Timothy syndrome is a rare genetic disorder characterized by QT prolongation (designated LQT8), arrhythmias and sudden death, structural heart disease, cognitive defects with autism, syndactyly (webbed fingers and toes), hypoglycemia, and immune deficiencies.\(^1,2\) A single mutation (G406R) in exon 8a of the cardiac L-type calcium channel (CACNA1C, Ca,1.2, \(\alpha_1 c\)) was shown to cause Timothy syndrome in multiple unrelated subjects, whereas mutations (G406R, G402S) in the alternatively spliced exon 8 (which is expressed at \(\approx 3\)-fold–higher levels than exon 8a) cause a similar syndrome lacking syndactyly.\(^3,4\) These 3 mutations decrease voltage-dependent inactivation of Ca,1.2, which is predicted to slow the inactivation of \(I_{\text{Ca,L}}\) during each action potential, prolong action potential and QT interval duration, increase the amplitude and duration of \(\text{Ca}^{2+}\) transients, and predispose to afterdepolarizations and arrhythmias.\(^3,5\)

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Calcium/calmodulin–dependent protein kinase II (CaMKII) is a part of a family of serine/threonine kinases regulated by calcium bound to calmodulin and is encoded by 4 genes (\(\alpha, \beta, \gamma, \delta\)).\(^6,7\) Six to 12 subunits homo- or heteromultimerize to form the active enzyme. Each subunit contains an N-terminal catalytic domain that binds ATP and substrate, a regulatory domain that includes an autoinhibitory domain, and a Ca/Calmodulin–binding domain, a C-terminal multimerization domain, and in some cases a nuclear localization signal.\(^8\) CaMKII functions as a local calcium sensor in the heart. In the absence of elevated intracellular \(\text{Ca}^{2+}\), the autoinhibitory domain prevents substrate binding to the enzyme. During each heartbeat, intracellular \(\text{Ca}^{2+}\) rises because of transmembrane influx through L-type \(\text{Ca}^{2+}\) channels or the Na/Ca exchanger and release from internal stores such as the sarcoplasmic reticulum (SR). The \(\text{Ca}^{2+}\) binds to calmodulin, and the \(\text{Ca}^{2+}/\text{calmodulin}\) complex binds to the regulatory domain of CaMKII and removes the inhibition of the autoinhibitory domain. In addition, the activated CaMKII can be autophosphorylated, which allows the enzyme to remain active in the absence of elevated intracellular \(\text{Ca}^{2+}\).

A number of cardiac effects related to cytoplasmic CaMKII activation have been identified using in vitro expression systems, CaMKII inhibitors in vivo, and transgenic mice. CaMKII mediates \(\text{Ca}^{2+}\)-dependent facilitation of the L-type \(\text{Ca}^{2+}\) channel, an increase in \(I_{\text{Ca,L}}\) at increased stimulation frequencies due to an increase of peak current, and slowing of inactivation.\(^9\) In addition, CaMKII phosphorylates the ryanodine receptor to increase SR leak.\(^10\) Both of these actions lead to increased intracellular \(\text{Ca}^{2+}\), which can further enhance CaMKII activity along with promoting afterdepolarizations and arrhythmias. CaMKII can also affect the activity of other ion channels. Transgenic mice overexpressing the CaMKII inhibitory peptide AC3-I have shorter action potential durations and QT intervals as a result of upregulation of the inward rectifier current \(I_{\text{K1}}\) and the transient outward current \(I_{\text{to,f}}\), with no significant changes in channel RNA or protein expression.\(^11\) CaMKII expression may also play a role in heart failure. Transgenic mice overexpressing CaMKII in the heart develop dilated cardiomyopathy.\(^12\) CaMKII suppression by KN93 or by overexpression of the CaMKII inhibitory peptide AC3-I eliminates \(\text{Ca}^{2+}\)-dependent facilitation, improves cardiac function, and suppresses arrhythmias in a calcineurin overexpression mouse heart failure model or after myocardial infarction.\(^13,14\)

In this issue of the *Circulation*, Thiel et al used isolated rat cardiomyocytes infected with dihydropyridine-resistant wild type and G406R Ca,1.2 channels to study the mechanisms underlying proarrhythmia in Timothy syndrome.\(^15\) As expected, myocytes infected with mutant G406R channels had action potential prolongation, afterdepolarizations, and decreased voltage-dependent inactivation. In addition, the Timothy syndrome mutation increased CaMKII autophosphorylation, enhanced \(I_{\text{Ca,L}}\) facilitation, and slowed \(I_{\text{Ca,L}}\) inactivation. Of note, inhibition of CaMKII with the AC3-I inhibitory peptide normalized action potential duration, prevented afterdepolarizations, and normalized \(I_{\text{Ca,L}}\) facilitation and inactivation. Moreover, myocytes infected with mutant channels had larger but briefer \(\text{Ca}^{2+}\) transients, no change in SR \(\text{Ca}^{2+}\) content, and an increase in \(\text{Ca}^{2+}\) spark frequency. The authors concluded that although changes in voltage-dependent inactivation may initiate the cardiac abnormalities in Timothy syndrome, downstream changes mediated by CaMKII on \(\text{Ca}^{2+}\)-dependent facilitation and SR \(\text{Ca}^{2+}\) release channels (ryanodine receptors) are required for the arrhythmia phenotype.

The findings by Thiel et al are potentially important for several reasons. First, the mechanistic link between changes in \(I_{\text{Ca,L}}\) and arrhythmia susceptibility in Timothy syndrome appears more complex than previously thought. Second, this is the first evidence that CaMKII directly participates in arrhythmia susceptibility in a human inherited channelopathy. Third, although Timothy syndrome is rare, abnormalities in \(\text{Ca}^{2+}\) handling may cause arrhythmias and sudden death in common cardiac conditions including heart failure.\(^12,14\) CaMKII-mediated changes in \(I_{\text{Ca,L}}\) facilitation and ryanodine receptor leak can be studied in these other pathological states,
and the potential efficacy of CaMKII inhibition can be assessed. Finally, the findings raise the intriguing possibility that CaMKII-related proteins may contribute to the extracardiac phenotypes including autism.

A number of additional studies are required to confirm the conclusions. As pointed out by the authors, the findings need to be replicated in myocytes from a larger animal with cardiac action potentials more similar to those of humans. Although canine wedge studies have been performed using the Ca\(^{2+}\) channel opening drug BayK8644 to model increases in ICa,L,16 additional studies using intact hearts and whole animal models expressing mutant channels are necessary. In addition, long-term expression of the mutation may lead to different findings than short-term viral exposure, as seen with the effects on K\(^+\) channel expression caused by transgenic overexpression of the AC3-I peptide.11 Studies using G406R Ca,L.2 knockin mice could address many of these questions.

During the last 15 years, ion channelopathies have been shown to cause a number of inherited arrhythmia syndromes, including long-QT syndrome (K\(^+\), Na\(^+\), and Ca\(^{2+}\) channels), short-QT syndrome (K\(^+\) channels), Brugada syndrome (Na\(^+\) channels), short-QT and Brugada syndrome (Ca\(^{2+}\) channels), and catecholaminergic polymorphic ventricular tachycardia (SR Ca\(^{2+}\) release channels).17 Given the importance of ICa,L to cardiac electrical and mechanical function, it is surprising how few pathogenic mutations have been identified. Two potential causes could underlie this finding: Mutations could be rare because they are highly lethal, or they could be only rarely identified because they are well tolerated. The evidence suggests that both may be true. Marked increases in ICa,L occur as a result of physiological \(\beta\)-adrenergic stimulation; similarly, L-type Ca\(^{2+}\) channel blockers are well tolerated but do not prevent arrhythmias or sudden cardiac death despite decreasing intracellular Ca\(^{2+}\) load. The mutations that lead to Timothy syndrome are all located in the 8th exon and affect voltage-dependent inactivation. Similarly, only a few mutations characterized by marked loss of Ca\(^{2+}\) channel function have been identified in individuals with an overlap syndrome consisting of short-QT intervals and Brugada-like ECG abnormalities.18 Thus, it is possible that whereas modest changes in the amplitude of ICa,L are well tolerated, changes in its time course disrupt important signaling mechanisms including CaMKII and are poorly tolerated. If true, heterozygous mutations that alter channel number or current amplitude would have a limited phenotype, whereas rare mutations that alter channel properties in specific ways would lead to highly lethal arrhythmia syndromes.

In summary, the study by Thiel et al has expanded our understanding of the pathophysiological mechanisms underlying Timothy syndrome. In addition, it may ultimately provide new insights into the role of CaMKII in arrhythmia susceptibility, into Ca\(^{2+}\) handling in other organs including the brain, and into the regulatory pathways that are controlled by cardiac Ca\(^{2+}\) channels. In addition, at least some hope exists that these findings could ultimately lead to novel therapeutic options to treat or prevent life-threatening arrhythmias.

Disclosures
None.

References

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