Intracellular Calcium Handling Dysfunction and Arrhythmogenesis
A New Challenge for the Electrophysiologist

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Catecholaminergic polymorphic ventricular tachycardia (CPVT) is an inherited arrhythmogenic disease characterized by the development of adrenergically mediated bidirectional and polymorphic ventricular tachycardia in individuals with a normal heart. Although this disease was initially described by Counsell in the seventies, it was only after the identification of its genetic substrate that interest about this uncommon clinical condition has extended beyond pediatric cardiology to involve a broader spectrum of clinicians and basic scientists.

CPVT is caused by mutations in 2 genes encoding calsequestrin and the cardiac ryanodine receptor; ie, 2 proteins strongly implicated in the regulation of intracellular calcium. The currently incomplete understanding of calcium homeostasis in the heart under normal settings as well as in disease states has led to consideration of CPVT as a simplified human and experimental model that may help to clarify intracellular calcium regulation.

Since the clinical description of CPVT, it was noted that the bidirectional VT that is the distinguishing manifestation of the disease resembles the VT observed in patients with digitalis intoxication. For that reason it has been speculated that DAD-mediated triggered activity would be the most likely electrophysiologic mechanism for arrhythmia initiation in CPVT. As of today, a conclusive demonstration of this hypothesis is lacking, and this is why studies like the one presented by Jiang et al in this issue of Circulation Research are of major relevance.

Jiang et al have investigated in vitro the functional characteristics of different point mutations identified in patients with CPVT: their study is not the first of this kind; yet it brings novel insight and provides new arguments that help addressing controversial aspects in the field. In this editorial we will examine the areas of debate in the understanding of CPVT and will discuss the data reported by Jiang et al in the context of the leading speculations that have been elaborated to account for arrhythmogenesis in CPVT.

What Is the Role of FKBP12 in the Pathogenesis of CPVT?

The results presented by Jiang et al address an important unresolved dispute that is present in the literature about the molecular mechanism that links a mutation in RYR2 protein and the development of tachyarrhythmias. In the last few years Wehrens et al have elaborated a converging theory to explain arrhythmogenesis in heart failure and in CPVT. Based on the evidence that arrhythmias in the failing heart are likely to be initiated by triggering rhythms, these authors have proposed that a common final pathway for arrhythmogenesis in CPVT and HF is provided by the reduced affinity of RyR2 for the FKBP12.6 protein. Functional characterization of RyR2 performed by investigators of this group has provided experimental evidence suggesting that RyR2 mutations reduce the affinity of the ryanodine receptor for FKBP12.6 and that the same effect is produced by the disease process occurring in the failing heart. Additional compelling evidence to link FKBP12.6 binding to RyR2 and arrhythmias has come from studies based on a FKBP12.6 knock-out model in which reduced FKBP12.6 binding, assessed by FKBP12.6-RYR2 communoprecipitation, has been linked to the development of adrenergically-mediated polymorphic VTs that resemble those occurring in CPVT patients. In the present study, however, Jiang et al further extend their previous observations and contest the data by Wehrens showing that CPVT mutations of RyR2 do not alter the binding of FKBP12.6 (see also George et al).

This debate is not limited to a theoretical interest, as it has remarkable practical implications: the hypothesis advanced by Wehrens et al had been accompanied by a remarkable effort of these authors to develop a novel pharmacological approach to restore FKBP12.6 binding. These authors tested a novel compound called JTV519 that increases affinity of FKBP12.6 for RyR2, and they demonstrated that in vivo administration of JTV519 is able to restore binding of FKBP12.6 to RyR2 to levels observed in controls and to prevent the development of adrenergically-mediated arrhythmias. These results raised hope of having identified a new pharmacological strategy for the treatment of CPVT: this achievement would represent a major clinical finding. CPVT patients are incompletely protected by therapy with beta blockers, and the implant of an ICD, although life-saving, is certainly associated with reduction of the quality of life in this pediatric population that is more susceptible to device-related complications. The data presented here by Jiang et al raise the concern that, in carriers of mutations of RyR2, the pharmacological approach proposed by Wehrens et al may...
not be applicable. To provide a more conclusive answer to the issue, it is expected that the recently developed knock-in mouse model manifesting arrhythmias identical to those observed in patients will be a better preclinical setting to test the hypothesis of Wehrens and to assess the role of JTV in preventing the CPVT arrhythmic phenotype.

**CPVT or ARVC?**

One intriguing aspect of the phenotype linked to RYR2 mutations is related to the fact that, whereas most of the investigators have reported that RyR2 is the gene for CPVT (ie, adrenergically mediated arrhythmias in the normal heart), one single group has supported the view that RyR2 is the gene for right ventricular cardiomyopathy of type 2. Because right ventricular cardiomyopathy is a disease of adhesion molecules, the identification of one variant of ARVC caused by mutations in a calcium-controlling protein has raised substantial interest and scientific debate. Tiso et al have proposed that mutations in the amino terminus of the ryanodine receptor gene cause ARVC2 and that these mutations cause different functional derangements than those associated with CPVT. Tiso et al had in fact suggested that at least one amino-terminal mutation of RYR2 reduces the affinity of the ryanodine receptor for the regulatory protein FKBP12.6; the same authors reported that CPVT mutations would instead increase this affinity. The data presented by Jiang et al on the contrary clearly show that, irrespective of their position on the putative topology of the protein, all mutations identified in patients with polymorphic and bidirectional VT lead to a similar “gain of function” that sensitizes the ryanodine receptor to a premature release of calcium from the intracellular stores. In light of these data it seems prudent to call for a reappraisal of the diagnosis of ARVC and to assess the consequences of genetic abnormalities present in cardiac cells in vitro. Surrogate experiments, like the one presented by Jiang et al, suggest that mutant RyR2 may lead to the development of DADs; however, further support of this hypothesis in adult myocytes is needed before the mechanism for arrhythmogenesis in CPVT can be conclusively demonstrated.

Another open issue that will have to be addressed in knock-in animal models of CPVT whenever a role for DADs is confirmed will be to define whether DADs in vivo originate in the ventricular myocytes or in the Purkinje fibers. For several years this debate has remained unsettled, and only few studies have supported the concept that DADs originating in the ventricular tissue may propagate the entire heart and elicit ventricular tachycardia. If the development of triggered activity in myocytes isolated from transgenic models of CPVT will confirm the presence of DADs, it will become possible to devise mapping studies to identify the site of origin of the triggered beats and also to explain why the tachycardia often has its typical bidirectional morphology. Such a contribution will extend beyond the pathophysiology of CPVT and will contribute to shed new light on the role of triggered activity in the human heart.

**Do RyR2 Mutations Alter Calcium Handling at Rest?**

A final controversy that is addressed by Jiang et al concerns the role of RYR2 mutations in modifying intracellular calcium control in the absence of adrenergic stimulation. The group of Chen has thus far been the only laboratory reporting the presence of abnormalities in calcium release from intracellular store in resting conditions (ie, without caffeine administration or beta adrenergic stimulation). The issue is not marginal, as in clinical settings it may have major relevance to know if abnormalities are constantly present in the heart of CPVT patients and that they are exacerbated by adrenergic stimulation, or if the heart of patients affected by CPVT have normal calcium control at rest which becomes altered only in response to excitatory stimulation. The fact that beta-blockers afford only an incomplete protection and that they seem more effective in slowing ventricular tachycardia rather than abolishing it would favor the concept that abnormalities are present already in resting conditions. Once more the availability of an animal model of CPVT may help sort this issue: if the presence of abnormal SOIRC would be confirmed in vivo, the therapeutic strategy for CPVT should explore means of preventing SOIRC and stabilizing RyR2 rather than simply blocking the trigger for calcium release as currently done with the use of beta blockers.

**Does Triggered Activity Initiate Arrhythmias in CPVT?**

The study by Jiang et al reinforces the hypothesis that delayed after-depolarisations (DADs) trigger arrhythmia initiation in CPVT. The role of triggered activity in vivo is still debated, and whether DADs originating in the myocardium may initiate ventricular tachycardia is still unresolved. Because RyR2 is a very large gene, it has not been possible so far to transduce adult myocytes with the mutant RyR2 gene and assess the consequences of genetic abnormalities present in CPVT in cardiac cells in vitro. Surrogate experiments, like the one presented by Jiang et al, suggest that mutant RyR2 may lead to the development of DADs; however, further support of this hypothesis in adult myocytes is needed before the mechanism for arrhythmogenesis in CPVT can be conclusively demonstrated.
clinical models in which severe arrhythmic phenotypes are caused by 1 amino acid replacement that we may gain invaluable and largely unexpected insight in the fine processes controlling the heart rhythm.

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References


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