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Functional characterization of CRISPR-gene editing methods in canine fibroblasts

Approximately 10% of all dogs in the United States have some form of heart disease which can progress to heart failure. In canines, DCM is the 3rd most frequent type of heart disease and is a significant health issue for many breeds. The Doberman Pinscher (DP) is affected by a specific form of DCM, inherited as an autosomal dominant trait with incomplete penetrance (1). The prevalence of the disease in the DP is higher than that of other breeds, and ranges in incidence from 45 to 63% (2, 3). DPs have a high mortality rate, with mean survival times less than 6 months following the first episode of congestive heart failure (4). DCM in canines have been linked to two major genetic defects, the most recent being defects in the gene Titin which is involved in structural integrity of cells (5). To improve outcomes of DCM, novel therapeutic options are needed for familial DCM, particularly those associated with titin, to address the significant morbidity and mortality associated with heart failure. DPs can be genotyped for the titin mutation to predict the risk of developing DCM (6, 7). We have developed fibroblast cultures containing either the wildtype, heterozygous, or homozygous Titin mutation. The overarching goal of a larger collaborative project is to design CRISPR gene editing tools to modify the titin gene in DPs. This *student-led* project will develop functional assays to determine whether CRISPR gene editing approaches are successful once developed. ***Specific Aim 1*** will determine relative expression levels of gene biomarkers in fibroblast cells derived from the three genotypes for Titin. *The objectives* are to determine whether fibroblasts for the three different genotypes express dysfunctional gene indicators of structural damage and myopathy. We hypothesis that homozygous DPs for the Titin gene will exhibit altered expression patterns for *titin, desmin, α-β crystallin, myotilin, α-actinin matrix metalloproteinase-2, dystrophin,* and *calpain 1,2,3.* Calpains are a family of Ca2+-dependent intracellular cysteine proteases that increase under pathological conditions such as myocardial infarction (MI) and other conductions related to cardiac disease. These transcripts are biomarkers for damaged cell structure. These data will inform us on background expression levels for key transcripts being pursued in the project and will generate fundamental data for assessing CRISPR gene editing approaches from a functional and molecular perspective. In addition to gene expression, ***Specific Aim 2*** will measure calpain activity, an enzyme that is critical for the function of titin. The calpain small subunit regulates cell-substrate mechanical interactions in fibroblasts and other cell types. Based on previous studies, we hypothesize that there will be higher calpain activity levels in cells exhibiting titinopathies. Gene therapy approaches using gene editing tools promises to be a significant therapeutic approach for genetic diseases. The significance of this research project is that functional assessment tools will be optimized to evaluate gene editing corrections and help guide conclusions as to whether gene editing of Titin can restore cells to a wild time phenotype.

The significance for Veterinary Medicine and the student. CRISPR/Cas9 gene editing has emerged as a powerful approach to understanding the roles of genes in all animals and humans. The student will learn the basics of cell culture, gene expression analysis, and how to measure enzyme activity in cell homogenates. SYBR green gene expression assays will be conducted to quantify transcription levels in all three cell types. These assays will follow standard protocols optimized in the Martyniuk laboratory. We have developed a small project which has a testable hypothesis and one that will move our larger project forward. We currently have a resident developing CRISPR technologies (Dr. L Shen) and the veterinary student will interact significantly with our team and with Dr. Shen to learn about gene editing approaches in small animal medicine. Conducting both gene expression analysis and an enzyme activity is feasible within the 3-month internship and Dr. Martyniuk and his team will work closely with the student to ensure success. We encourage trainees to present their research at the UF Genetics Institute symposium in the Fall. Students will develop their own posters and present to the wider UF community, representing our College in research on campus which is very important.