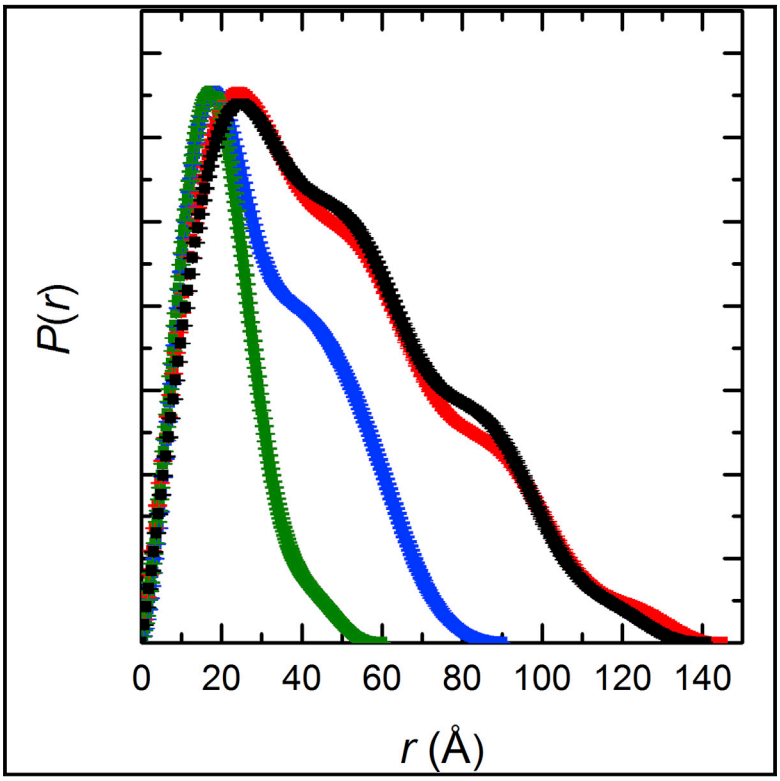
Article

Structure

Clinically Linked Mutations in the Central Domains of Cardiac Myosin-Binding Protein C with Distinct Phenotypes Show Differential Structural Effects

Graphical Abstract



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In Brief

This study of the structural effects of three mutations in cardiac myosin-binding protein C (cMyBP-C) that are linked to hypertrophic cardiomyopathy, the most common forms of inherited heart disease, contributes to our understanding of the role of cMyBP-C in healthy and diseased heart function.

Highlights

- Mutations in cMyBP-C linked to familial HCM have distinct structural consequences
- Mutations impeding domain folding are linked to late-onset severe hypertrophy
- A mutation linked to early-onset HCM may only interfere with protein interactions
- Bioinformatics and modeling largely predicted the experimentally observed results

Nadvi et al., 2016, Structure *24*, 105–115
January 5, 2016
http://dx.doi.org/10.1016/j.str.2015.11.001

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Clinically Linked Mutations in the Central Domains of Cardiac Myosin-Binding Protein C with Distinct Phenotypes Show Differential Structural Effects

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http://dx.doi.org/10.1016/j.str.2015.11.001

SUMMARY

The structural effects of three missense mutations clinically linked to hypertrophic cardiomyopathy (HCM) and located in the central domains of cardiac myosin-binding protein C (cMyBP-C) have been determined using small-angle scattering, infrared spectroscopy, and nuclear magnetic resonance spectroscopy. Bioinformatics and modeling were used to initially predict the expected structural impacts and assess the broader implications for function based on sequence conservation patterns. The experimental results generally affirm the predictions that two of the mutations (D745G, P873H) disrupt domain folding, while the third (R820Q) is likely to be entirely solvent exposed and thus more likely to have its impact through its interactions within the sarcomere. Each of the mutations is associated with distinct disease phenotypes, with respect to severity, stage of onset, and end phase. The results are discussed in terms of understanding key structural features of these domains essential for healthy function and the role they may play in disease development.

INTRODUCTION

The objective of this study was to determine the structural effects of three disease-linked missense mutations (D745G, R820Q, and P873H) located in the central domains of cardiac myosin-binding protein C to gain insight into their key structural features essential for healthy function, and potentially some insight also into the molecular causes for disease development. The mutations chosen for study are all linked to clinical cases of hypertrophic cardiomyopathy (HCM), a restrictive disease of the left heart ventricle that has been estimated to affect as many as 1 in 200 individuals (Mamidi et al., 2014). While a large proportion of inherited HCM cases are linked to mutations in cMyBP-C (recently reviewed in Carrier et al., 2015), the relationship between genotype and phenotype has been elusive, and frustrated by the fact that the disease has a highly heterogeneous phenotypic and genetic profile (Harris et al., 2011; Lee et al., 2015).

Isoforms of myosin-binding protein C (MyBP-C) are found in vertebrate striated muscle located in the cross-bridge-bearing C zone of the A band in the sarcomere. The name of the protein reflects the early recognition of its myosin-binding properties, but it also has been found to interact with thin filament actin. The highly modular architecture of cMyBP-C comprises 11 domains (C0 through C10) (Carrier et al., 1997), recently reviewed in Sadayappan and de Tombe (2014); eight having high similarity to immunoglobulin (Ig)-like domains (C0 through C5, plus C8 and C10) with the remaining three (C6, C7, C9) highly similar to fibronectin (Fn)-like domains. Between the C1 and C2 domains is a sequence of approximately 100 amino acids called the "motif," which in vitro studies find contains some helical structure (Tanner et al., 2014) but is otherwise largely unstructured. The cardiac isoform of MyBP-C (cMyBP-C) has a number of distinctive features, including the cardiac-specific N-terminal C0 domain, a nine-residue insertion in the motif that is a key site for phosphorylation, and two insertions in the C5 domain that affect its fold and stability (Idowu et al., 2003). The full-length cMyBP-C is composed of 1,274 amino acids encoded by the *MYBPC3* gene.

We have previously reported small-angle X-ray scattering (SAXS) studies of full-length cMyBP-C and a number of multi-domain constructs (Jeffries et al., 2011); however, a detailed structural study of the full-length protein is challenging due to its large size and flexibility. There are nuclear magnetic resonance (NMR) and crystal structures of individual domains, including the cardiac-specific C5 (Idowu et al., 2003), but no high-resolution structures of multi-domain constructs. Many studies of cMyBP-C to date have focused on the N-terminal modules that are proposed to interact with actin and with the lever arm domain of myosin (S2) proximal to the myosin head (S1) to regulate contraction, and to a lesser extent on the C-terminal region that anchors the protein to thick filament and titin.

The central domains inclusive of C5 through C7 are interesting for a number of reasons. Firstly, they include the cardiac isoform-specific C5 Ig-like domain and two adjacent Fn-like domains (C6, C7), a unique arrangement in the protein. The cardiac-specific insertions in C5 are the ~30-residue insertion in the middle of the domain referred to as the CD loop and an additional ten-residue insertion between the C4 and C5 domains that forms an integral part of the C5 fold. NMR studies show that the CD loop is highly unstructured and dynamic, resulting in the lower stability of C5 compared with other Ig domains (Idowu et al.,