IVF-vs-control group showed slower maximum rates for Na^+/Ca^{2+} exchanger (V_{NCX}), SERCA (V_{SERCA}), and plasma membrane Ca^{2+} ATPase (V_{PMCA}), and lower conductance for the funny current (G_f), and larger maximum conductances of rapid delayed rectifier K^+ current (G_{Kr}), background Ca^{2+} current (G_{bCa}), and L-type Ca^{2+} current (G_{CaL}) in IVF vs control models. Additionally, IVF models exhibited a left shift in the steady-state activation variable of L-type Ca^{2+} current (CaL_{Vshift}), and a greater EC50 for sarcoplasmic reticulum (SR) Ca^{2+}-dependent activation of ryanodine receptor release (RyR_{EC50SR}) compared with the control group (Figure 1D).

DISCUSSION

The challenge with IVF is that without a genetic marker from which to build a hypothesis of arrhythmia mechanism, a new paradigm must be designed. To solve this problem, we created "reverse cellular phenotyping": starting with the end phenotype of IVF (after all attempts are made to assign an arrhythmia syndrome diagnosis) and working backwards from the signals and features of their iPSC-CMs to identify the abnormal arrhythmic mechanisms (Central Illustration). As a proof-of-concept study, we show the utility of 2 analytic methods to better understand the arrhythmic phenotype of IVF: single-cell electrophysiological optical mapping and genetic computational modeling. For the present IVF cell line, we found that CaT duration measured at 0.5 Hz is significantly longer for IVF compared to control. Mechanistically, this suggests that Ca^{2+} reuptake into the SR and/or extrusion of Ca^{2+} via the plasma membrane (through Na^+/Ca^{2+}-exchanger and/or PMCA) is impaired. Computational analysis of the IVF transients complemented these studies by showing a parallel mechanism with reductions in maximal SERCA2, PMCA, and NCX rates. Nevertheless, CaT duration was shorter for IVF compared to control at 1 Hz. We attribute this difference to a rate-dependent increase Ca^{2+}-dependent extrusion (despite diminished transport rates) owing to enhanced Ca^{2+} loading promoted by the increase in the maximal conductance of L-type (which voltage dependence of activation is negatively shifted) and background Ca^{2+} currents. To counteract these changes, IVF models also exhibit increased G_{Kr}, which limit the Ca^{2+} influx via shortening the action potential duration, and reduced luminal Ca^{2+} activation of ryanodine receptors and G_f, which likely reduce automaticity in the presence of enhanced Ca^{2+} loading. An important future direction will be to test whether the specific ionic alterations predicted from modeling can be experimentally validated by targeted ion channel survey, and their functional effects confirmed by modulating them in the IVF iPSCs to show restoration of normal Ca^{2+} handling. The recent delineation of IVF sub-phenotypes[9,10] will benefit this type of characterization, as common feature sets are likely to be recognized between IVF sub-phenotypes. Our future work will incorporate these deep phenotyping subtypes into larger sets of IVF patient-derived cell lines to further validate the disease phenotype and compare arrhythmogenic mechanisms using

deep-cellular phenotypic characterization including membrane voltage, voltage mapping, current clamp, and multiomics.

STUDY LIMITATIONS.

We recognize that use of iPSC-CMs has limitations in terms of electrical and metabolic maturity. However, at present use of patient-specific cells represents the gold standard for the study of a complex disease for which primary tissue acquisition or a mechanistically inspired murine model are not feasible, such as IVF. In this study, iPSC-CMs were derived and purified using the latest methodologies to reduce the effect of such limitations.

CONCLUSIONS

Here, we have developed a novel platform for elucidating molecular mechanisms of complex arrhythmic syndromes. This work serves as a proof-of-concept that we can recapitulate a provocative cellular phenotype using patient iPSCs: starting at the end to return to the beginning. Further, this concept can also serve as a testing bed for antiarrhythmic drug therapy.

FUNDING SUPPORT AND AUTHOR DISCLOSURES

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ABBREVIATIONS AND ACRONYMS

CaT	calcium transient
iPSC-CMs	induced pluripotent stem cell–derived cardiomyocytes
IVF	idiopathic ventricular fibrillation
SCD	sudden cardiac death

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE:

IVF is difficult to treat without a mechanistic understanding of the disease. Our work outlines a new paradigm for identifying functional and molecular targets for arrhythmia suppression in patients with IVF.

TRANSLATIONAL OUTLOOK:

The cellular mechanisms of IVF remain unsolved. We designed paradigm-shifting methodology to approach IVF investigation using “inversion” phenotyping and uncovered some altered Ca^{2+}-handling and upstream disease determinants. Our approach will not only uncover cellular mechanisms involved in IVF arrhythmogenesis but will also serve as a testbed for novel or repurposed therapeutics.

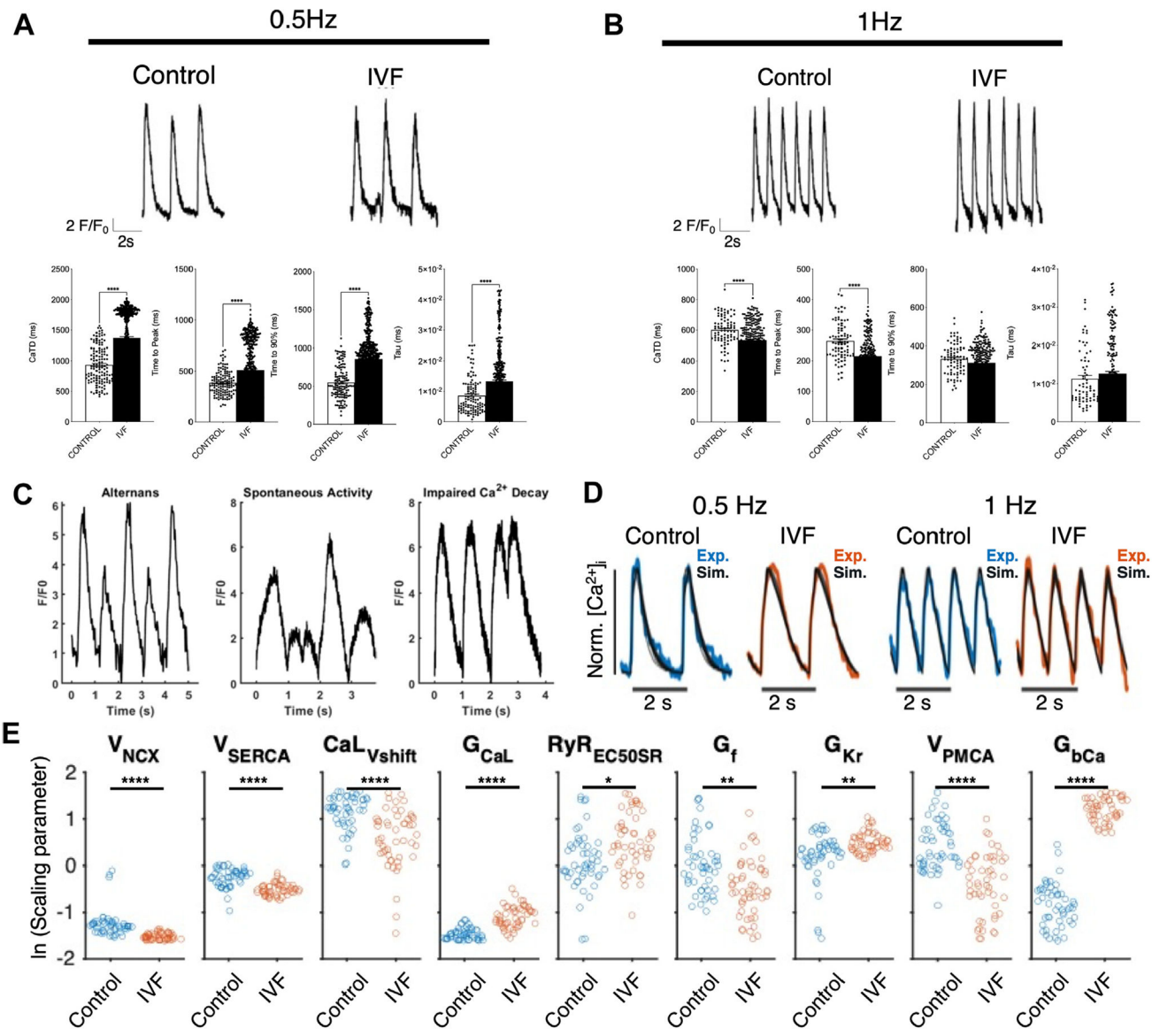
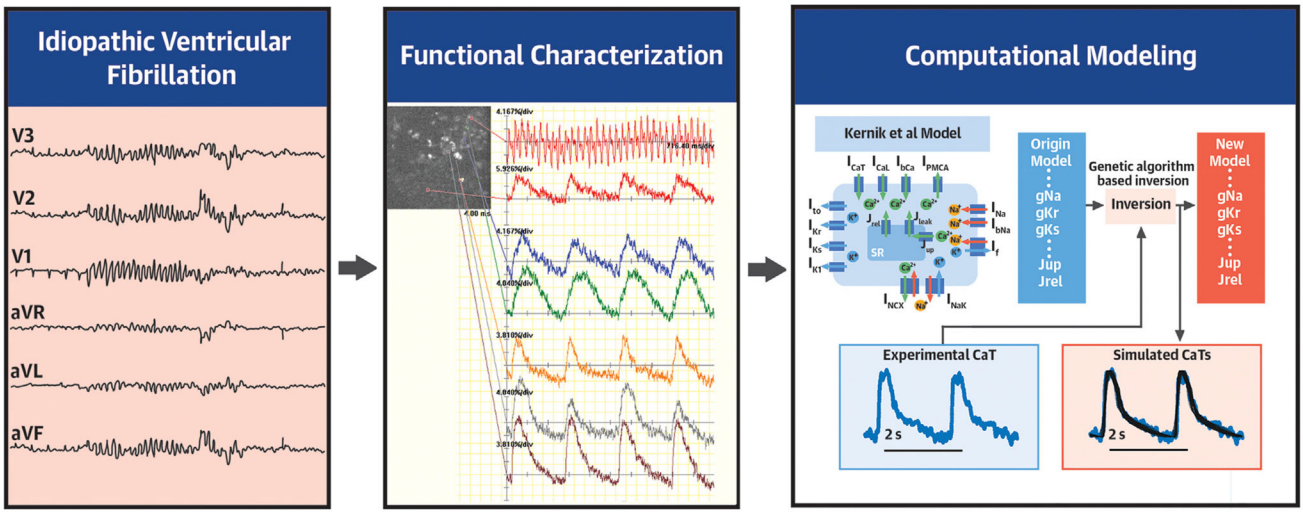


FIGURE 1.

IVF iPSC-CMs Show Altered Calcium Handling, Triggered Activity, and Decrease Ion Conductances

(A) Representative traces from 0.5Hz pacing (upper panel) and mean data from parameters measured (lower panel). (B) Representative traces from 1-Hz pacing (upper panel) and mean data from parameters measured (lower panel). (C) Representative traces from idiopathic ventricular fibrillation (IVF) induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) showing spontaneous triggered activity. (D, top) Genetic algorithm identifies differential model parameters between control and IVF iPSC-CMs. Simulated (Sim.) CaTs of iPSC-CMs are superimposed with experimental (Exp.) Ca^{2+} transient (CaT). (E, bottom) Model analysis reveals significant difference between parameters for control (n = 50) and IVF (n = 50). V_{NCX}, V_{SERCA}, V_{PMCA}: maximum rate for Na^+/Ca^{2+} exchanger, SERCA, and plasma membrane Ca^{2+} ATPase, respectively; G_{CaL}, G_f, G_{Kr}, G_{bCa}: maximum conductance

for L-type Ca^{2+} current, funny current, rapid delayed rectifier K^+ current, and background Ca^{2+} current, respectively; CaL_{Vshift}: voltage shift in $V_{1/2}$ of the steady-state activation variable for L-type Ca^{2+} current; RyR_{EC50SR}: median effective dose for sarcoplasmic reticulum (SR) Ca^{2+}-dependent activation of ryanodine receptor release. Statistical analysis was performed using a two-sided Wilcoxon rank sum test with a Bonferroni correction for multiple comparison. *$P \leq 0.05$. ** $P \leq 0.01$. **** $P \leq 0.0001$.



CENTRAL ILLUSTRATION.

Study Design

CaT = calcium transient.