Too light to be essential? Insights from FHC-related mutations in essential myosin light chains

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This editorial refers to ‘Mutations of ventricular essential myosin light chain disturb myosin binding and sarcomeric sorting’ by J. Lossie et al., pp. 390–396, this issue.

Familial hypertrophic cardiomyopathy (FHC) is an autosomal dominant disease affecting one in 500 individuals that is characterized by left ventricular hypertrophy, interstitial fibrosis, and myocyte disarray. Moreover, the disease is associated with arrhythmias and may progress to heart failure or sudden cardiac death, especially in young adults. Clinical diagnosis and genetic analysis of FHC have been performed over the past two decades, but the underlying pathomechanisms are still not entirely understood. FHC is a disease of the sarcomere caused by numerous mutations in proteins of the contractile machinery. Among them are the thin filament proteins troponin I and T; yet, the majority of FHC-related mutations have been identified in the genes of myosin heavy chain (MYH or MHC) and MHC-associated proteins such as myosin-binding protein C or myosin light chains.

While the role of MHC in driving muscle contraction is well established, less is known about the physiological role of MHC-associated proteins. Studying FHC-related mutations in myosin-binding proteins therefore provides a chance to not only unravel the pathomechanisms of inherited cardiomyopathies but also to improve our basic understanding of the functional role of these proteins in the sarcomeric setting.

Lossie et al. address this topic by using several biochemical and cell-biological methods to investigate the functional relevance of FHC-related mutations in the essential myosin light chain (ELC). ELC, in tandem with the regulatory light chain (RLC), binds to the actin-associated lever arm domain of MHC. In smooth muscle cells, the light chains are part of the regulatory ‘on and off’ machinery of the phosphorylation-induced actin–myosin interaction. The role of ELC/RLC in cardiac and striated muscle function is still under debate and may involve stabilization of the MHC lever arm as well as modulation of the speed and force of cardiac muscle contraction. To date, five FHC-associated mutations are linked to the human ventricular isoform of ELC (hVLC-1). The disease phenotype ranges from asymptomatic septal hypertrophy (mutation hVLC-1E143K), mid-cavitary, or apical hypertrophy (hVLC-1M149Y, hVLC-1E134K) to a non-symptomatic course of the disease (hVLC-1E134K). No clinical data are available for the fifth hVLC-1 mutation, E56G (hVLC-1E56G). The structure of VLC-1 comprises an actin-binding N-terminus, a spacer region, and a highly conserved C-terminal domain with four EF-hand motifs that bind to the lever arm of MHC. Although the EF-hand motifs of VLCs have lost their ability to bind divalent cations, the fact that all FHC-related mutations were found in this specific region suggests an important role in maintaining myosin function.

In their current study, Lossie et al. applied surface plasmon resonance spectroscopy and CD spectroscopy and found that mutated hVLC-1s bind approximately three-fold weaker to the myosin lever arm than normal hVLC-1. Despite an unchanged secondary structure of the mutant protein, a 30-fold reduced binding to the myosin lever arm was observed for hVLC-1E56G, explaining why the authors focused on this mutation. By applying a double epitope-tagging competition method, Lossie et al. show that hVLC-1E56G almost completely lacks the ability to integrate into sarcomeres of rat cardiomyocytes when co-expressed with wild-type hVLC-1. Interestingly, without simultaneous expression of wild-type hVLC-1, the mutated isoform was properly incorporated. This finding raises the question whether the low affinity of hVLC-1E56G for MHC is sufficient to explain the lack of integration in co-transfected cells. The authors explain this with a recently published hypothesis of the same group suggesting an intracompartamental sorting of myosin light chains prior to integration into the sarcomeres. It will be interesting to see whether the same concept holds true for the other hVLC-1 mutations that also showed a significantly reduced MHC-binding affinity and that have been characterized clinically in more detail.

The idea of a precisely regulated sarcomeric sorting process is tempting; however, some important questions remain to be considered. FHC-associated mutations are often inherited as a heterozygous dominant disease with co-expression of wild-type and mutated protein in the affected hearts, and previous studies often raised the question of a putative gene dose effect that determines the FHC phenotype. In a heterozygous disease setting, the data by Lossie et al. imply that incorporation of hVLC-1E56G should be prevented by the intracompartamental sorting process; thus, myofilament

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function should be largely preserved. In contrast, in the case of homozygous expression of the mutation, one would expect a rather lethal phenotype of the affected individuals considering the dramatically low binding affinity of hVLC-1E56G to MHC. Unfortunately, whether hVLC-1E56G is inherited in a homozygous or heterozygous manner is unknown.3

In addition to the biochemical data presented by Lossie et al., four transgenic animal models of FHC-associated hVLC-1 mutations have been reported to date. However, only one fully recapitulated an FHC-related phenotype.3,6 Nevertheless, the transgenic mouse model of the A57G mutation recently reported by Muthu et al.8 provides some interesting aspects. The mutation significantly increased rigor stiffness of papillary muscle preparations, and X-ray diffraction studies revealed a reduction in the interfilament lattice spacing of the cardiac myofilaments.8 It would be important to understand what consequences this structural modulation has on the contractile function and whether changes in the lattice spacing are common to all hVLC-1 mutations, provided that they are integrated into the sarcomere. Unfortunately, the transgenic mice showed only mild cardiac fibrosis and signs of myofilament disarray, and thus do not recapitulate the more severe FHC phenotype reported for patients carrying the A57G mutation.6,8 This again highlights the difficulties in translating results from animal models to the clinical situation in humans in which FHC develops over many years, and potential compensatory mechanisms may influence the clinical outcome.

Taken together, the recent publications in this field demonstrate how little we still know about the complex process of sarcomere formation and the development of myofilament dysfunction in FHC.3,8 In the light of the difficulties experienced with transgenic models of hVLC-1 mutations, the approach by Lossie et al. to study hVLC-1 function in basic biochemical in vitro assays seems promising. Future studies combining such biochemical methods with biomechanical analyses, such as in vitro motility assays or active and passive force measurements, may have a strong potential to shed light on the question how ‘essential’ VLC-1 actually is for sarcomeric function.

Conflict of interest: none declared.

References