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Abstract

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 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]. Doberman Pinschers 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, including one gene called pyruvate dehydrogenase kinase 4 (PDK4). Doberman Pinscher can be genotyped for PDK4 to predict risk of heart disease. There are currently >300 DPs genotyped and phenotyped in Florida. Due to the degree of genetic heterogeneity in Doberman Pinscher, along with the high mortality rates, novel therapeutic strategies are urgently needed to treat DCM. New approaches in CRISPR gene editing show promise for treating cardiomyopathies. For example, homology- independent target integration (HITI) can be used to insert a corrected sequence into the homozygous (PDK4 del/del) cell, resulting in cells that exhibit wildtype phenotypes. We have optimized fibroblast cultures containing either the wildtype, heterozygous, or homozygous PDK4 mutation. The overarching goal of this large collaborative project is to design CRISPR gene editing tools to modify the PDK4 gene in Doberman Pinchers. This ***student-led*** project will develop and screen transfection strategies for CRISPR gene editing approaches in fibroblast cells. These investigations have broader implications for many other susceptible breeds that have increased risk of heart disease (e.g., Irish Wolfhounds, Great Danes, Boxers, and Bulldogs.

Specific Aim 1 Screen and test efficacy of sgRNA probes in fibroblast cells.

The objectives of the study will include the following: (1) Culture fibroblast from Doberman Pinschers that differ in genotype (2) Verify mutants with DNA sequencing and develop guide RNAs to target sites for gene editing of PDK4. The present study will contribute to the development of future CRISPR techniques to mitigate the prevalence of DCM in Doberman Pinschers.

Approach: We will aim to identify combinations of Cas9 enzymes and candidate sgRNA that target regions of interest for a one and two site HITI approach. One goal is to utilize an AAV vector for delivery in vivo, and we will also pursue smaller Cas9 enzymes including saCAS9, cjCAS9, and nme2Cas9, which have a more limited set of PAM sites than the extensively modified spCAS9. We will use several computational tools to identify candidate Cas9/sgRNA combinations for systematic evaluation. Candidate sgRNAs that target the PDK4 regions of interest will be matched with corresponding all-in-one AAV vectors. We will subsequently clone all sgRNAs into the corresponding vectors and sequence verify the clones. We will then test sgRNAs both in vitro and in vivo for targeting efficiency in both Doberman Pinscher fibroblasts and a new cell line recently obtained, A72 canine fibroblast cells. Cells will be transfected with each vector and CRISPR targeting efficiency assessed. To assess targeting efficiency, we will consider TIDER, which uses DNA sequencing around the corresponding targeted region and Sanger sequencing of the targeted region followed by analysis with ICE algorithm.

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 and how to conduct cell transfections. 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 (Dr. L Shen) developing CRISPR technologies 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.