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Contractile imbalance as trigger for HCM pathogenesis: evidence from mutations in different sarcomeric proteins

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Funding Acknowledgement: German Research Foundation (DFG)

Hypertrophic cardiomyopathy (HCM) is mostly caused by mutations in sarcomeric proteins. About 90% of mutation-positive patients have mutations in one of four proteins: the β-myosin heavy chain (β-MyHC, MYH7), cardiac myosin binding protein C (cMyBP-C, MYBPC3), cardiac troponin I (cTnI, TNNI3), and cardiac troponin T. Almost all patients are heterozygous; they express the wildtype and the mutant protein isoform. For patients with β-MyHC missense mutations we have reported previously that individual cardiomyocytes show a significant variability in force generation and calcium-sensitivity, ranging from essentially donor–like to highly altered function. We provided evidence that the MYH7-alleles are switched on and off stochastically and independently from each other in each cell. This burst-like expression leads to highly variable fractions of mutant and wildtype mRNA between the cardiomyocytes, presumably causing variable fractions of mutant protein. We assume that this variability underlies the determined contractile imbalance leading to stronger cells that overcontract and over-stretch weaker cells. This could trigger development of HCM-hallmarks like myocyte disarray, fibrosis and hypertrophy.

To test whether contractile imbalance may provide a common mechanism of HCM-development, we extended our analysis to additional sarcomeric proteins with HCM-mutations. Analysis of cardiomyocytes from a patient with missense mutation R145W in cTnI revealed highly variable calcium-sensitivity between individual cardiomyocytes, substantially higher than for donor cardiomyocytes. This functional heterogeneity was associated with highly variable fractions of mutant TNNI3-mRNA from cell-to-cell. This suggests that not only missense mutations in β-MyHC but also in cTnI induce hallmarks of HCM via the contractile imbalance mechanism.

In contrast to missense mutations, truncation mutations in cMyBP-C presumably cause HCM via haploinsufficiency. Degradation of truncated proteins causes a lack of functional cMyBP-C and thereby alters function of the sarcomere. We hypothesized that different levels of haploinsufficiency from cell-to-cell may also cause contractile imbalance. Therefore we examined a patient with truncation mutation c.927–2A>G in cMyBP-C. Western blot analysis revealed no truncated protein and reduced levels of wildtype-cMyBP-C, consistent with haploinsufficiency. We also observed a significantly higher variability in fluorescence intensity ratio (MyBPC/Alpha-Actinin) for cardiomyocytes of the HCM-patient than in donor cardiomyocytes. The patchy distribution of cMyBP-C in histological tissue section indicated variable levels of functional protein from cell-to-cell. Functional analysis revealed significantly more variable isometric force generation from cell to cell of patient cardiomyocytes compared to donor, suggesting contractile imbalance.

We conclude that contractile imbalance may be a potential common mechanism of HCM pathogenesis.