

The Role of Calcium/Calmodulin-Dependent Protein Kinase II Activation in Hypertrophic Cardiomyopathy

Article, see p 1738

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The classic definition of hypertrophic cardiomyopathy (HCM) as originally described by Teare¹ is deceptively simple: “left ventricular hypertrophy in the absence of any identifiable cause.” This definition fits the majority of referral patients who are identified by either their symptoms or incidental findings on noninvasive testing. Longitudinal studies, however, including a seminal study performed by Frank and Braunwald² in 1964, clearly described the disorder much as we know it today: a complex, progressive, and highly variable cardiomyopathy. Subsequent linkage studies in the early 1990s established mutations in the components of the cardiac sarcomere as the molecular cause of the familial form of HCM. Since then, the growing accessibility of genetic screening and advent of specialized centers of care have greatly expanded our understanding of what is now considered to be a genetic cardiomyopathy until proven otherwise.³

Even with this sophisticated understanding of the genetic etiology of the disorder, many clinically relevant questions remain regarding the basic mechanisms whereby mutations in sarcomeric proteins lead to the variable degrees of myocardial remodeling that define HCM. In other words, how does disrupted myofilament function trigger a myocellular response that alters the geometry of the heart leading to arrhythmias, impaired relaxation, hyperdynamics, and, in some cases, left ventricular outflow tract obstruction? Recent longitudinal studies and a pilot clinical trial of genotyped HCM cohorts have helped to refine and integrate these important questions.^{4,5} In particular, the observation that patients carrying sarcomeric mutations (sarc-positive HCM) have worse outcomes that may not necessarily be defined by the degree of hypertrophy has refocused research efforts on both the preclinical state and temporal development of the disorder.⁶ Thus, we now know that sarc-positive patients may exhibit impaired relaxation before the onset of symptoms or overt left ventricular hypertrophy, that they exhibit a nonlinear trajectory and focal patterns of ventricular remodeling, and, finally, that diltiazem can slow the initial development of left ventricular hypertrophy in some cohorts.^{4,5,7} This far more nuanced clinical understanding of sarc-positive HCM establishes a framework for testable hypotheses that will finally allow for proactive management and perhaps even genotype-driven care instead of the current symptom palliation.

Building on these central observations and previous results from transgenic animal models of HCM demonstrating early activation of calcium/calmodulin-dependent protein kinase II (CaMKII δ) activity, in the current issue of *Circulation*, Helms et al⁸ utilized a robust set of myomectomy samples from patients with extensively phenotyped and fully genotyped hypertrophic cardiomyopathy to delineate the potential role of CaMKII δ activation in disease pathogenesis. The study is specifically focused on identifying pathways known to be involved in the regulation of myocellular calcium homeostasis that may distinguish the natural history of HCM patients with sarcomeric gene mutations (sarc-positive HCM) from those who do not carry sarcomeric mutations (sarc-negative

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HCM). Extensive transcriptional profiling of the known components of the Ca^{2+} regulatory machinery, CaMKII isoforms, and the mediators of hypertrophic growth revealed only a decrease in sarco/endoplasmic reticulum Ca^{2+} -ATPase (SERCA2A) transcripts in both HCM groups compared with controls. It is important to note that the SERCA2A results were validated at the protein level where the SERCA2A/phospholamban (PLN) ratio was decreased for both HCM cohorts, indicative of extensive myocellular remodeling. Of particular note, CaMKII δ expression was differentially upregulated only in the sarc-positive HCM samples. This isolated upregulation suggested that CaMKII δ may act as a nodal point for the often aggressive cardiac remodeling observed in sarc-positive HCM patients.

To address this hypothesis, the phosphorylation states of CaMKII δ and its relevant substrates were measured, revealing a significant level of auto-phosphorylated CaMKII δ and PLN only in the sarc-positive HCM samples and a trend toward an increase in histone deacetylase 4 phosphorylation in both HCM cohorts (sarc-positive and sarc-negative) compared with controls. The phosphorylation of histone deacetylase 4 by CaMKII δ is an important component of the signaling cascade that drives pathologic cardiac remodeling, linking myocellular Ca^{2+} dysregulation to hypertrophic growth.⁹ Subsequent probing of the downstream targets of histone deacetylase 4, nuclear factor of activated T cells, and myocyte enhancer factor-2, however, revealed no induction of transcriptional activity in the HCM samples. Similarly, the increase in phosphorylated PLN observed in the sarc-positive HCM group did not translate into a change in the Ca^{2+} sensitivity of SERCA2A uptake capacity despite a decrease in the activity and total capacity of SERCA2A for Ca^{2+} in all HCM groups. Finally, to determine the potential contribution of the calcium release axis to myocellular Ca^{2+} dysregulation in HCM, ryanodine receptor 2 abundance, phosphorylation state, and ryanodine binding capacity were assessed. Although a trend toward a decrease in ryanodine receptor 2 abundance and a concomitant small decrease in [^3H]ryanodine binding were noted for the sarc-positive HCM subset, no significant changes were noted in ryanodine receptor 2 phosphorylation potential nor was any response to oxidation or nitrosylation observed for any group.

It is illustrative to compare the current results to those of Coppini et al,¹⁰ who utilized combined sarc-positive and sarc-negative HCM myectomy samples to explore the roles of dysregulated signaling cascades in arrhythmogenicity and impaired relaxation. They found a marked decrease in Ca^{2+} kinetics (both time to peak and decay) and a consistent increase in intracellular diastolic [Ca^{2+}]. Similar to the current findings, the SERCA2A/PLN ratio was decreased, and both the amount and phosphorylation potential of CaMKII (and several downstream targets) was increased in HCM samples, leading to the conclusion that CaMKII likely occupies a central role in disease pathogen-

esis. In the current study, by expanding their sample size to facilitate a comparison of sarc-positive versus sarc-negative HCM and incorporating ^{45}Ca uptake to directly measure SERCA2A activity, Helms et al confirmed and significantly extended the previous results with the intriguing observation that the increase in auto-activated CaMKII δ was specific for sarc-positive HCM alone. Surprisingly, no additional functional effect on SERCA2A activity and only minor effects on other downstream CaMKII substrates were observed. Although these results, coupled with previous studies in murine models, are strikingly concordant regarding a likely central role of CaMKII in the pathogenesis of HCM, the trajectory of the complex remodeling cascade over time is unclear.¹¹ This lack of clarity is inherent to the limitations in using explanted tissue to study heart failure. Myectomy samples (representing established, treated disease) constrain the ability to identify early disease mechanisms, especially those involving signaling cascades. The use of genotyped HCM samples represents a significant advance in that, unlike heart failure tissue, the primary biophysical mechanism is known and, as shown in the current study, can both identify potential modulators of discrete disease subsets and provide an important framework for developing novel hypotheses of disease progression.

Perhaps given the near protean complexity of CaMKII signaling in myocardial remodeling, this lack of clarity in the context of a progressive and highly variable genetic cardiomyopathy is to be expected.¹² Pathogenic insight into complex cardiomyopathic disorders that develop over decades requires multiple levels of investigation before robust connections can be established. It is illustrative to frame the question of how mutations in the cardiac sarcomere cause HCM in the context of timescales. The timescales involved in disease pathogenesis range from nanosecond dynamics that drive the allosteric interactions between sarcomeric proteins to the milliseconds required for the calcium transient and myofilament activation, to the beat-to-beat regulation of cardiac function by the autonomic nervous system and the decades required to remodel the myocardium and disrupt function. Inherent in this system are constants and variables. For example, the presence of the primary mutation and the effect of this mutation on sarcomeric dynamics is a constant that likely triggers a variable myocellular response that can wax and wane dependent on hemodynamic demand and external stressors. Based on recent advances in our understanding of myocellular CaMKII biology, it may be uniquely positioned to play this nodal role in sarc-positive HCM remodeling.¹² It is known that sarcomeric mutations alter Ca^{2+} dissociation kinetics at the myofilament level, a change that could directly alter local nanodomains where CaMKII resides, regulated by tight spatiotemporal controls.^{13,14} Although the initial molecular response may be compensatory, prolonged, or even variable, local mechanical strain may lead to auto-activation of CaMKII and pathogenic remodeling.¹⁵ An important question remains: How do we

take into account the lack of downstream activation of hypertrophic signaling, ryanodine receptor 2 Ca²⁺ release, and SERCA2A Ca²⁺ uptake activity in the current study? As noted by the authors, it is likely due to the established disease state of the tissue. Moreover, in the context of the often-described nonlinear cardiac remodeling in sarc-positive HCM, an important avenue to investigate is whether cyclic activation and deactivation of CaMKII-responsive pathways over time underlies these periods of accelerated remodeling, an important therapeutic target.

In the main, this benchmark translational study establishes an important role for CaMKII regulation in the pathogenesis of HCM. Of course, many questions remain, again bridging the wide temporal divide that characterizes this unique disorder, sharing some but perhaps not many molecular aspects of heart failure. Although these future discoveries will require multiple methodologies to overcome the limitations of myomectomy tissue, the close concordance with previous work from mouse models of HCM and the potential application of engineered heart tissue systems can be leveraged to fill the gaps.¹⁶ The long-term goal for the management of HCM remains to identify the earliest stages of the disorder by genetic screening and to use targeted and eventually even genotype-specific therapies to alter the natural history of disease. The current study brings us closer to that future.

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FOOTNOTES

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REFERENCES

- Teare D. Asymmetrical hypertrophy of the heart in young adults. *Br Heart J*. 1958;20:1–8.
- Frank S, Braunwald E. Idiopathic hypertrophic subaortic stenosis: clinical analysis of 126 patients with emphasis on the natural history. *Circulation*. 1968;37:759–788.
- Gersh BJ, Maron BJ, Bonow RO, Dearani JA, Fifer MA, Link MS, Naidu SS, Nishimura RA, Ommen SR, Rakowski H, Seidman CE, Towbin JA, Udelson JE, Yancy CW, American College of Cardiology Foundation/American Heart Association Task Force on Practice G, American Association for Thoracic S, American Society of E, American Society of Nuclear C, Heart Failure Society of A, Heart Rhythm S, Society for Cardiovascular A, Interventions and Society of Thoracic S. 2011 ACCF/AHA guideline for the diagnosis and treatment of hypertrophic cardiomyopathy: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. *Circulation*. 2011;124:e783–e831.
- Ho CY, Cirino AL, Lakdawala NK, Groarke J, Valente AM, Semsarian C, Colan SD and Orav EJ. Evolution of hypertrophic cardiomyopathy in sarcomere mutation carriers. *Heart*. 2016;102:1805–1812. doi: 10.1136/heartjnl-2016-310015.
- Ho CY, Lakdawala NK, Cirino AL, Lipshultz SE, Sparks E, Abbasi SA, Kwong RY, Antman EM, Semsarian C, González A, López B, Diez J, Orav EJ, Colan SD, Seidman CE. Diltiazem treatment for pre-clinical hypertrophic cardiomyopathy sarcomere mutation carriers: a pilot randomized trial to modify disease expression. *J Am Coll Cardiol. Heart Fail*. 2015;3:180–188. doi: 10.1016/j.jchf.2014.08.003.
- Lopes LR, Rahman MS, Elliott PM. A systematic review and meta-analysis of genotype-phenotype associations in patients with hypertrophic cardiomyopathy caused by sarcomeric protein mutations. *Heart*. 2013;99:1800–1811. doi: 10.1136/heartjnl-2013-303939.
- Spirito P, Maron BJ, Chiarella F, Bellotti P, Tramarin R, Pozzoli M, Vecchio C. Diastolic abnormalities in patients with hypertrophic cardiomyopathy: relation to magnitude of left ventricular hypertrophy. *Circulation*. 1985;72:310–316.
- Helms AS, Alvarado FJ, Yob J, Tang VT, Pagani F, Russell MW, Valdivia HH, Day SM. Genotype-dependent and -independent calcium signaling dysregulation in human hypertrophic cardiomyopathy. *Circulation*. 2016;134:1738–1748. doi: 10.1161/CIRCULATIONAHA.115.020086.
- Backs J, Backs T, Neef S, Kreuzer MM, Lehmann LH, Patrick DM, Grueter CE, Qi X, Richardson JA, Hill JA, Katus HA, Bassel-Duby R, Maier LS, Olson EN. The delta isoform of CaM kinase II is required for pathological cardiac hypertrophy and remodeling after pressure overload. *Proceedings of the National Academy of Sciences*. 2009;106:2342–2347.
- Coppini R, Ferrantini C, Yao L, Fan P, Del Lungo M, Stillitano F, Sartiani L, Tosi B, Suffredini S, Tesi C, Yacoub M, Olivetto I, Belardinelli L, Poggesi C, Cerbai E, Mugelli A. Late sodium current inhibition reverses electromechanical dysfunction in human hypertrophic cardiomyopathy. *Circulation*. 2013;127:575–584. doi: 10.1161/CIRCULATIONAHA.112.134932.
- Guinto PJ, Haim TE, Dowell-Martino CC, Sibinga N, Tardiff JC. Temporal and mutation-specific alterations in Ca²⁺ homeostasis differentially determine the progression of cTnT-related cardiomyopathies in murine models. *Am J Physiol Heart Circ Physiol*. 2009;297:H614–H626. doi: 10.1152/ajpheart.01143.2008.
- Couchonnal LF, Anderson ME. The role of calmodulin kinase II in myocardial physiology and disease. *Physiology (Bethesda)*. 2008;23:151–159. doi: 10.1152/physiol.00043.2007.
- Bers DM. Ca²⁺-calmodulin-dependent protein kinase II regulation of cardiac excitation-transcription coupling. *Heart Rhythm*. 2011;8:1101–1104. doi: 10.1016/j.hrthm.2011.01.030.
- Williams MR, Lehman SJ, Tardiff JC, Schwartz SD. Atomic resolution probe for allosteric in the regulatory thin filament. *Proceedings of the National Academy of Sciences*. 2016;113:3257–3262.
- Jian Z, Han H, Zhang T, Puglisi J, Izu LT, Shaw JA, Onofriok E, Erickson JR, Chen YJ, Horvath B, Shimkunas R, Xiao W, Li Y, Pan T, Chan J, Banyasz T, Tardiff JC, Chiamvimonvat N, Bers DM, Lam KS, Chen-Izu Y. Mechanochemotransduction during cardiomyocyte contraction is mediated by localized nitric oxide signaling. *Sci Signal*. 2014;7:ra27. doi: 10.1126/scisignal.2005046.
- Stöhr A, Friedrich FW, Flenner F, Geertz B, Eder A, Schaaf S, Hirt MN, Uebeler J, Schlossarek S, Carrier L, Hansen A, Eschenhagen T. Contractile abnormalities and altered drug response in engineered heart tissue from Mybpc3-targeted knock-in mice. *J Mol Cell Cardiol*. 2013;63:189–198. doi: 10.1016/j.jmcc.2013.07.011.

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