RESEARCH PROGRESS REPORT SUMMARY

Grant 02945-MOU: Understanding the Genetic Basis of Addison's Disease in Portuguese Water Dogs

Principal Investigator: Steven Friedenberg, DVM, PhD

Research Institution: University of Minnesota

Grant Amount: $209,381.00

Start Date: 5/1/2021 **End Date:** 4/30/2025

Progress Report: FINAL

Report Due: 4/30/2025

(The content of this report is not confidential and may be used in communications with your organization.)

Original Project Description:

Addison's disease (AD) is a common, life-threatening disorder in dogs characterized by the immune-mediated destruction of portions of the adrenal gland. This damage prevents the adrenal gland from synthesizing hormones that are necessary for normal cell metabolism, kidney function, and maintenance of the immune system. Dogs with AD are also highly predisposed to succumbing to a life-threatening adrenal crisis. AD is most common in Portuguese Water Dogs (PWDs), which have a 29-fold greater risk of developing the disease compared to other dog breeds, indicating a strong genetic component. To date, no genetic variants have been associated with AD in PWDs. This lack of knowledge has prevented the development of a genetic test that would allow for prediction of a dog's disease risk and the development of informed breeding practices related to AD. In this study, investigators will use state-of-the-art scientific tools to understand the genetic basis of AD in PWDs. The data generated here will provide the foundation for the development of a genetic test for AD in PWDs, enabling early diagnosis and treatment, as well as maintenance of genetic diversity within the breed while helping to decrease disease incidence.

Funding for the research is provided through the collaborative efforts and generosity of the Portuguese Water Dog Foundation, Inc. The AKC Canine Health Foundation supports the funding of this effort and will oversee grant administration and scientific progress.

Publications:

We recently published a manuscript related to environmental exposures that might be associated with Addison's disease in Portuguese Water Dogs and Standard Poodles. This is indirectly related our work on this grant. The paper can be found at this link: DOI: 10.1111/jvim.16896.

We also just submitted a manuscript related to the genetics of Addison's disease in Standard Poodles to Scientific Reports, which is related to the work we are doing in PWDs. We plan to submit a manuscript related to our PWD findings by the first quarter of 2025

Presentations:

Dr. Friedenberg gave a 50-minute talk at the Portuguese Water Dog National Specialty on September 29, 2021 in Norfolk, VA. This talk discussed the pathophysiology of Addison's disease, Dr. Friedenberg's research strategy for the funded grant, as well as some preliminary data. There was significant audience engagement via Q&A throughout the talk.

Dr. Friedenberg also participated in a Q&A webinar through the CARE website on September 18, 2021. While this webinar was not solely related to this study, he did discuss it and encourage qualifying dogs to enroll in the study.

We gave a webinar on Addison's disease and our research to a seminar sponsored by the PWDF/PWDCA on 3/28/2023. The seminar was recorded and can be viewed at this link: https://www.pwdfoundation.org/project/understanding-the-genetic-basis-of-addisons-disease-in-pwds/

We will be presenting some initial findings from our work studying the genetics of Addison's disease at the International Conference on Canine and Feline Genetics and Genomics in Helsinki, Finland in early June. We also plan to give a presentation to the Portuguese Water Dog Foundation in October 2024 to provide an update on our results.

We presented findings from our work studying the genetics of Addison's disease in Standard Poodles and PWDs at the International Conference on Canine and Feline Genetics and Genomics in Helsinki, Finland in early June 2024. We also presented our findings to date at several internal seminars at the University of Minnesota and the University of Georgia. We also gave presentations to the Portuguese Water Dog Foundation in April 2023 and February 2025 to provide an update on our results. In all presentations, we acknowledged the support of the AKC-CHF and the PWDF.

Report to Grant Sponsor from Investigator:

Over the course of this study, we collected DNA samples from hundreds of affected and unaffected PWDs. We performed high-coverage whole-genome sequencing of 16 PWDs (and used publicly available resources to acquire an additional 20 high-coverage samples); low-pass whole-genome sequencing of 101 PWDs, and class II MHC genotyping of the same 101 PWDs. We focused our principal analyses on the 101 low-pass/MHC PWDs because these dogs formed the least-related population out of the hundreds of DNA samples collected.

One of the main goals of this study was to perform a genome-wide association study to look for regions of the genome associated with Addison's disease in the breed. We performed this GWAS using a variety of approaches with the imputed low-pass data, but unfortunately, we were unable to find a genomic region significantly associated with Addison's disease. This implies that within PWDs, Addison's may be: (1) fixed, meaning all PWDs are equally predisposed; (2) highly polygenic, meaning that many genomic regions are responsible for Addison's disease, however our study did not have enough samples to identify these multiple regions; (3) caused by mutations that are not easily detectable by the methods we used, such as structural genomic variants; (4) caused by a combination of these factors.

We also thoroughly interrogated various major histocompatibility complex genes, which are important and diverse genes associated with the immune system. We found one group of MHC genes are associated with Addison's in the breed, and another group of genes appears to be protective. These findings reinforce (but do not prove) the idea that Addison's disease has an immune-mediated component in PWDs. That said, these associations have a small effect size, meaning that they only increase or decrease the risk of developing Addison's disease by a small amount. This suggests that other genetic or environmental factors are at play.

To interrogate the potential for fixed effects, we are in the process of undertaking several different analyses on the data we have already collected. However, it is difficult to definitively prove the impact of fixed effects because, in this scenario, all dogs have the potential to carry deleterious genetic variants. These analyses are actively underway and will continue despite the conclusion of this grant. We are also continuing to collect DNA samples that are minimally related to our existing study population from around the world, and we are working to better understand larger structural variants that may be associated with Addison's disease in PWDs. We hope to have a publication submitted regarding our findings within the next year.