CALMing Down Arrhythmogenic Calmodulinopathies via a Precision Medicine Approach

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In this issue, Limpitikul et al. 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). The main LQTS mechanism is via a loss in Ca2+ affinity of CaM, resulting in a loss of Ca-dependent inactivation of L-type Ca current (ICa) and hence prolongation of the ventricular action potential duration (Figure). Several other human CaM mutations have been linked with LQTS and seem likely to function at ICa in the same way (D96V, N98I, A103V, D130V, D134H, E141G, F142L). Indeed, all of these mutations are in the third and fourth Ca2+ binding domains of CaM, which are known to be critical in ICa inactivation. 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 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 (INaL; CaM-141G)5 and some degree of target overlap exists. For example, N98S and A103V promote both ICa and RyR2-mediated Ca leak, and E141G has effects on both ICa and INaL. Given the ubiquitous role of CaM in cellular Ca2+-dependent signaling, this is likely to be the only 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).

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. 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 ICa 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 [Ca2+], the mutant CaMs outcompete WT CaM in binding to the L-type Ca channel and RyR2. Then when local [Ca2+] rises, the associated CaM fails to bind Ca2+ or induce ICa 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 Ca2+ bound) to the channel is an important aspect because if the channel interaction required Ca-CaM to bind to the channel, these weak Ca2+-binding CaMs might have little effect. One of the CPVT mutant CaMs (N54I) does not alter Ca2+ 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 Ca2+ fluxes may cause the relatively high functional penetrance in young patients.5 Limpitikul et al. 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...
actual LQTS patient (QTc=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 ICa inactivation and myocyte Ca2+ 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 ICa 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), 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 Ca2+-CaM regulates Cama channel gating at the molecular and biophysical level. This is a wonderful study that extends David’s phenomenal body of work in such an Irving and complete 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

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

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 Ca2+ affinity, but higher Ca2+ channel (yellow) affinity vs normal CaM. This results in a disproportionate fraction of Ca2+ channels with mutant CaM, greater inward ICa (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 ICa gating and APD.
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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
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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:
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