RESEARCH PROGRESS REPORT SUMMARY

Grant 02772: Identifying Early Stage Ultra-rare Mutations as Predictive Biomarkers of Lymphoma in High-risk versus Low-risk Breeds Within the Dog Aging Project

Principal Investigator: Daniel Promislow, PhD

Research Institution: University of Washington

Grant Amount: $75,600

Start Date: 3/1/2020 **End Date:** 6/30/2022

Progress Report: Final Report

Report Due: 6/30/2022 **Report Received:** 8/8/2022

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Original Project Description:

The most common type of cancer in dogs is lymphoma, with ~80,000 cases diagnosed annually in the United States. Breeds vary in their risk of lymphoma, but it is unclear why there is variation despite considerable effort to identify the genetics of cancer risk and progression in dogs. Cancer typically arises from the accumulation of non-inherited (i.e. somatic) mutations. However, variation among breeds in cancer risk could be due to breed-specific variation in the types of mutations, the rate of accumulation of mutations, or the downstream effects of mutations in healthy dogs. This study will use novel sequencing technology to test the hypothesis that breed-specific lymphoma risk is due to variation in the frequency and type of rare precancerous mutations. Normally, measuring these low-frequency mutations has been beyond the range of standard sequencing technology, which is limited to detecting mutations present in >1% of cells. The new technology applied here represents a >10,000-fold improvement in accuracy, enabling the investigators to accurately detect a precancerous mutation present at a single site at a frequency of just one out of every 10 million DNA base pairs. By determining if mutation frequency in blood of healthy high-risk and low-risk dogs can predict lymphoma risk, this work could lead to the development of novel tests for the early diagnosis and prognosis of canine lymphoma. This work has the potential to shed light on the mechanisms that underlie breed-specific variation in lymphoma risk, and in the long term, could lead to the development of novel tests for the early diagnosis and prognosis of canine lymphoma.

Publications: None at this time.

Presentations:

March 23, 2022.
Daniel Promislow. Predicting Lymphoma in Dogs. Online presentation to the AKC Canine Health Foundation's Cancer Webinar Series.

Oct 7-9, 2022.
Dr. Promislow will be giving a keynote address at Canine Science Conference 2022, to be held at Colgate University in Hamilton, NY in October 2022. This will provide an opportunity to introduce this first canine duplex sequence analysis to an expert audience of dog researchers.

Report to Grant Sponsor from Investigator:

The primary goal of this project is to test the hypothesis that breeds vary in risk of lymphoma due, at least in part, to breed-specific variation in rates of mutation in genes relevant to lymphoma. To test this hypotheses, we needed to gather blood samples from breeds at high risk and low risk of lymphoma, and from individuals of different ages within those breeds. We would then measure the frequency of rare mutations in several genes, and ask if this frequency is higher in breeds at relatively high risk of lymphoma.

Genes are made up of strings of four bases, which we call A, T, G and C. Every time a cell divides, there is a risk of one of these bases mutating to become another. Our goal is to identify when that happens, and to ask if it happens more often in breeds that have high risk of lymphoma, and secondarily, to ask if mutation frequency increases with age, which might explain why risk of lymphoma increases with age.

To identify novel mutations in blood cells, we sequence the DNA within each cell. Normal gene sequencing methods make mistakes at a rate of about 1 in 1000. A mistake would mean that we would think a particular base pair had mutated (say, from A to T), when it fact it had not. The actual frequency of rare mutations is less than one in a million. Thus, to measure the real mutations above the noise of mistakes, we need a method that is far more accurate at sequencing. Standard sequencing methods sequence the entire genome (2.4 billion base pairs in a dog), and for each sample, typically repeat the sequencing about 30 times ("30X") to capture most of the DNA. It is important to note that with Duplex Sequencing, we have sequenced just a few genes, but at extremely high depth (~10,000X). We need to do this to be sure that we sequence enough DNA, from enough different cells, that we will see those one-in-a-million mutations.

With the support of this grant, we were able to collect and sequence DNA in white blood cells from 117 dogs representing eight different breeds, five at low risk of lymphoma, and three at high risk. All sequencing is complete, and we are now in the process of analyzing the data with the goal of writing and submitting a manuscript to a scientific journal during the coming academic year.

The work funded by this grant was delayed considerably by the COVID19 pandemic. With all samples and sequencing data now in hand, we are well placed to carry out the last steps needed to complete what we are confident will be a high-profile scientific manuscript, laying the groundwork for a new and powerful approach to study cancer risk in companion dogs.