SPECIFIC AIMS

Truncating variants in the *TTN* gene (TTNtvs) are strongly associated with non-ischemic cardiomyopathy (NICM), a common presentation of heart failure. Titin, the largest known protein in humans, plays an essential role in sarcomere assembly and force generation. In patients with a TTNtv, truncated titin may disrupt cellular turnover mechanisms, leading to accumulation of cytosolic waste (lipofuscin), and improper titin integration into sarcomeres. However, the mechanisms by which TTNtvs contribute to NICM remain poorly understood. This project aims to investigate how TTNtvs disrupt RNA and protein turnover, lipofuscin accumulation, and sarcomeric titin integration, leading to cardiac dysfunction in affected individuals.

Innate turnover pathways exist at the DNA, RNA, and protein levels to limit penetrance of genomic mutations. Two key players in RNA quality control are Up-Frameshift Protein 1 (UPF1) and Exosome Complex 10 (EXOSC10), which are involved in nonsense-mediated and exosomal decay pathways, respectively. At the protein level, damaged proteins are tagged with K48-linked polyubiquitin chains for proteasomal recognition and degradation. Titin's atypical size may overwhelm these RNA and protein checkpoints, resulting in accumulation of ubiquitinated titin and lipofuscin.

Working with Dr. Campbell et al., I have developed experiments to test the **global hypothesis that TTNtvs contribute to NICM pathogenesis by overloading RNA surveillance and proteasomal degradation systems, leading to defective protein turnover, lipofuscin accumulation, and incorporation of truncated titin into sarcomeres.** To test this hypothesis, procured human myocardium from 3 groups will be used: (1) organ donors (control), (2) patients with NICM, and (3) patients with NICM and a TTNtv. Presence of a NICM phenotype and TTNtvs will be confirmed using matched clinical data and whole exome sequencing results, respectively.

Aim 1: Test the hypothesis that samples with a TTNtv have higher UPF1 and EXOSC10 abundance. Homogenized left ventricular tissue will be analyzed using Western blotting to quantify UPF1 and EXOSC10 abundance. 2,2,2-trichloroethanol will be used for total protein normalization, and bands will be quantified using peer-reviewed software developed by our lab.

Aim 2: Test the hypothesis that samples with a TTNtv have higher K48-linked polyubiquitinated titin and lipofuscinogenesis. (*2.1*) Homogenized left ventricular tissue will be analyzed using agarose gel electrophoresis to resolve titin. After transfer to a PVDF membrane, K48-linked polyubiquitinated titin will be quantified with immunoblotting. Oriole fluorescent staining will be used for total protein normalization, and bands will be quantified peer-reviewed software developed by our lab. (*2.2*) Left ventricular tissue will be cryosectioned (10-μm thickness) and immunostained for alpha actinin. A laser within lipofuscin's autofluorescent excitation spectrum (~650 nm) will be used to excite lipofuscin during imaging with fluorescent microscopy. Total alpha actinin will be used to calculate the relative proportion of lipofuscin. Images will be analyzed using custom analysis software that I have trained to write.

Aim 3: Test the hypothesis that samples with TTNtv incorporate truncated titin filaments into sarcomeres and exhibit lower intracellular passive and maximal isometric forces. (*3.1*) Left ventricular tissue will be cryosectioned (10-μm thickness) and immunhistochemically stained for the M-9 epitope of titin, which is near the sarcomeric M-line, and for alpha actinin. Cryosections will be imaged with super-resolution microscopy. Total alpha actinin will be used to calculate the relative proportion of titin. Images will be analyzed using custom analysis software that I have trained to write. (*3.2*) Triton-permeabilized left ventricular tissue will be anchored between a force transducer and length controller to evaluate passive tension and maximal isometric force. Myofilament destabilizing solutions KCl and KI will be used to parse out extracellular and intracellular contribution to passive tension.

Overall Impact

Heart failure remains a leading cause of hospitalization, with a significant gap in understanding the molecular mechanisms underlying NICM, particularly those involving TTNtv. This proposal directly assesses human myocardial function to uncover how TTNtvs disrupt RNA and protein turnover, lipofuscin accumulation, and sarcomeric titin integration. By bridging molecular, cellular, and mechanical analyses, this research could identify new biomarkers and therapeutic targets, offering potential advances in personalized treatments for TTNtv-associated heart failure. I have developed these experiments to extend our understanding of heart failure's multimodal disease onset and have worked with Dr. Campbell to formulate a training plan that will help me develop into a distinguished principal investigator at the forefront of clinical advancement.