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COMMENTARY

Mechanistic complexity of contractile dysfunction in hypertrophic cardiomyopathy

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Hypertrophic cardiomyopathy (HCM) is a relatively common disease in which the muscular wall of the heart becomes thickened. It is estimated to affect 1 in every 500 people—men and women equally—and can cause cardiac arrest in young people, including athletes. In 1990, a genetic linkage between a mutated protein found within cardiac muscle sarcomeres and the HCM phenotype was described (Geisterfer-Lowrance et al., 1990). Since then, the familial form of hypertrophic cardiomyopathy (fHCM) has been ascribed to mutations found in at least ten different sarcomere proteins within cardiac muscle (Seidman and Seidman, 2011; Haas et al., 2015). Although ∼80% of fHCM mutations identified as causal are found in thick filament proteins (myosin heavy chain, myosin regulatory light chain, and myosin binding protein C), a significant number have also been identified in thin filament proteins that regulate cardiac muscle contraction (troponin and tropomyosin; Tardiff, 2011). Of the mutations identified thus far in the cardiac troponin complex, ∼60% occur in the troponin T (cTnT) subunit, with the remainder primarily in the troponin I (cTnI) subunit and a few putative mutations in troponin C (cTnC; Cheng et al., 2015). In a recent issue of the *Journal of General Physiology*, Reda and Chandra investigated the effect of a recently identified fHCM mutation in cTnT on the contractile function of cardiac muscle fibers.

Biophysical characterization can confirm when an identified fHCM mutation may be pathogenic and provide mechanistic insight into the disease by determining how it alters function at scales that range from individual cardiac proteins to the contractile apparatus, whole cells, and tissues. Characterization of contractile dysfunction for disease-related mutations has been performed in animal, tissue, and cell models. Until recently, most emphasis has been on the later, or end stages, of heart failure. Perhaps the most common measurement in characterizing how sarcomere protein mutations influence contractile properties is the amount of steady-state force or ATPase produced by skinned (chemically demembranated) cardiac muscle with varying levels of extracellular Ca^{2+} in the activation solutions. The "Ca^{2+} sensitivity" of the contractile apparatus is usually defined as the pCa_{50} (negative log concentration of Ca^{2+} level that produces half-maximal force) of this force–pCa relationship. For many, but certainly not all fHCM mutations studied, there is an increase in the Ca^{2+} sensitivity of force development (Harris et al., 2011; Tardiff, 2011; Cheng et al., 2015, 2016) These measurements, although relatively easy to make, provide a very limited picture of contractile performance and do not present a clear picture of how ventricular function is compromised. Several studies have also examined the effect of mutations on the kinetics of contractile activation and relaxation, as well as other indices of myosin–actin cross-bridge cycling and cellular contractile behavior. For several fHCM-associated mutations, there are reports that relaxation kinetics are altered and that the effects of PKA-mediated phosphorylation of myofilament proteins (β-adrenergic mechanisms) are reduced or blunted (Belus et al., 2008; Witjas-Paalberends et al., 2014a; Cheng et al., 2015, 2016).

An important feature of cardiac function is the Frank-Starling mechanism, which allows for changes in venous return to be matched by changes in stroke volume. This mechanism is often disrupted in cardiomyopathies (Schwinger et al., 1994; Vahl et al., 1998). The cellular basis for the Frank-Starling mechanism results from the length dependence of cardiac myofilament activation, which is often studied by measuring the dependence of the Ca^{2+} sensitivity of force development on sarcomere length (SL). In the Reda and Chandra (2018) study, the effect of an fHCM-associated mutation in the central region of cTnT (F88L) on the SL dependence of cardiac myofilament activation was investigated. Reconstituted recombinant troponin, containing cTnT with or without F88L, was exchanged into skinned guinea pig cardiac muscle preparations (papillaries). They then measured the Ca^{2+} dependence of force, ATPase production, and instantaneous force increase with rapid stretch at short (1.9 µm) and long (2.3 µm) SL. An important aspect of the study is that guinea pigs express predominantly the β-myosin heavy chain (β-MHC) isoform in ventricular myocytes (similar to humans) as opposed to

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the α-myosin heavy chain isoform (α-MHC) that predominates in mice and rats. Myosin containing β-MHC has slower ATPase cycling and longer attachment times to thin filaments than myosin containing α-MHC and thus has significant effects on the contractile function of cardiac muscle. It has been demonstrated that both SL-dependent activation and the influence of cTnT mutations on contractile properties may be dependent on the MHC isoform (Fitzsimons and Moss, 1998; Ford et al., 2012).

Reda and Chandra (2018) found that the F88L cTnT mutation increased the Ca^{2+} sensitivity of force development at both short and long SL, but that the increase was greater at short SL, thus diminishing the SL dependence of contractile activation. There was also some loss of the apparent cooperativity of force generation, as determined from the slope of the force–pCa relationship. The decrease in SL-dependent activation and loss of apparent cooperativity has been seen for other mutations in troponin that result in enhanced Ca^{2+} binding. For example, an engineered mutation in cardiac troponin C (L48Q) also causes a greater increase in the Ca^{2+} sensitivity of force generation at short SL than at long SL and therefore reduces the SL dependence of contractile activation (Korte et al., 2012). Interpretation of the results from rapid stretch measurements led Reda and Chandra (2018) to conclude that at least part of the greater increase in Ca^{2+} sensitivity at short SL resulted from a reduced impact of negatively strained cross-bridges on positive force–generating cross-bridges, as well as an enhanced cross-bridge to thin filament–cooperative activation mechanism mediated by the F88L mutation. Further analysis suggested that cross-bridge detachment rate and tension cost were not affected by the mutation at either SL. This would seem to indicate that, for this cTnT mutation, there is no uncoupling between work and energy utilization. Other mutations in cTnT have been reported to increase tension-dependent ATP consumption in transgenic mice, as have thick filament protein mutations in animal models and human fHCM (Chandra et al., 2005; He et al., 2007; Ferrantini et al., 2009; Witjas-Paalberends et al., 2014a,b; Wilder et al., 2015; Birch et al., 2016). The results of the Reda and Chandra (2018) study suggest that uncoupling between contractile function and energetics may not be a universal feature of fHCM, though this needs to be further investigated.

Reda and Chandra (2018) conclude that the F88L cTnT mutation might have a negative impact on the Frank-Starling mechanism. This may be true, but may translate to a decrease in cardiac performance only during exercise, when greater venous return results in longer SL at the end of diastole. Indeed, this mutation may result in increased basal myocardial performance, when cardiomyocytes are operating at shorter SLs, and thus could be a compensatory mechanism. Although cross-bridge recruitment was measured only during maximal Ca^{2+} activation, the authors also concluded that, at physiological levels of Ca^{2+}, an increase in cross-bridge recruitment at longer SL may result in greater systolic muscle force development. This would prolong systolic ejection time, leading to a delay in relaxation and greater resistance to ventricular filling during diastole. There are two concepts here that merit further thought and investigation. The first is that some mutations may not allow complete deactivation of thin filaments at the end of systole, thus increasing a cross-bridge component of increased wall stiffness during diastole that resists ventricular filling. This has been reported by others for mutations in cardiac and skeletal muscle (Greenberg et al., 2009; Racca et al., 2015). The second is that fHCM mutations in troponin proteins may alter the kinetics of relaxation during basal function and/or during stress (exercise), as mentioned above (Cheng et al., 2015, 2016).

In conclusion, the work of Reda and Chandra (2018) agrees well with other recent publications demonstrating that the mechanism underlying contractile dysfunction resulting from fHCM-associated mutations is more complex than simple changes in the Ca^{2+} sensitivity of contractile activation. Mutation-related alterations in cross-bridge recruitment and cycling kinetics, impaired relaxation, and the SL dependence of these properties likely all play a role. There is also increasing evidence that unique differences in dysfunctional sarcomere behavior may depend on whether mutations are located in thick versus thin filament proteins, or even when mutations are located in different regions of the same protein (Montgomery et al., 2001; He et al., 2007; Tardiff, 2011). Better understanding of the complexity and diversity of mutation-mediated changes in sarcomere structure and function are needed to develop new and more specific targeting strategies for treating fHCM patients.

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References

Belus, A., N. Piroddi, B. Scellini, C. Tesi, G. D'Amati, F. Girolami, M. Yacoub, F. Cecchi, I. Olivotto, and C. Poggesi. 2008. The familial hypertrophic cardiomyopathy-associated myosin mutation R403Q accelerates tension generation and relaxation of human cardiac myofibrils. *J. Physiol.* 586:3639–3644. https://doi.org/10.1113/jphysiol.2008.155952

Birch, C.L., S.M. Behunin, M.A. Lopez-Pier, C. Danilo, Y. Lipovka, C. Saripalli, H. Granzier, and J.P. Konhilas. 2016. Sex dimorphisms of crossbridge cycling kinetics in transgenic hypertrophic cardiomyopathy mice. *Am. J. Physiol. Heart Circ. Physiol.* 311:H125–H136. https://doi.org/10.1152/ajpheart.00592.2015

Chandra, M., M.L. Tschirgi, and J.C. Tardiff. 2005. Increase in tension-dependent ATP consumption induced by cardiac troponin T mutation. *Am. J. Physiol. Heart Circ. Physiol.* 289:H2112–H2119. https://doi.org/10.1152/ajpheart.00571.2005

Cheng, Y., V. Rao, A.Y. Tu, S. Lindert, D. Wang, L. Oxenford, A.D. McCulloch, J.A. McCammon, and M. Regnier. 2015. Troponin I Mutations R146G and R21C Alter Cardiac Troponin Function, Contractile Properties, and Modulation by Protein Kinase A (PKA)-mediated Phosphorylation. *J. Biol. Chem.* 290:27749–27766. https://doi.org/10.1074/jbc.M115.683045

Cheng, Y., S. Lindert, L. Oxenford, A.Y. Tu, A.D. McCulloch, and M. Regnier. 2016. Effects of Cardiac Troponin I Mutation P83S on Contractile Properties and the Modulation by PKA-Mediated Phosphorylation. *J. Phys. Chem. B.* 120:8238–8253. https://doi.org/10.1021/acs.jpcb.6b01859

Ferrantini, C., A. Belus, N. Piroddi, B. Scellini, C. Tesi, and C. Poggesi. 2009. Mechanical and energetic consequences of HCM-causing mutations. *J. Cardiovasc. Transl. Res.* 2:441–451. https://doi.org/10.1007/s12265-009-9131-8

Fitzsimons, D.P., and R.L. Moss. 1998. Strong binding of myosin modulates length-dependent Ca^{2+} activation of rat ventricular myocytes. *Circ. Res.* 83:602–607. https://doi.org/10.1161/01.RES.83.6.602

Ford, S.J., R. Mamidi, J. Jimenez, J.C. Tardiff, and M. Chandra. 2012. Effects of R92 mutations in mouse cardiac troponin T are influenced by changes

in myosin heavy chain isoform. *J. Mol. Cell. Cardiol.* 53:542–551. https://doi.org/10.1016/j.yjmcc.2012.07.018

Geisterfer-Lowrance, A.A., S. Kass, G. Tanigawa, H.P. Vosberg, W. McKenna, C.E. Seidman, and J.G. Seidman. 1990. A molecular basis for familial hypertrophic cardiomyopathy: a beta cardiac myosin heavy chain gene missense mutation. *Cell.* 62:999–1006. https://doi.org/10.1016/0092-8674(90)90274-I

Greenberg, M.J., J.D. Watt, M. Jones, K. Kazmierczak, D. Szczesna-Cordary, and J.R. Moore. 2009. Regulatory light chain mutations associated with cardiomyopathy affect myosin mechanics and kinetics. *J. Mol. Cell. Cardiol.* 46:108–115. https://doi.org/10.1016/j.yjmcc.2008.09.126

Haas, J., K.S. Frese, B. Peil, W. Kloos, A. Keller, R. Nietsch, Z. Feng, S. Müller, E. Kayvanpour, B. Vogel, et al. 2015. Atlas of the clinical genetics of human dilated cardiomyopathy. *Eur. Heart J.* 36:1123–1135. https://doi.org/10.1093/eurheartj/ehu301

Harris, S.P., R.G. Lyons, and K.L. Bezold. 2011. In the thick of it: HCM-causing mutations in myosin binding proteins of the thick filament. *Circ. Res.* 108:751–764. https://doi.org/10.1161/CIRCRESAHA.110.231670

He, H., M.M. Javadpour, F. Latif, J.C. Tardiff, and J.S. Ingwall. 2007. R-92L and R-92W mutations in cardiac troponin T lead to distinct energetic phenotypes in intact mouse hearts. *Biophys. J.* 93:1834–1844. https://doi.org/10.1529/biophysj.107.107557

Korte, F.S., E.R. Feest, M.V. Razumova, A.Y. Tu, and M. Regnier. 2012. Enhanced Ca2+ binding of cardiac troponin reduces sarcomere length dependence of contractile activation independently of strong crossbridges. *Am. J. Physiol. Heart Circ. Physiol.* 303:H863–H870. https://doi.org/10.1152/ajpheart.00395.2012

Montgomery, D.E., J.C. Tardiff, and M. Chandra. 2001. Cardiac troponin T mutations: correlation between the type of mutation and the nature of myofilament dysfunction in transgenic mice. *J. Physiol.* 536:583–592. https://doi.org/10.1111/j.1469-7793.2001.0583c.xd

Racca, A.W., A.E. Beck, M.J. McMillin, F.S. Korte, M.J. Bamshad, and M. Regnier. 2015. The embryonic myosin R672C mutation that underlies Freeman-Sheldon syndrome impairs cross-bridge detachment and cycling in adult skeletal muscle. *Hum. Mol. Genet.* 24:3348–3358. https://doi.org/10.1093/hmg/ddv084

Reda, S.M., and M. Chandra. 2018. Cardiomyopathy mutation (F88L) in troponin T abolishes length dependency of myofilament Ca^{2+} sensitivity. *J. Gen. Physiol.* 150:809–819. https://doi.org/10.1085/jgp.201711974

Schwinger, R.H., M. Böhm, A. Koch, U. Schmidt, I. Morano, H.J. Eissner, P. Uberfuhr, B. Reichart, and E. Erdmann. 1994. The failing human heart is unable to use the Frank-Starling mechanism. *Circ. Res.* 74:959–969. https://doi.org/10.1161/01.RES.74.5.959

Seidman, C.E., and J.G. Seidman. 2011. Identifying sarcomere gene mutations in hypertrophic cardiomyopathy: a personal history. *Circ. Res.* 108:743–750. https://doi.org/10.1161/CIRCRESAHA.110.223834

Tardiff, J.C. 2011. Thin filament mutations: developing an integrative approach to a complex disorder. *Circ. Res.* 108:765–782. https://doi.org/10.1161/CIRCRESAHA.110.224170

Vahl, C.F., T. Timek, A. Bonz, H. Fuchs, R. Dillman, and S. Hagl. 1998. Length dependence of calcium- and force-transients in normal and failing human myocardium. *J. Mol. Cell. Cardiol.* 30:957–966. https://doi.org/10.1006/jmcc.1998.0670

Wilder, T., D.M. Ryba, D.F. Wieczorek, B.M. Wolska, and R.J. Solaro. 2015. N-acetylcysteine reverses diastolic dysfunction and hypertrophy in familial hypertrophic cardiomyopathy. *Am. J. Physiol. Heart Circ. Physiol.* 309:H1720–H1730. https://doi.org/10.1152/ajpheart.00339.2015

Witjas-Paalberends, E.R., C. Ferrara, B. Scellini, N. Piroddi, J. Montag, C. Tesi, G.J. Stienen, M. Michels, C.Y. Ho, T. Kraft, et al. 2014a. Faster cross-bridge detachment and increased tension cost in human hypertrophic cardiomyopathy with the R403Q MYH7 mutation. *J. Physiol.* 592:3257–3272. https://doi.org/10.1113/jphysiol.2014.274571

Witjas-Paalberends, E.R., A. Güçlü, T. Germans, P. Knaapen, H.J. Harms, A.M. Vermeer, I. Christiaans, A.A. Wilde, C. Dos Remedios, A.A. Lammertsma, et al. 2014b. Gene-specific increase in the energetic cost of contraction in hypertrophic cardiomyopathy caused by thick filament mutations. *Cardiovasc. Res.* 103:248–257. https://doi.org/10.1093/cvr/cvu127