PROJECT SUMMARY/ABSTRACT

Heart failure affects 1 in 4 individuals during their lifetime, with non-ischemic cardiomyopathy being one of the least understood forms. Inherited variants account for ~30% of non-ischemic cardiomyopathy with truncating variants in the *TTN* gene (TTNtvs) being the most common sub-type. Titin is expressed in striated muscle cells and is essential for sarcomere formation and force generation. Since titin is also the largest known protein, I hypothesize that cellular control pathways can become overloaded in patients who have a TTNtv. This would lead to accumulation of damaged proteins, compromised sarcomere integrity, and diminished cardiac performance.

As an F31 Fellow, I will investigate how TTNtvs affect RNA and protein turnover, lipofuscin buildup, sarcomeric titin integration, and cardiac mechanics. The central hypothesis is that TTNtvs overload RNA surveillance and proteasomal systems, leading to defective protein turnover, accumulation of lipofuscin, and improper integration of truncated titin into sarcomeres. My experiments will leverage my lab's large biobank of human myocardium. I have identified 24 patients with TTNtvs and non-ischemic cardiomyopathy that are available for this project with many more samples from patients with non-genetic non-ischemic cardiomyopathies and organ donor controls.

Aim 1 focuses on how TTNtvs impact RNA degradation by analyzing nonsense-mediated and exosomal decay pathways in human samples. This will help determine whether TTNtvs upregulate the activity of RNA surveillance mechanisms to clear faulty transcripts.

Aim 2 will investigate the downstream effects of TTNtvs on proteasomal degradation and lipofuscin accumulation. Using immunoblotting and image segmentation techniques, I will measure K48-linked polyubiquitination of titin and quantify lipofuscin granules in affected myocardial tissue.

Aim 3 assesses the integration of truncated titin into sarcomeres and its impact on cardiac mechanics. Immunohistochemistry will illuminate truncated titin filaments in sarcomeric structures, and biophysical testing will measure maximal isometric and passive forces in triton-permeabilized fibers.

The outcomes will provide invaluable insights into how TTNtvs disrupt cellular protein quality control, promote disease progression, and alter myocardial mechanics. These findings could uncover biomarkers and therapeutic targets for TTNtv-associated non-ischemic cardiomyopathy, leading to potential treatments that enhance protein turnover and mitigate disease progression.