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COMMENTARY

Novel cardiac myosin inhibitor for hypertrophic cardiomyopathy

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In this issue of the *Journal of General Physiology*, researchers from the University of Washington, Illinois Institute of Technology, Bristol Myers Squibb Research, and Dalian Medical University in China report the discovery of a novel small molecule, RLC-1, an inhibitor of cardiac myosin (Kooiker et al., 2024). The authors demonstrate that the effectiveness of this myosin-specific drug hinges on the presence of the myosin regulatory light chain (RLC) bound to its partner, β-myosin heavy chain (β-MHC), hence the name RLC-1. Their compelling findings show that RLC-1 can modulate the force generated by β-MHC, presenting a promising new treatment avenue for hypertrophic cardiomyopathy (HCM).

HCM is a genetic condition characterized by the thickening of the heart muscle, particularly the ventricles that can lead to various complications, including heart failure, cardiac arrhythmia, and sudden cardiac death (Maron and Maron, 2013). The prevalence of HCM in the general population is ∼1 in 200 to 1 in 500, and about 30% of cases have a known genetic cause (Semsarian et al., 2015; Ciabatti et al., 2020). Dominant pathogenic variants in HCM genes are the most common causes of HCM with the two predominant genes responsible for approximately half of the familial HCM cases being *MYH7* (β-MHC) and *MYBPC3* (cardiac myosin binding protein C) (Alcalai et al., 2008). Mutations in *MYL2* or *MYL3*, encoding the ventricular myosin RLC or ELC (essential light chain), respectively, are rare but are often implicated in malignant HCM outcomes (Yadav et al., 2019a). Many additional mutations in sarcomeric actin and regulatory proteins, such as tropomyosin, and troponins T, I, and C, have also been linked to HCM (Van Driest et al., 2003; Garfinkel et al., 2018).

Traditional therapies for HCM have been well established for decades. These treatments include β-blockers (such as Metoprolol and Atenolol) and calcium channel blockers (such as Diltiazem and Verapamil), which help manage symptoms by reducing heart rate, blood pressure, myocardial oxygen demand, and heart muscle contractility (Bickel et al., 2019). Although these interventions can effectively manage the disease in the short term, they do not provide a long-term solution as the disease progresses. More advanced options, such as the implantation of a cardioverter–defibrillator device, myectomies for obstructive HCM, and heart transplantation, are available. However, each of these options carries significant risks for patients in heart failure.

Recent research has increasingly focused on identifying pharmacological agents that can alleviate the symptoms and progression of HCM by specifically targeting the dysfunction of key sarcomeric proteins, particularly ventricular myosin, the molecular motor of the heart. The primary objective of this research is to develop myosin-specific therapeutics, such as small molecules that can modulate the force generated by the β-MHC, which currently represents the most promising treatment avenue for HCM. Recently approved by the US Food and Drug Administration, mavacamten is a myosin inhibitor that reduces hypercontractility in HCM patients by modulating the interaction between HCM-mutant myosin and actin, thereby diminishing excessive muscle contraction (Green et al., 2016; Anderson et al., 2018; Margara et al., 2022). In patients, mavacamten (marketed as Camzyos) has demonstrated the ability to ameliorate hypercontractility without lowering heart rate. However, its benefits are limited to patients with left ventricular (LV) outflow tract obstruction, and its effect on arrhythmia is unknown (Ho et al., 2020; Olivotto et al., 2020). Another selective small-molecule inhibitor of cardiac myosin, aficamten (Chuang et al., 2021), is being evaluated for its potential to treat obstructive HCM in the SEQUOIA-HCM trial, which shows significantly improved exercise capacity and relief of symptoms in patients with obstructive HCM (Coats et al., 2024). Both myosin inhibitors, mavacamten and aficamten, offer a novel approach to mitigate the severity of HCM in patients (Kalinski et al., 2024). Recent x-ray crystallography studies revealed that these drugs bind to different sites on myosin S1 and inhibit actin-activated phosphate release, although the phenotypic effects on phosphate release differ between them (Auguin et al., 2024; Hartman et al., 2024). Mavacamten slows phosphate release by 5- to 10-fold, indicating that heads can still bind actin and complete phosphate release, while aficamten slows phosphate release to a greater

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extent and turns over ATP extremely slowly, reducing the number of active force generators and cardiac contractility (Auguin et al., 2024; Hartman et al., 2024).

Following the search for effective therapeutics targeting myosin, Kooiker et al. (2024) report the discovery of a novel cardiac myosin inhibitor, RLC-1, whose effectiveness depends on the presence of the myosin RLC. In a series of elegant experiments performed in skinned and intact cardiac muscle preparations, the authors demonstrate that RLC-1 inhibits the interaction between myosin and actin, reduces contractile force development and Ca^{2+} sensitivity of force, and increases the submaximal rate constant for tension redevelopment. They also observed a decrease in the baseline and maximal ratiometric Fura-2 calcium fluorescence in isolated rat cardiomyocytes, with no changes in $[Ca^{2+}]$ transient kinetics, suggesting subtle effects of RLC-1 on calcium handling within the cell. In both loaded and unloaded intact preparations, RLC-1 substantially decreased the magnitude of contraction and shortened the overall duration of contraction, suggesting RLC-1 could be effective in reducing hypercontractility associated with HCM and potentially restoring normal heart function (Kooiker et al., 2024).

Interestingly, even though the presence of myosin RLC was required for RLC-1 to inhibit the acto-myosin ATPase, the drug failed to bind to the recombinant cardiac myosin RLC protein, suggesting that its binding pocket is located somewhere in the myosin head in the vicinity of the RLC. The RLC-1 dose-dependent binding isotherms could be successfully obtained for RLC-containing heavy meromyosin (HMM) and were not affected by the presence of various nucleotides. The binding of RLC-1 was also insensitive to HMM phosphorylation. The insensitivity of the drug action to myosin RLC phosphorylation was somewhat surprising, as cardiac myosin light chain kinase (cMLCK)-dependent RLC phosphorylation is widely known to be a major regulatory mechanism of myosin function in vivo (Yadav and Szczesna-Cordary, 2017). An acute, inducible reduction of cMLCK in mice was shown to cause sarcomeric disorganization, fibrosis, reduced contractility, and cell death, resulting in severe systolic and diastolic dysfunction and progression to heart failure (Massengill et al., 2016). Studies by Yadav et al. (2019b) suggested that RLC phosphorylation could be used as a therapeutic modality for compromised heart function associated with the HCM-RLC mutation.

The authors also used small angle x-ray diffraction to examine the effect of RLC-1 on the position of myosin heads relative to the thick and thin filaments. They observed that RLC-1 promoted the movement of myosin heads away from the thick filament backbone and closer to the thin filaments. Specifically, they showed that RLC-1 increases the equatorial reflections intensity ratio, $I_{1,1}/I_{1,0}$, and reduces the intensity of the meridional M3 reflections, suggesting that more cross-bridges might be available for contraction. These findings contrast with high-resolution structural studies of the myosin inhibitor, mavacamten, which was shown to stabilize the myosin heads in the super-relaxed (SRX) state by forming so-called interacting heads motif (IHM) (Dutta et al., 2023). Unlike mavacamten, RLC-1 did not promote the structural transition from the DRX (disordered relaxed) to SRX state, and the myosin heads were shown to be closer to the thin filaments rather than the myosin backbone. These contrasting results between RLC-1 and mavacamten highlight the need for further investigation into their distinct mechanisms of action. High-resolution crystallographic studies, such as those conducted for mavacamten and aficamten (Auguin et al., 2024; Hartman et al., 2024) might shed light on the molecular mechanism of this promising RLC-1 molecule.

The search for drugs to mitigate cardiomyopathy is an area of active research and development, driven by continuous advancements in several key scientific fields. These advancements hold promise for more effective and targeted therapies for HCM. Techniques such as gene editing using CRISPR/Cas9 (Toepfer et al., 2019) or RNAi therapeutics (Zaleta-Rivera et al., 2019) directed towards human cardiomyopathies are promising steps toward targeted therapy for prevalent heart diseases. Additionally, advances in drug discovery technologies, such as high-throughput screening (as utilized in the discovery of RLC-1 (Kooiker et al., 2024)) and computational modeling, have enabled the identification and optimization of new drug candidates. These state-of-the-art techniques have allowed researchers to provide insights into developing targeted therapies that address the root causes of the disease at a molecular level. As our understanding of the disease continues to grow and new technologies emerge, there is hope for more effective and personalized treatments that can significantly improve the quality of life for individuals with HCM.

In summary, the authors of the RLC-1 discovery convincingly showed that RLC-1 could help alleviate and improve the contractile abnormalities associated with HCM. As such, it could be used to circumvent HCM-associated phenotypes, including hypercontractility, arrhythmias, and diastolic dysfunction. However, further research is needed to identify the precise mechanism by which RLC-1 inhibits myosin-related contractility, determine whether calcium regulation within the cell is involved, and elucidate the role of the RLC protein in these processes.

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References

Alcalai, R., J.G. Seidman, and C.E. Seidman. 2008. Genetic basis of hypertrophic cardiomyopathy: From bench to the clinics. *J. Cardiovasc. Electrophysiol.* 19:104–110. https://doi.org/10.1111/j.1540-8167.2007.00965.x

Anderson, R.L., D.V. Trivedi, S.S. Sarkar, M. Henze, W. Ma, H. Gong, C.S. Rogers, J.M. Gorham, F.L. Wong, M.M. Morck, et al. 2018. Deciphering the super relaxed state of human beta-cardiac myosin and the mode of action of mavacamten from myosin molecules to muscle fibers. *Proc. Natl. Acad. Sci. USA.* 115:E8143–E8152. https://doi.org/10.1073/pnas.1809540115

Auguin, D., J. Robert-Paganin, S. Rety, C. Kikuti, A. David, G. Theumer, A.W. Schmidt, H.J. Knolker, and A. Houdusse. 2024. Omecamtiv mecarbil and Mavacamten target the same myosin pocket despite opposite effects in heart contraction. *Nat. Commun.* 15:4885. https://doi.org/10.1038/s41467-024-47587-9

Bickel, T., P. Gunasekaran, G. Murtaza, R. Gopinathannair, S. Gunda, and D. Lakkireddy. 2019. Sudden cardiac death in famous athletes, lessons learned, heterogeneity in expert recommendations and pitfalls of contemporary screening strategies. *J. Atr. Fibrillation.* 12:2193. https://doi.org/10.4022/jafib.2193

Chuang, C., S. Collibee, L. Ashcraft, W. Wang, M. Vander Wal, X. Wang, D.T. Hwee, Y. Wu, J. Wang, E.R. Chin, et al. 2021. Discovery of aficamten (CK-274), a next-generation cardiac myosin inhibitor for the treatment of hypertrophic cardiomyopathy. *J. Med. Chem.* 64:14142–14152. https://doi.org/10.1021/acs.jmedchem.1c01290

Ciabatti, M., C. Fumagalli, M. Beltrami, E. Vignini, L. Martinese, A. Tomberli, M. Zampieri, A. Bertini, G. Carrassa, A. Marchi, et al. 2020. Prevalence, causes and predictors of cardiovascular hospitalization in patients with hypertrophic cardiomyopathy. *Int. J. Cardiol.* 318:94–100. https://doi.org/10.1016/j.ijcard.2020.07.036

Coats, C.J., M.S. Maron, T.P. Abraham, I. Olivotto, M.M.Y. Lee, M. Arad, N. Cardim, C.S. Ma, L. Choudhury, H.D. Dungen, et al. 2024. Exercise capacity in patients with obstructive hypertrophic cardiomyopathy: SEQUOIA-HCM baseline characteristics and study design. *JACC Heart Fail.* 12:199–215. https://doi.org/10.1016/j.jchf.2023.10.004

Dutta, D., V. Nguyen, K.S. Campbell, R. Padron, and R. Craig. 2023. Cryo-EM structure of the human cardiac myosin filament. *Nature*. 623:853–862. https://doi.org/10.1038/s41586-023-06691-4

Garfinkel, A.C., J.G. Seidman, and C.E. Seidman. 2018. Genetic pathogenesis of hypertrophic and dilated cardiomyopathy. *Heart Fail. Clin.* 14:139–146. https://doi.org/10.1016/j.hfc.2017.12.004

Green, E.M., H. Wakimoto, R.L. Anderson, M.J. Evanchik, J.M. Gorham, B.C. Harrison, M. Henze, R. Kawas, J.D. Oslob, H.M. Rodriguez, et al. 2016. A small-molecule inhibitor of sarcomere contractility suppresses hypertrophic cardiomyopathy in mice. *Science*. 351:617–621. https://doi.org/10.1126/science.aad3456

Hartman, J.J., D.T. Hwee, J. Robert-Paganin, C.Y. Chuang, E.R. Chin, S. Edell, K.H. Lee, R. Madhvani, P. Paliwal, J. Pernier, et al. 2024. Aficamten is a small-molecule cardiac myosin inhibitor designed to treat hypertrophic cardiomyopathy. *Nat. Cardiovasc. Res.* https://doi.org/10.1038/s44161-024-00505-0

Ho, C.Y., I. Olivotto, D. Jacoby, S.J. Lester, M. Roe, A. Wang, C.B. Waldman, D. Zhang, A.J. Sehnert, and S.B. Heitner. 2020. Study design and rationale of EXPLORER-HCM: Evaluation of mavacamten in adults with symptomatic obstructive hypertrophic cardiomyopathy. *Circ. Heart Fail.* 13:e006853. https://doi.org/10.1161/CIRCHEARTFAILURE.120.006853

Kalinski, J.K., B. Xu, R. Boyd, N. Tasseff, K. Rutkowski, S. Ospina, N. Smedira, M. Thamilarasan, Z.B. Popovic, and M.Y. Desai. 2024. Novel cardiac myosin inhibitor therapy for hypertrophic cardiomyopathy in adults: A contemporary review. *Am. J. Cardiovasc. Drugs*. https://doi.org/10.1007/s40256-024-00667-z

Kooiker, K., Q.F. Gan, M. Yu, N. Sa, S. Mohran, Y. Cheng, G. Flint, S. Neys, C. Gao, D. Nissen, et al. 2024. Mechanisms of a novel regulatory light chain-dependent cardiac myosin inhibitor. *J. Gen. Physiol.* 156:e202313503. https://doi.org/10.1085/jgp.202313503

Margara, F., Y. Psaras, Z.J. Wang, M. Schmid, R. Doste, A.C. Garfinkel, G.G. Repetti, J.G. Seidman, C.E. Seidman, B. Rodriguez, et al. 2022. Mechanism based therapies enable personalised treatment of hypertrophic cardiomyopathy. *Sci. Rep.* 12:22501. https://doi.org/10.1038/s41598-022-26889-2

Maron, B.J., and M.S. Maron. 2013. Hypertrophic cardiomyopathy. *Lancet*. 381:242–255. https://doi.org/10.1016/S0140-6736(12)60397-3

Massengill, M.T., H.M. Ashraf, R.R. Chowdhury, S.M. Chrzanowski, J. Kar, S.A. Warren, G.A. Walter, H. Zeng, B.H. Kang, R.H. Anderson, et al. 2016. Acute heart failure with cardiomyocyte atrophy induced in adult mice by ablation of cardiac myosin light chain kinase. *Cardiovasc. Res.* 111:34–43. https://doi.org/10.1093/cvr/cvw069

Olivotto, I., A. Oreziak, R. Barriales-Villa, T.P. Abraham, A. Masri, P. Garcia-Pavia, S. Saberi, N.K. Lakdawala, M.T. Wheeler, A. Owens, et al. 2020. Mavacamten for treatment of symptomatic obstructive hypertrophic cardiomyopathy (EXPLORER-HCM): A randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet*. 396:759–769. https://doi.org/10.1016/S0140-6736(20)31792-X

Semsarian, C., J. Ingles, M.S. Maron, and B.J. Maron. 2015. New perspectives on the prevalence of hypertrophic cardiomyopathy. *J. Am. Coll. Cardiol.* 65:1249–1254. https://doi.org/10.1016/j.jacc.2015.01.019

Toepfer, C.N., A. Sharma, M. Cicconet, A.C. Garfinkel, M. Mucke, M. Neyazi, J.A.L. Willcox, R. Agarwal, M. Schmid, J. Rao, et al. 2019. SarcTrack. *Circ. Res.* 124:1172–1183. https://doi.org/10.1161/CIRCRESAHA.118.314505

Van Driest, S.L., E.G. Ellsworth, S.R. Ommen, A.J. Tajik, B.J. Gersh, and M.J. Ackerman. 2003. Prevalence and spectrum of thin filament mutations in an outpatient referral population with hypertrophic cardiomyopathy. *Circulation*. 108:445–451. https://doi.org/10.1161/01.CIR.0000080896.52003.DF

Yadav, S., Y.H. Sitbon, K. Kazmierczak, and D. Szczesna-Cordary. 2019a. Hereditary heart disease: Pathophysiology, clinical presentation, and animal models of HCM, RCM, and DCM associated with mutations in cardiac myosin light chains. *Pflugers Arch.* 471:683–699. https://doi.org/10.1007/s00424-019-02257-4

Yadav, S., and D. Szczesna-Cordary. 2017. Pseudophosphorylation of cardiac myosin regulatory light chain: A promising new tool for treatment of cardiomyopathy. *Biophys. Rev.* 9:57–64. https://doi.org/10.1007/s12551-017-0248-8

Yadav, S., C.C. Yuan, K. Kazmierczak, J. Liang, W. Huang, L.M. Takeuchi, R.M. Kanashiro-Takeuchi, and D. Szczesna-Cordary. 2019b. Therapeutic potential of AAV9-S15D-RLC gene delivery in humanized MYL2 mouse model of HCM. *J. Mol. Med.* 97:1033–1047. https://doi.org/10.1007/s00109-019-01791-z

Zaleta-Rivera, K., A. Dainis, A.J.S. Ribeiro, P. Cordero, G. Rubio, C. Shang, J. Liu, T. Finsterbach, V.N. Parikh, S. Sutton, et al. 2019. Allele-specific silencing ameliorates restrictive cardiomyopathy attributable to a human myosin regulatory light chain mutation. *Circulation*. 140:765–778. https://doi.org/10.1161/CIRCULATIONAHA.118.036965