Editorial

*CALM*ing Down Arrhythmogenic Calmodulinopathies via a Precision Medicine Approach

Donald M. Bers

In this issue, Limpitikul et al[1] used the novel CRISPR interference (CRSPRi) strategy to suppress the expression of an arrhythmogenic mutant calmodulin (CaM) gene in human induced pluripotent cell-derived cardiomyocytes (iPSC-CM). This mutant CaM (D130G) is linked with long-QT syndrome (LQTS).[2] The main LQTS mechanism is via a loss in Ca^{2+} affinity of CaM, resulting in a loss of Ca-dependent inactivation of L-type Ca current (I_{Ca}) and hence prolongation of the ventricular action potential duration (Figure).[2–4] Several other human CaM mutations have been linked with LQTS and seem likely to function at I_{Ca} in the same way (D96V, N98I, A103V, D130V, D134H, E141G, F142L).[5] Indeed, all of these mutations are in the third and fourth Ca^{2+} binding domains of CaM, which are known to be critical in I_{Ca} inactivation.[6] Human CaM mutations have also been linked to catecholaminergic polymorphic ventricular tachycardia (CPVT), a phenotype that is mediated by inappropriate diastolic SR Ca release channel (ryanodine receptor 2 [RyR2]) activity (CaM-N54I, N98S, and A103V)[7–9] and also by the promotion of late Na current (I_{NaL}; CaM-141G)[5] and some degree of target overlap exists. For example, N98S and A103V promote both I_{Ca} and RyR2-mediated Ca leak, and E141G has effects on both I_{Ca} and I_{NaL}. Given the ubiquitous role of CaM in cellular Ca^{2+}-dependent signaling, this is likely to be only the tip of the iceberg for CaM mutant effects, albeit highly apparent because of the strong arrhythmogenic phenotype. Many other CaM-signaling pathways might also be altered by human CaM mutations, but direct linkages have yet to be made (and may be more challenging to demonstrate).

Article, see p 39

One of the intriguing aspects of the recently emergent group of calmodulinopathies is that there are 3 genes (*CALM1*, *CALM2*, and *CALM3*) that all encode the identical CaM protein, and all three genes are expressed in heart. Indeed, cardiac arrhythmia–linked mutations as above occur in each of the 3 genes and exhibit autosomal dominance. That means that these mutant CaMs can produce their functional effects when 5 other *CALM* alleles are producing wild-type (WT) CaM (Figure). The relative expression levels of *CALM1*, *CALM2*, and *CALM3* mRNA in human heart are ≈13%, 25%, and 61% respectively.[2] Although relative protein levels are unknown, only 1 of the 17 reported arrhythmogenic CaM mutations is in *CALM3*. That suggests that as little as 7% to 13% of mutant CaM is sufficient to cause a penetrant phenotype in patients. One can appreciate that real consequences could result if only a small fraction of I_{Ca} fails to inactivate during the action potential, or a small fraction of RyR2s exhibit diastolic SR Ca leak. However, for those mutants studied, the LQTS-linked mutant CaMs have higher affinity versus WT CaM for the Ca channel,[3] and the same is true for the CPVT-linked mutants and RyR2.[7] Thus, even at low diastolic $[Ca]_i$, the mutant CaMs outcompete WT CaM in binding to the L-type Ca channel and RyR2. Then when local $[Ca^{2+}]_i$ rises, the associated CaM fails to bind Ca^{2+} or induce I_{Ca} inactivation (or RyR2 closure). This creates a disproportionately large functional effect and fraction of L-type Ca channel and RyR2 that are dysfunctional versus the fractional expression levels of the isoform allele (Figure). It should be noted that this potent prebinding of apo-CaM (without Ca^{2+} bound) to the channel is an important aspect because if the channel interaction required Ca-CaM to bind to the channel, these weak Ca^{2+}-binding CaMs might have little effect. One of the CPVT mutant CaMs (N54I) does not alter Ca^{2+} affinity, but this Apo-CaM binds to RyR2 (even with an 8-fold excess of WT CaM), and increases RyR2 opening (unlike WT CaM than suppresses opening).[7] These amplified gain-of-function effects on arrhythmogenic Ca^{2+} fluxes may cause the relatively high functional penetrance in young patients.[5]

Limpitikul et al[1] took advantage of the redundancy among CaM produced by the 3 *CALM* gene isoforms and used a variant of the CRISPR/Cas9 technology to knockdown CaM expressed only from the mutant *CALM2* gene. *CALM2* mRNA was reduced by >50%, whereas overall CaM expression was near normal, because of the unaltered expression of *CALM1* and *CALM3* gene products. They used the CRISPR interference strategy, which binds specifically to the CALM2 gene to inhibit transcription of that gene. Importantly, the suppressor construct does not cleave the DNA because the incorporated Cas9 is catalytically dead, but the design causes partial suppression of both CALM2 alleles. This could be an advantage (versus a direct mutation-site-targeted suppression) in that the same vector could, in principle, be used to knockdown any *CALM2*-linked mutant CaM (and *CALM2* is responsible for roughly half of the arrhythmogenic phenotypes). The authors also show that a similar approach could selectively suppress either *CALM1* or *CALM3* gene expression. Thus, this strategy, with 3 optimized suppressors could be extended to precision therapy for all CaM-opathies.

This study also makes excellent use of iPSC-CMs to test this precision medicine strategy in cardiac myocytes from an

The opinions expressed in this article are not necessarily those of the editors or of the American Heart Association.

From the Department of Pharmacology, University of California, Davis.

Correspondence to Donald M. Bers, PhD, Department of Pharmacology, University of California, Davis, 451 Health Science Dr, Davis, CA 95616. E-mail dmbers@ucdavis.edu

(*Circ Res*. 2017;120:3-4.
DOI: 10.1161/CIRCRESAHA.116.310216.)

© 2017 American Heart Association, Inc.

Circulation Research is available at http://circres.ahajournals.org
DOI: 10.1161/CIRCRESAHA.116.310216

Downloaded from http://circres.ahajournals.org/ by guest on March 23, 2017

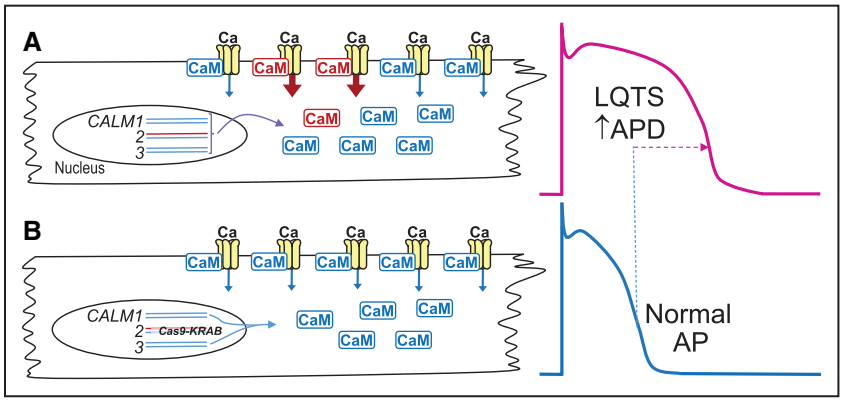

Figure. Long-QT syndrome (LQTS)–linked calmodulin (CaM) mutations cause action potential (AP) prolongation. A, Myocyte schematic showing nuclear *CALM1*, *CALM2*, and *CALM3* genes (5 normal blue alleles and 1 mutant red allele) that produce proportional amounts of normal (blue) and mutant CaM (red). Mutant CaM has weaker Ca^{2+} affinity, but higher Ca^{2+} channel (yellow) affinity vs normal CaM. This results in a disproportionate fraction of Ca^{2+} channels with mutant CaM, greater inward I_{Ca} (because of reduced Ca-dependent inactivation) and AP duration (APD) prolongation. **B**, Knockdown of *CALM2* gene expression by the Cas9–KRAB fusion protein limits mutant CaM expression, with normalization of I_{Ca} gating and APD.

actual LQTS patient (QT_C=740 ms) with the D130G point mutation in *CALM2*. While iPSC-CM function imperfectly matches that in adult ventricular myocytes, the authors demonstrate nicely that this patient's iPSC-CMs exhibit the LQTS phenotype with long action potential durations, slowed I_{Ca} inactivation and myocyte Ca^{2+} alterations (versus a WT control). Finally they show that lentiviral expression of the CRISPR interference in iPSC-CMs from this patient caused action potential duration shortening and accelerated I_{Ca} inactivation, nearly normalizing these properties toward WT iPSC-CM (parallel to the down-regulation of *CALM2* mRNA).

This is an exciting new therapeutic strategy that is exemplar of what we think about with respect to precision medicine. That is, it takes a cluster of clinical arrhythmia phenotypes (LQTS, CPVT and idiopathic ventricular fibrillation[10]), determines the causative mutation, and provides a therapeutic strategy that directly targets the mutant gene that can be tailored to specific subgroups of patients (ie, where 1 of the 3 *CALM* genes can be selectively suppressed). Of course, before this gets to patients, this important proof-of-principle strategy will require further development in terms of suppressor optimization and the usual challenges of delivery, efficacy, and safety of gene therapy. But this is an exciting strategy and study.

On a personal note, the late David T. Yue was a true pioneer whose group critically developed our current understanding of exactly how Ca^{2+}-CaM regulates Ca channel gating at the molecular and biophysical level. This is a wonderful study that extends David's phenomenal body of work in a truly translational manner and may pave the way to a novel precision medicine approach in the exact field of his focus.

Acknowledgments

We thank Dr David J. Segal for useful discussions.

Sources of Funding

This study was supported by National Institutes of Health R01-030077 and R01-HL092097.

Disclosures

None.

References

1. Limpitikul WB, Dick IE, Tester DJ, Boczek NJ, Limphong P, Yang W, Choi MH, Babich J, DiSilvestre D, Kanter RJ, Tomaselli GF, Ackerman MJ, Yue DT. A precision medicine approach to the rescue of function on malignant calmodulinopathic long-QT syndrome. *Circ Res*. 2017;120:39–48. doi: 10.1161/CIRCRESAHA.116.309283.
2. Crotti L, Johnson CN, Graf E, et al. Calmodulin mutations associated with recurrent cardiac arrest in infants. *Circulation*. 2013;127:1009–1017. doi: 10.1161/CIRCULATIONAHA.112.001216.
3. Limpitikul WB, Dick IE, Joshi-Mukherjee R, Overgaard MT, George AL Jr, Yue DT. Calmodulin mutations associated with long QT syndrome prevent inactivation of cardiac L-type Ca(2+) currents and promote proarrhythmic behavior in ventricular myocytes. *J Mol Cell Cardiol*. 2014;74:115–124. doi: 10.1016/j.yjmcc.2014.04.022.
4. Yin G, Hassan F, Haroun AR, Murphy LL, Crotti L, Schwartz PJ, George AL, Satin J. Arrhythmogenic calmodulin mutations disrupt intracellular cardiomyocyte Ca2+ regulation by distinct mechanisms. *J Am Heart Assoc*. 2014;3:e000996. doi: 10.1161/JAHA.114.000996.
5. Boczek NJ, Gomez-Hurtado N, Ye D, et al. Spectrum and prevalence of CALM1-, CALM2-, and CALM3-encoded calmodulin variants in long QT syndrome and functional characterization of a novel long QT syndrome-associated calmodulin missense variant, E141G. *Circ Cardiovasc Genet*. 2016;9:136–146. doi: 10.1161/CIRCGENETICS.115.001323.
6. Alseikhan BA, DeMaria CD, Colecraft HM, Yue DT. Engineered calmodulins reveal the unexpected eminence of Ca2+ channel inactivation in controlling heart excitation. *Proc Natl Acad Sci U S A*. 2002;99:17185–17190. doi: 10.1073/pnas.262372999.
7. Hwang HS, Nitu FR, Yang Y, Walweel K, Pereira L, Johnson CN, Faggioni M, Chazin WJ, Laver D, George AL Jr, Cornea RL, Bers DM, Knollmann BC. Divergent regulation of ryanodine receptor 2 calcium release channels by arrhythmogenic human calmodulin missense mutants. *Circ Res*. 2014;114:1114–1124. doi: 10.1161/CIRCRESAHA.114.303391.
8. Nyegaard M, Overgaard MT, Søndergaard MT, Vranas M, Behr ER, Hildebrandt LL, Lund J, Hedley PL, Camm AJ, Wettrell G, Fosdal I, Christiansen M, Børglum AD. Mutations in calmodulin cause ventricular tachycardia and sudden cardiac death. *Am J Hum Genet*. 2012;91:703–712. doi: 10.1016/j.ajhg.2012.08.015.
9. Gomez-Hurtado N, Boczek NJ, Kryshtal DO, Johnson CN, Sun J, Nitu FR, Cornea RL, Chazin WJ, Calvert ML, Tester DJ, Ackerman MJ and Knollmann BC. Novel CPVT-associated calmodulin mutation in CALM3 (CALM3-A103V) activates arrhythmogenic Ca waves and sparks. *Circ Arrhythm Electrophysiol*. 2016;9.
10. Marsman RF, Barc J, Beekman L, Alders M, Dooijes D, van den Wijngaard A, Ratbi I, Sefiani A, Bhuiyan ZA, Wilde AA, Bezzina CR. A mutation in CALM1 encoding calmodulin in familial idiopathic ventricular fibrillation in childhood and adolescence. *J Am Coll Cardiol*. 2014;63:259–266. doi: 10.1016/j.jacc.2013.07.091.

Key Words: Editorials ■ arrhythmias, cardiac ■ calcium ■ calcium channels ■ calmodulin ■ long QT syndrome

Downloaded from http://circres.ahajournals.org/ by guest on March 23, 2017

Circulation Research
Journal of the American Heart Association

*CALM*ing Down Arrhythmogenic Calmodulinopathies via a Precision Medicine Approach

Donald M. Bers

Circ Res. 2017;120:3-4
doi: 10.1161/CIRCRESAHA.116.310216
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2017 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/120/1/3

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in *Circulation Research* can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to *Circulation Research* is online at:
http://circres.ahajournals.org//subscriptions/

Downloaded from http://circres.ahajournals.org/ by guest on March 23, 2017