



European Heart Journal (2017) **38**, 2132–2136
doi:10.1093/eurheartj/ehw574

EJH BRIEF COMMUNICATION

Arrhythmia/electrophysiology

Optogenetic termination of ventricular arrhythmias in the whole heart: towards biological cardiac rhythm management

Emile C.A. Nyns¹, Annemarie Kip¹, Cindy I. Bart¹, Jaap J. Plomp²,
Katja Zeppenfeld¹, Martin J. Schalij¹, Antoine A.F. de Vries^{1†}, and
Daniël A. Pijnappels^{1*†}

¹Laboratory of Experimental Cardiology, Department of Cardiology, Heart Lung Center Leiden, Albinusdreef 2, 2300 RC Leiden, The Netherlands; and ²Department of Neurology and Neurophysiology, Leiden University Medical Center, Albinusdreef 2, 2300 RC Leiden, The Netherlands

Received 6 August 2016; revised 3 October 2016; editorial decision 7 November 2016; accepted 7 November 2016; online publish-ahead-of-print 23 December 2016

Aims

Current treatments of ventricular arrhythmias rely on modulation of cardiac electrical function through drugs, ablation or electroshocks, which are all non-biological and rather unspecific, irreversible or traumatizing interventions. Optogenetics, however, is a novel, biological technique allowing electrical modulation in a specific, reversible and trauma-free manner using light-gated ion channels. The aim of our study was to investigate optogenetic termination of ventricular arrhythmias in the whole heart.

Methods and results

Systemic delivery of cardiotropic adeno-associated virus vectors, encoding the light-gated depolarizing ion channel red-activatable channelrhodopsin (ReaChR), resulted in global cardiomyocyte-restricted transgene expression in adult Wistar rat hearts allowing ReaChR-mediated depolarization and pacing. Next, ventricular tachyarrhythmias (VTs) were induced in the optogenetically modified hearts by burst pacing in a Langendorff setup, followed by programmed, local epicardial illumination. A single 470-nm light pulse (1000 ms, 2.97 mW/mm²) terminated 97% of monomorphic and 57% of polymorphic VTs vs. 0% without illumination, as assessed by electrocardiogram recordings. Optical mapping showed significant prolongation of voltage signals just before arrhythmia termination. Pharmacological action potential duration (APD) shortening almost fully inhibited light-induced arrhythmia termination indicating an important role for APD in this process.

Conclusion

Brief local epicardial illumination of the optogenetically modified adult rat heart allows contact- and shock-free termination of ventricular arrhythmias in an effective and repetitive manner after optogenetic modification. These findings could lay the basis for the development of fundamentally new and biological options for cardiac arrhythmia management.

Keywords

Ventricular arrhythmias • Optogenetics • Anti-arrhythmic • Gene Therapy • Adeno-associated virus vector • Channelrhodopsin

Introduction

Ventricular arrhythmias are a large and growing problem worldwide, with high annual mortality and morbidity rates.¹ Despite significant

progress in therapeutic strategies, the current treatment options for ventricular arrhythmias remain suboptimal. In brief, drug treatment is rather ineffective, while catheter ablation may cause irreversible complications and generally has a modest long-term efficiency.

* Corresponding author: Tel: 003171-5265330, Fax: 003171-5266809, Email: d.a.pijnappels@lumc.nl

† The last two authors contributed equally to the study.

© The Author 2016. Published by Oxford University Press on behalf of the European Society of Cardiology.

This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/4.0/>), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited. For commercial re-use, please contact journals.permissions@oup.com

Electroshock therapy, on the other hand, is effective in terminating ventricular arrhythmias and has shown to reduce mortality as represented by the implantable cardiac defibrillator (ICD). However, the high-voltage shocks delivered by these devices are traumatizing, especially when given inappropriately, as they are associated not only with severe pain, anxiety and depression,² but also with myocardial tissue damage.³

In contrast, optogenetics is an emerging biological technique to electrically modulate cells, tissue and organs in a specific, reversible and shock-free manner using light-gated ion channels.⁴ Thus, optogenetics may provide a new incentive for the development of biological and pain-free treatment options for cardiac arrhythmias. Recent studies have shown that cardiac optogenetics allows for optical pacing of the whole heart and light-induced arrhythmia termination in cell cultures.^{5–8} The aim of our present study was to investigate whether optogenetic modification of the adult rat heart would enable light-mediated termination of ventricular arrhythmias.

Methods

Experimental procedures are described in detail in Supplementary material online, *Supplementary material*.

Animal studies

All animal experiments were approved by the Animal Experiments Committee of the Leiden University Medical Centre and conformed to the Guide for the Care and Use of Laboratory Animals as stated by the US National Institutes of Health.

Optogenetic pacing and arrhythmia termination

Four to six weeks after systemic injection of cardiotropic adeno-associated virus (AAV) vectors encoding red-activatable channelrhodopsin (ReaChR),⁹ the hearts of anesthetized adult Wistar rats were rapidly excised, cannulated and subsequently perfused using a Langendorff apparatus ($n = 8$). Optical pacing was performed by exposure for 20 ms to 470-nm light-emitting diode (LED) light. Ventricular tachyarrhythmia's (VTs) were induced by irregular electrical burst pacing protocols with a cycle length varying between 20 and 80 ms. When the VTs lasted for at least 5 s, they were considered sustained and the hearts were subsequently exposed to a 1000-ms light pulse. VTs were considered to be optogenetically terminated when ventricular arrhythmias stopped within 2 s after the start of illumination. When optogenetic termination failed, bipolar electrical defibrillation was performed (20 ms, 80 V, 12 mA). To evaluate the effect of action potential duration (APD) shortening on optogenetic termination efficiency, 3 μ l of 10 mM of the K_{ATP} channel opener P1075 was directly injected into the aortic cannula. Following an incubation period of 1 min, the hearts were subjected to the same arrhythmia induction and optogenetic termination protocol as used prior to P1075 infusion. P1075 was considered washed out after 15 min following administration as APD was then normalized to standard values.

Results

Systemic delivery of cardiotropic AAV vectors encoding ReaChR resulted in widespread transduction of the cardiomyocytes in adult rat hearts with an average transduction rate of 93% (SD 4%) (Figure 1A).

Functionality of these light-gated depolarizing ion channels was confirmed by sharp-electrode measurements in cardiac tissue slices, revealing significant and sustained depolarization of the membrane potential during 470-nm LED illumination for 1000 ms (Figure 1B).

Upon excision and preparation of the hearts in the Langendorff setup for stimulation and readout (Figure 1C), part of the epicardial surface was exposed to short (i.e. 20-ms) 470-nm light pulses (0.97 mW/mm²). This allowed optical pacing up to 4.5 Hz, thereby producing QRS complexes similar to those induced by electrical stimulation (Figure 1D). Following the induction of sustained VTs by electrical burst pacing, a single 470-nm light pulse (1000 ms, 2.97 mW/mm²) was given illuminating circa 125 mm² of the ventricular surface. Such pulses led to an average successful arrhythmia termination rate of 97% for monomorphic VTs (corresponding to 26 VTs in 8 hearts) and 57% of polymorphic VTs (corresponding to 19 VTs in 6 hearts). (Supplementary material online, Video S1). Without illumination, none of these arrhythmias were terminated ($n = 6$ for both mono- and polymorphic VTs) (Figure 1E and F).

Optical voltage mapping was performed in order to obtain more mechanistic insight into light-induced arrhythmia termination. Activation maps confirmed global high-frequency activation of the ventricles resembling reentrant activity (Figure 1G). Optical recordings showed that the 1000-ms light pulses resulted in arrhythmia termination within 420.9 ms (SD 189.7 ms, range 185–734 ms). Furthermore, based on the optical signals, significant prolongation of APD was noted just before arrhythmia termination (58.6 ms (SD 7.3 ms) vs. 72.3 ms (SD 16.3 ms) for APD₈₀ before and during illumination, $P = 0.021$, $n = 7$). (Figure 1H and I). As this finding indicated an important role for APD prolongation in optogenetic arrhythmia termination in these hearts, we next evaluated the effects of APD shortening on termination efficiency. For this purpose, the optogenetically modified hearts were treated with a single bolus of P1075, a K_{ATP} channel opener ($n = 2$). As a result, APD₈₀ during the arrhythmia was significantly shortened (62.9 ms (SD 11.8 ms) vs. 44.0 ms (SD 13.7 ms) before and after P1075 administration, $P = 0.018$). Using the same arrhythmia induction and optogenetic termination protocol, none of monomorphic VTs (0 out of 5) and only 1 out of 9 (11%) polymorphic VTs could be terminated. After a washout period of 15 min, and normalization of APD₈₀, optogenetic termination efficiency increased to 80% for monomorphic VTs (4 out of 5) and 71% for polymorphic VTs (5 out of 6) (Figure 1J).

Discussion

Here, we demonstrate that forced expression of a light-gated depolarizing ion channel (ReaChR) in the adult rat heart allows contact- and shock-free termination of VTs through brief local illumination of the ventricular surface, i.e. without relying on conventional drugs, tissue ablation or electroshocks. Both mono- and polymorphic VTs could be terminated in an effective and repetitive manner by a light-induced electrical current driven by natural electrochemical gradients, providing proof-of-concept for biological arrhythmia termination.

The unique properties of optogenetics make it possible to modulate cardiac electrical activity by applying depolarizing or hyperpolarizing photocurrents with high specificity and unmatched spatiotemporal precision.⁴ The finding that optogenetic arrhythmia

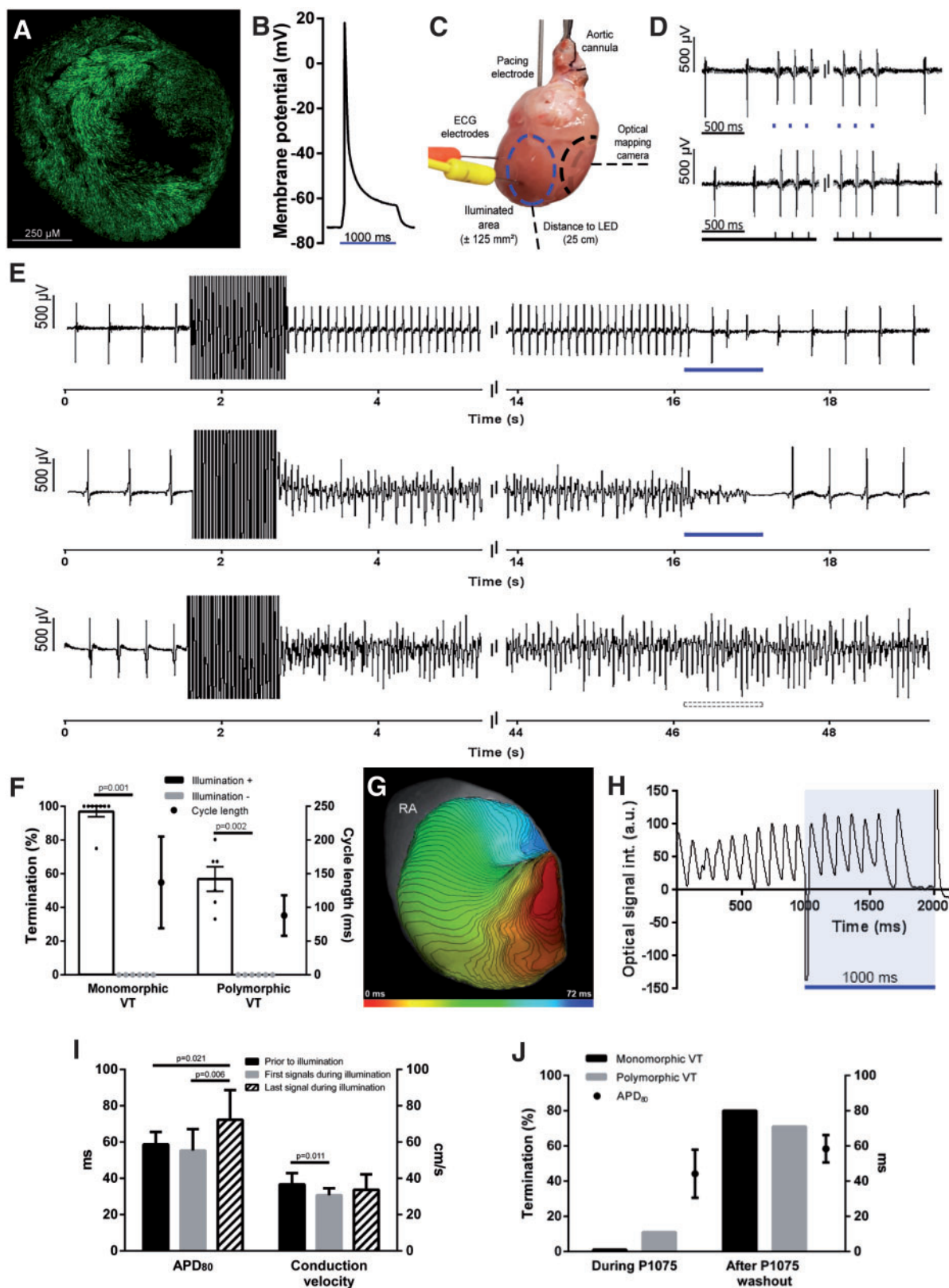


Figure 1 (A) Mid-ventricular transversal slice of an adult rat heart transduced with a cardiotropic AAV vector encoding ReaChR fused to citrine showing global transgene expression 6 weeks after vector injection. (B) Confirmation, by sharp-electrode measurement, of light-induced and sustained depolarization in a ReaChR-expressing ventricular tissue slice. (C) Schematic overview of a ReaChR-expressing heart in the Langendorff setup equipped for electrical stimulation and recording, in which the area of LED illumination and optical mapping are indicated by the blue and black dotted

termination is feasible in the intact heart could have an impact on the development of novel treatment options for cardiac arrhythmias, as there may be several advantages of biological anti-arrhythmic therapy over conventional therapy. These advantages could relate to the specificity of the intervention, which is superb for optogenetics and relatively poor for drugs.¹⁰ In addition, optogenetic interventions allow temporary modulation of electrical function, as opposed to tissue ablation, which results in permanent alteration of the electrical properties of cardiac tissue.¹¹ Importantly, optogenetic arrhythmia termination obviates the use of high-voltage electroshocks. These shocks are not only known to be painful and to cause anxiety and stress in a significant percentage of patients wearing an ICD², but also to cause myocardial tissue damage.³ In theory, these disadvantages could be avoided if the heart itself would be able to generate, on cue, the electrical current for arrhythmia termination, as with cardiac optogenetics. Obviously, many hurdles need to be overcome before clinical translation can be considered, but the concept of biological arrhythmia management seems worth further investigation considering the unique potential benefits.

Our data suggests that APD prolongation plays an important role in optogenetic arrhythmia termination. Such prolongation has been associated with destabilization of arrhythmic electrical activity, thereby favouring its termination.¹² However, as the exact mechanisms remain incompletely understood, further studies are needed in order to improve mechanistic insight, including evaluation of the minimum requirements for effective optogenetic termination, such as strength, area, and location of illumination. Moreover, further development and optimization of optogenetic tools and light delivery would help to improve optogenetic termination efficiency and, together with advances in gene transfer technology, will potentially aid the translation to *in vivo* applications.

It is expected that potential *in vivo* applications are hindered by poor light penetration, and that therefore only a small fraction of the total number of light-gated ion channels would be activated.¹³ An important finding of this study is that light-induced arrhythmia termination was already successful by illuminating only a small area of the epicardial surface. This finding at least suggests that it may not be necessary to illuminate the whole heart. Hence, the challenging aspect of illumination might be overcome by focusing the light on a relatively small, but in terms of arrhythmia maintenance, critical area. In addition, this finding also suggests that regional genetic modification by local delivery of viral vectors^{14,15} or optogenetically modified cells into cardiac tissue may already be sufficient for effective optogenetic

modulation, which would be of practical benefit. Furthermore, as heart size may play an important role in determining the efficiency of arrhythmia termination via optogenetics, further studies in larger hearts are needed.¹⁶

Taken together, our study demonstrates that the heart itself is able to produce an electrical current for arrhythmia termination upon forced expression of light-gated depolarizing ion channels and their activation by brief and local epicardial illumination. Such biological arrhythmia termination enables precise and shock-free control over disturbed cardiac rhythm, allowing normal rhythm to regain. Although further and more detailed studies are certainly needed, the findings presented in this brief communication do provide proof-of-principle for optogenetic termination of arrhythmias in the whole heart and may thereby pave the way for the design and development of fundamentally novel strategies of cardiac arrhythmia management.

Supplementary material

Supplementary material is available at *European Heart Journal* online.

Acknowledgements

We would like to thank Dongsheng Duan (University of Missouri, Columbia, MO), Brent French (University of Virginia, Charlottesville, VA) and Roger Tsien (University of California, San Diego, CA) for kindly providing DNA constructs, Annelies van der Laan (Department of Molecular Biology, LUMC) for imaging assistance, and Niels Harlaar, Iolanda Feola, Rupamanjari Majumder and Alexander Teplenin for their technical assistance and useful discussions (Department of Cardiology, LUMC).

Funding

This work was supported by the Netherlands Organization for Scientific Research (NWO, Vidi grant 91714336 to D.A.P.) and the Netherlands Heart Institute (ICIN grant 230.148-04 to A.A.F.d.V.).

Conflict of interest: none declared.

References

1. John RM, Tedrow UB, Koplan BA, Albert CM, Epstein LM, Sweeney MO, Miller AL, Michaud GF, Stevenson WG. Ventricular arrhythmias and sudden cardiac death. *Lancet* 2012;**380**:1520–1529.
2. Magyar-Russell G, Thombs BD, Cai JX, Baveja T, Kuhl EA, Singh PP, Montenegro Braga Barroso M, Arthurs E, Roseman M, Amin N, Marine JE, Ziegelstein RC. The prevalence of anxiety and depression in adults with implantable cardioverter defibrillators: a systematic review. *J Psychosom Res* 2011;**71**:223–231.

line, respectively. (D) Optical (top panel) and electrical (bottom panel) pacing of a ReaChR-expressing heart showing 1:1 capture with similar electrocardiographic signals for both modes of stimulation. (E) Typical intra-cardiac electrogram readouts demonstrating successful termination of a monomorphic (top panel) and polymorphic VT (middle panel) with a single 1000-ms local light pulse (blue line) onto the epicardial surface, while the arrhythmias are sustained without (bottom panel) such illumination (dotted black line). (F) Quantification of light-induced termination of mono- and polymorphic VTs expressed as a percentage of successful attempts averaged for all hearts (error bar represents one standard error of the mean) and of the average arrhythmia cycle length prior to illumination (error bar represents one standard deviation). (G) Electrical activation map of ReaChR-expressing heart, derived from voltage mapping, showing a reentrant conduction pattern. (H) Typical trace of optical voltage signals showing prolongation of the last voltage signal prior to VT termination by local epicardial illumination. (I) Quantification of APD₈₀ and conduction velocity based on optical voltage signals at different stages of optogenetic arrhythmia termination (error bar represents one standard deviation). (J) The effect of the K_{ATP} channel opener P1075 on the APD₈₀ and success of light-induced termination of mono- and polymorphic VTs, showing almost complete failure of optogenetic termination of VTs upon APD shorting by P1075 and recovery of photocurrent-mediated VT termination ability upon P1075 washout (error bar represents 1 SD).

3. Sham'a RA, Nery P, Sadek M, Yung D, Redpath C, Perrin M, Sarak B, Birnie D. Myocardial injury secondary to ICD shocks: insights from patients with lead fracture. *Pacing Clin Electrophysiol* 2014;**37**:237–241.
4. Deisseroth K. Optogenetics. *Nat Methods* 2011;**8**:26–29.
5. Arrenberg AB, Stainier DY, Baier H, Huisken J. Optogenetic control of cardiac function. *Science* 2010;**330**:971–974.
6. Nussinovitch U, Gepstein L. Optogenetics for in vivo cardiac pacing and resynchronization therapies. *Nat Biotechnol* 2015;**33**:750–754.
7. Vogt CC, Bruegmann T, Malan D, Ottersbach A, Roell W, Fleischmann BK, Sasse P. Systemic gene transfer enables optogenetic pacing of mouse hearts. *Cardiovasc Res* 2015;**106**:338–343.
8. Bingen BO, Engels MC, Schaliij MJ, Jangsangthong W, Neshati Z, Feola I, Ypey DL, Askar SF, Panfilov AV, Pijnappels DA, de Vries AA. Light-induced termination of spiral wave arrhythmias by optogenetic engineering of atrial cardiomyocytes. *Cardiovasc Res* 2014;**104**:194–205.
9. Lin JY, Knutsen PM, Muller A, Kleinfeld D, Tsien RY. ReaChR: a red-shifted variant of channelrhodopsin enables deep transcranial optogenetic excitation. *Nat Neurosci* 2013;**16**:1499–1508.
10. Hilleman DE, Bauman AL. Role of antiarrhythmic therapy in patients at risk for sudden cardiac death: an evidence-based review. *Pharmacotherapy* 2001;**21**:556–575.
11. Bohnen M, Stevenson WG, Tedrow UB, Michaud GF, John RM, Epstein LM, Albert CM, Koplak BA. Incidence and predictors of major complications from contemporary catheter ablation to treat cardiac arrhythmias. *Heart Rhythm* 2011;**8**:1661–1666.
12. Hondeghem LM, Carlsson L, Duker G. Instability and triangulation of the action potential predict serious proarrhythmia, but action potential duration prolongation is antiarrhythmic. *Circulation* 2001;**103**:2004–2013.
13. Boyle PM, Williams JC, Ambrosi CM, Entcheva E, Trayanova NA. A comprehensive multiscale framework for simulating optogenetics in the heart. *Nat Commun* 2013;**4**:2370.
14. Prasad KM, Smith RS, Xu Y, French BA. A single direct injection into the left ventricular wall of an adeno-associated virus 9 (AAV9) vector expressing extracellular superoxide dismutase from the cardiac troponin-T promoter protects mice against myocardial infarction. *J Gene Med* 2011;**13**:333–341.
15. Kikuchi K, McDonald AD, Sasano T, Donahue JK. Targeted modification of atrial electrophysiology by homogeneous transmural atrial gene transfer. *Circulation* 2005;**111**:264–270.
16. Bruegmann T, Boyle PM, Vogt CC, Karathanos TV, Arevalo HJ, Fleischmann BK, Trayanova NA, Sasse P. Optogenetic defibrillation terminates ventricular arrhythmia in mouse hearts and human simulations. *J Clin Invest* 2016;**126**:3894–3904.