Role of truncating titin mutations in dilated cardiomyopathy

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Dilated cardiomyopathy (DCM) is a highly-prevalent heart muscle disorder that is characterized by dilatation of the ventricles and contractile impairment. Genetic factors are an important cause of DCM, yet mutations in known disease genes account for only a minority of cases and disease mechanisms are incompletely understood. Titin is a giant sarcomeric protein that is a major determinant of the contractile and signaling properties of heart and skeletal muscle. Recently, mutations in the TTN gene that result in a shortened titin protein ("truncating" TTN variants [TTNtv]) were identified in approximately 1 in 4 patients with adult-onset DCM (Herman *et al.*, 2012; Roberts *et al.*, 2015). These findings suggest that TTNtv are the single most common genetic cause of DCM, with a prevalence equivalent to that of all other known genetic causes of DCM combined. Importantly, however, TTNtv were also found in 1-3% of individuals in the general population. With the rapidly accelerating use of personal genome evaluation, TTNtv will not only be identified in many patients with DCM but will also arise as incidental findings in people without DCM. Interpreting the clinical significance of TTNtv is thus a major conundrum facing health professionals and families alike. We hypothesize that TTNtv are sufficient to cause DCM, and that location of truncation in the titin protein, as well as "second hit" genetic and/or acquired factors can modify disease onset and severity. To investigate this hypothesis, we are taking a two-pronged approach using family studies and zebrafish models of titin truncation. We have screened our large cohort of familial DCM cases and identified 32 patients who carry TTNtv. In ongoing work, we are performing detailed clinical and genetic analysis of the relatives of these individuals. This will enable us to determine the concordance between DCM and TTNtv status in family members, as well as identifying potential exacerbating or protective acquired factors. These genotype-phenotype and risk factor data are lacking from published studies that have evaluated cohorts of unrelated single DCM cases. To evaluate the effects of titin deficiency in the heart, we are using zebrafish mutants with titin truncations in different locations, including two lines that carry specific TTNtv found in our DCM families. Heart function can be readily assessed in embryonic zebrafish by videomicroscopy. However, the loss of transparency with age necessitates alternative methods in adult zebrafish. We have developed a suite of cutting-edge tools, including high frequency echocardiography, that allow us, for the first time, to look at the hearts of adult zebrafish. We are performing serial assessment of cardiac structure and function in titin-deficient fish using echocardiography, ECG, histopathological and molecular analyses. Genetically-modified zebrafish carrying titin truncations are a highly valuable disease model to assess the role of TTNtv as a primary cause of DCM, the effects of second hit factors, and for determining whether the location of a truncation in the titin protein might explain why DCM is seen in some TTNtv carriers but not others. These studies will provide new perspectives on the impact of titin deficiency in the heart and have direct relevance to the clinical conundrum of the role of TTNtv in human DCM.

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