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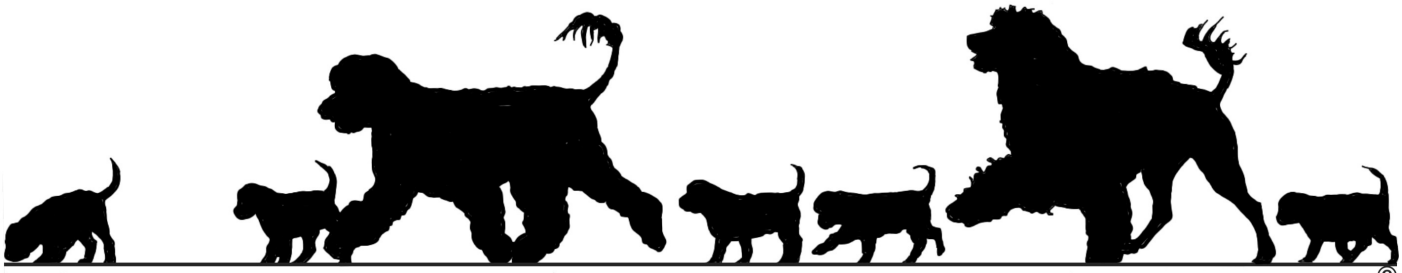

The Portuguese Water Dog Foundation, Inc.©

We are dedicated to funding canine medical research focused on issues that affect the health and well-being of Portuguese Water Dogs everywhere.

Inside this issue

What can a Million Dollars Buy?

Happy, healthy PWDs!

We are delighted to announce that The Foundation will end 2020 having funded ONE MILLION dollars in research to improve the health of our dogs! Thanks to all our generous supporters who recognize the importance of The Foundation's mission to provide a separate and permanent entity focused on supporting health research for the benefit of our dogs.

Since that small group of founders formed The Foundation in 1997, your generosity has resulted in a total of about $1.3 million in donations. We celebrate our community of supporters as we realize this important milestone. Your donations go directly to funding research that will benefit the breed we all love and cherish.

Our celebration will be marked later in the Fall by the availability of a logo commemorating the ONE MILLION dollar in research funding designed by longtime Foundation supporter, Kris Cofiell. We will offer logo apparel available for purchase by late November. Keep an eye out for more information and news about the release of our limited-time-only apparel sale.

The Foundation funds research projects which focus on diseases/conditions that will benefit Portuguese Water Dogs primarily through the Canine Health Foundation (CHF) and Morris Animal Foundation (MAF). The Foundation's selection process is to identify CHF and MAF projects that study diseases or conditions that are a concern to the Portuguese Water Dog. Although some research being conducted may use other breeds — all dogs with that particular condition will benefit from results. Our goal is to focus on the research and how it applies to health concerns within the Portuguese Water Dog breed, as well as the potential for improved outcome in the health and longevity of our dogs.

The progress of many of our studies this year has been delayed due to the impact of COVID-19 on the research community. Many veterinary facilities and institutions were closed in the Spring to non-essential or non-emergency activities. Those facilities have begun to reopen and research activities are resuming. Recent progress reports appear on the following pages and on our website.

As always, we are grateful for your continuing support. We have so much to look forward to as we close out this tumultuous and unpredictable year.

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Canine Hemangiosarcoma (HSA)

Strategic Prevention: Lifetime Follow-Up (Shine On)

Jamie Modiano, VMD, PhD Research Institution: University of Minnesota

The Shine On project is designed to utilize complementary technologies to reduce the impact of hemangiosarcoma in companion dogs. This novel, potentially disruptive approach is the first of its kind where artificial intelligence applied to the results of a blood test will be used to assign dogs to a risk category for the development of hemangiosarcoma. The test, called the Shine On Suspicion (SOS) Test is designed to detect hemangiosarcoma at its earliest stages of development before it becomes a clinically-detectable disease. Dogs that are considered to be at high risk based on the SOS Test results will be eligible to receive the drug eBAT for strategic prevention; that is, to eliminate emergent hemangiosarcoma tumors before they form. eBAT is a rationally designed drug developed in the laboratory to attack the cells that initiate and maintain the cancer, as well as to make the environment inhospitable for their growth.

For the initial phase of the Shine On project, investigators developed and refined the SOS Test and the artificial intelligence methods to assign dogs to specific diagnostic categories and started to establish the utility of the test in early detection in a group of 209 presumably healthy, pedigreed Golden Retrievers, Boxers, and Portuguese Water Dogs, 6 years of age or older.

In this continuation phase of the Shine On project, this group of dogs that had the SOS Test will be followed for their lifetimes to identify any diagnosis of cancer or another chronic disease, the cause of death, and date of death. In addition, a subset of dogs determined to be at high risk using the SOS Test will receive eBAT in the setting of prevention and also followed over their lifetime to establish their outcomes. This project expects to develop firm proof of concept to support larger clinical trials, and eventual deployment of this approach to the veterinary community setting for all dogs at risk of developing hemangiosarcoma.

Prevalence of Bartonella in dogs with Cardiac and Splenic HSA in Certain Geographic Locations

Edward Breitschwerdt, DVM, North Carolina State University

Hemangiosarcoma (HSA) accounts for the majority of canine malignant splenic tumors and occurs in many large dog breeds, including mixed breeds. A less common site of HSA localization is the heart (cardiac HSA). Risk factors for both cardiac and splenic HSA remain unclear, confounding development of preventative strategies. The investigators recently reported a high prevalence of species of the bacterial genus Bartonella in dogs with HSA from North Carolina, suggesting a potential role in the initiation and/or progression of this cancer. Bartonella species exist worldwide and are transmitted by blood-sucking arthropods (e.g. ticks, fleas) and their presence in splenic tissue could potentially be explained by the fact that the spleen is primarily responsible for removal of blood-borne parasites from the systemic circulation.

Investigators have completed all Year I study aims, with the exception of immunohistochemistry and FISH localization of Bartonella organisms within various cell types. An unanticipated complication arose that the mouse monoclonal antibody was no longer being made commercially. B. henselae specific FISH probes have been designed and validation of FISH probes are in-progress. IHC is also in-progress. All qPCR and ddPCR have been completed at this time and samples are waiting for FISH and IHC analysis. They are very excited with the qPCR and ddPCR results obtained from the fresh frozen hemangiosarcoma tissues provided by the NIH-CCOGC. The results strongly support a role forBartonella spp. in the etiopathogenesis of hemangiosarcoma in dogs. The regional study should provide additional insight as to the issue of potential causation.

Clinical Trial for Evaluation of Propranolol and Doxorubicin in the Treatment of HSA

Erin Dickerson, PhD, and Antonella Borgatti, DVM, MS, University of Minnesota

As of June 23, 2020, we enrolled six dogs in the study. Importantly, we were able to enroll dogs in all three dose cohorts (0.8 1.0, 1.3 mg/kg), and no dose limiting toxicities were noted in any of the dogs enrolled.Propranolol and doxorubicin levels in the plasma from four of the dogs has been analyzed, and analysis of samples from the fifth and sixth dogs are pending.The study has been delayed by approximately four months due to the COVID-19 pandemic. Plans are to continue to screen and enroll dogs into the study with the goal of enrolling another 8-10 dogs within the next 6 months.

Additional Progress Reports

Investigation into Diet-Associated Dilated Cardiomyopathy in Dogs

Darcy Adin, DVM, University of Florida

Dilated cardiomyopathy (DCM) is a serious disease of the heart muscle whereby the heart becomes enlarged with weak contractions. DCM can result in abnormal heart rhythms, congestive heart failure or sudden death. In dogs, DCM most often occurs in large-and giant-breeds, such as Doberman Pinschers, Boxers, Irish Wolfhounds, and Great Danes; in these dogs, survival time after diagnosis is often only months, even with aggressive medical therapy.

Recently, veterinary cardiologists have recognized DCM more frequently in all breeds of dogs including mixed breeds, and even those not usually associated with DCM. There is suspicion that the disease in some dogs is associated with boutique, exotic ingredient, or grain-free (BEG) diets. Some affected dogs on such diets have shown reversal or improvement of their disease after changing their diet, supporting a potential association between consumption of a BEG diet and development of DCM. A specific cause, however, has not been identified, despite extensive nutritional testing of the dog foods and the canine patients. Moreover, the extent of the problem is unknown because only dogs that are symptomatic for DCM have been reported. It is possible that more dogs may be affected but not yet showing signs of heart disease.

To investigate the extent of diet-associated heart problems in dogs, this multi-institutional team of veterinary cardiologists and nutritionists will prospectively screen a large population of apparently healthy dogs for DCM and compare important cardiac disease measures, including ultrasound of the heart, blood biomarker and taurine concentrations, and the frequency of DCM in dogs eating BEG versus non-BEG diets.

The study titled "Investigation into Subclinical Diet-Associated Dilated Cardiomyopathy in Four Dog Breeds" is progressing on schedule. Enrollment for the first part of the study is complete and we are in the midst of statistical analysis of the data in preparation for submission of a publication within the next few months.

We are also following dogs enrolled at UF that have bloodwork or echocardiographic abnormalities for a year after a diet change is enacted, to determine if any of the abnormalities will improve with nutritional intervention. We do not know if any or all of these abnormalities in these dogs are related to food and so follow-up is critical to this assessment. The number of dogs being followed at this time is approximately 20% of the total enrolled at UF. The team is in the process of preparing a manuscript for submission to the *Journal of Veterinary Internal Medicine.* They anticipate that the follow-up data will result in a second publication.

Landmark Clinical Trial to Establish the Evidence-Based Use of Regenerative Medicine to Treat Tendon Injury in Dogs

Jennifer Barrett, DVM, PhD, Virginia-Maryland Regional College of Veterinary Medicine

This study will evaluate the effectiveness of Platelet-Rich Plasma (PRP) and stem cells in the treatment of the most common sporting injury in dogs: supraspinatus tendinopathy (similar to the rotator cuff injury in humans). Tendon injuries in dogs often progress undiagnosed and result in chronic lameness and pain. Ultimately, unassisted tendon healing results in scar formation and reduced function of the joint and surrounding muscle tissue. PRP and stem cell therapies aim to accelerate and promote healing through tissue regeneration and reduced scarring. The investigators will conduct a randomized, placebo-controlled clinical trial evaluating the effectiveness of PRP, adipose-derived, cultured stem cells (ASC) and commonly used stromal vascular fraction (SVF) cells to directly compare efficacy of intratendinous injection of ASC versus SVF, both of which are currently commercially available despite having limited scientific evidence of efficacy. The investigators hope to identify an effective treatment to supraspinatus tendon injury.

Tendon injury is common, oftenprogresses undiagnosed, and results in reduced function, lameness and pain in both companion dogs and canine athletes. Failed healing and recurrence frequently occur because unassisted tendon healing results in scar formation with inferior mechanical properties.

Supraspinatus tendon injury of the shoulder is readily diagnosed, healing can be followed with objective measurements to evaluate efficacy, and the injury does not heal without intervention. Thus, it is an excellent tendon to study in a clinical trial. Regenerative therapies aim to accelerate and promote healing through tissue regeneration rather than scarring. There are several types of cells that promote healing, including platelets from blood and stem cells from adipose tissue. Platelets from blood can be concentrated and used as a vehicle for stem cells. Adipose tissue can either be a source of concentrated adipose stem cells (ASC) grown in a cell culture facility or can be used to prepare a mixture of various cells called the stromal vascular fraction (SVF). We propose to conduct the first randomized controlled clinical trial evaluating the effectiveness of stem cell and platelet therapy for the treatment of naturally occurring injury in dogs. Further this will be the first study to directly compare efficacy of intratendinous injection of ASC versus SVF, both of which are currently commercially available despite having limited scientific evidence of efficacy. Demonstrating an effective treatment for supraspinatus tendon injury will have profound impact on the treatment of musculoskeletal conditions as well as other types of injuries affecting dogs.

Discovery of Novel Biomarkers of Canine Atopic Dermatitis through Lipid Profiling

Harm HogenEsch, DVM, PhD

Canine atopic dermatitis (CAD) is a common allergic skin disease of dogs with a strong genetic basis. Evidence from human studies suggests that several variants of AD exist with different mechanisms and responses to treatment.

Current diagnosis of CAD requires time-consuming procedures that involve a considerable cost to the owner. Therefore, new approaches to identify molecular markers that can help with better diagnosis and management of the disease are warranted. In this study, we are using our tailored methodology for lipid biomarker discovery in CAD. 30 atopic dogs and 30 healthy dogs have been recruited. Patients are males and females of several different breeds and ages with seasonal or year-round itch. (*continued next page*)

Additional Progress Reports

Discovery of Novel Biomarkers of Canine Atopic Dermatitis through Lipid Profiling (continued)

CAD patients are being treated with either Apoquel®, Cytopoint® or prednisone and followed for 2 months to evaluate the lipid changes in their skin and blood. Using non-invasive sampling procedures, we have collected samples from the skin of healthy controls and from affected and non-affected areas of the skin of CAD patients, as well as blood. Preliminary statistical analysis demonstrates that lipid fingerprints of the blood and skin accurately classify samples from healthy dogs and CAD patients, whereas more overlap appears to be present in the lipid profile of lesional and non-lesional skin of CAD patients. MRM-profiling approach allows an unbiased analysis of the lipids that may result in new diagnostic biomarkers to classify disease phenotypes that will drive the development of new therapies.

Examination of the Effects of Cannabidiol on Canine Neoplastic Cell Apoptosis/Autophagy and Potential for Chemotherapy Resistance or Sensitivity

Joseph Wakshlag, DVM, PhD, Cornell University

Currently the use of cannabidiol (CBD) rich extracts for canine oncology patients is common, yet there is no data in canine oncology regarding the effects of CBD on canine cancer cells. Oncologists are wary of CBD use in their patients due to a lack of knowledge regarding the effects of CBD during chemotherapy. Initial studies on cytotoxicity by the research team show that CBD has cytotoxic activity on a variety of canine cancer cell lines at modest concentrations in the laboratory. These effects cause apoptosis, or programmed cell death, within a very short time frame, suggesting a discrete mechanism. The objective of this study is two-fold; 1) to determine if co-treatment of cancer cells with a common chemotherapeutic (doxorubicin) and CBD at varying concentrations affects chemotherapeutic cytotoxicity, and 2) to examine the molecular framework of the cell death response looking at the most commonly implicated pathways targeted in canine cancer treatment, including mechanisms of cell signaling and autophagy (removal of damaged cells).

We have completed a lot of the proposed work related to the effects of CBD on cell death an autophagy and the upregulation of the MAP kinase pathway which appears to be involved, however the main mechanism of apoptosis has not been elucidated which may just be directly related to the autophagy induction. This is a global response in all of the cell lines and has real implication for us tolook at other G protein signaling which is not within the scope of this project. The other area that we were in the middle of investigating is the induction of mitochondrial toxicity before the COVID epidemic hit. Once we are back up and running in the laboratory,we will be looking into mitochondrial insult using some standard assays including DHR free radical damage and mitochondrial permeability testing. This will complete the project and we are hoping it will lead tofurther publication.That said,we have completed the other aspect of the project related to doxorubicin co-treatment and have also done vincristine co-treatment showing some synergy between vincristine and CBD in the cell culture system.

Targeting the Cancer Epigenome: The Effect of Specific Histone Lysine Methyltransferase Inhibition in Canine B-Cell Lymphoma

Angela McCleary-Wheeler, DVM, PhD, University of Missouri

Lymphoma, particularly the large, B-cell subtype, is one of the most common malignancies in dogs. Canine lymphoma can be treated, but it is rarely cured. Novel therapeutic strategies are necessary to improve outcomes in dogs diagnosed with lymphoma. Recently, advances in the understanding of human lymphomas have focused on the area of epigenetics. One area of this research involves understanding how genes are turned on or off based on different modifications to histone proteins, a specific group of proteins that interact with DNA. Specific enzymes that modify these histone proteins have altered activity that can lead to lymphoma development in human lymphomas. One of these enzymes is EZH2. Increased activity of EZH2 has been shown to play an important role in the development of some human lymphomas.

Data from a Phase I study of an EZH2 inhibitor, tazemetostat, in relapsed or refractory human B-cell, non-Hodgkin lymphoma has shown to be a safe, oral therapy with potential clinical benefit. The role of EZH2, however, has not been evaluated in canine B-cell lymphomas to date. Given the similarity between human and canine B-cell lymphoma, we seek to investigate whether EZH2 activity plays a role in canine B-cell lymphoma. To do this, we use canine lymphoma cells and specific EZH2 inhibitors, including the tazemetostat used in early human studies, to evaluate the effect of EZH2 inhibition on cell growth and survival. Our data suggest that this inhibitor is highly potent and effective for inhibiting EZH2 effects on histone modification in canine lymphoma. This is important as this inhibitor is an orally bioavailable drug with a good toxicity profile in humans, making this inhibitor a candidate for clinical trials in dogs with lymphoma.

Initial data suggests that EZH2 inhibition may not impede lymphoma cell proliferation or survival. However, we have confirmed that some genes that regulate the ability of canine lymphoma cellsto replicate are altered with EZH2 inhibition. Specifically, one gene, CDKN1a, is turned back on when EZH2 is inhibited. The activation of CDKN1a is repeatable and profound. We will be continuing this work with a sequencing approach to further understand what genes are regulated by EZH2 in canine B-cell lymphoma cells. Additionally, evaluating the presence of some of these markers in banked tumor tissues will further our understanding of the role of this process in canine lymphoma.

Our findings are suggesting an importance for EZH2 in canine lymphoma and for continued investigations into cell cycle regulators that may be abnormal. By understanding the genes regulated by EZH2, we can characterize the role this histone modifying enzyme has in canine lymphoma. Moreover, we can assess how inhibition of EZH2 activity may be utilized clinical in dogs with lymphoma.

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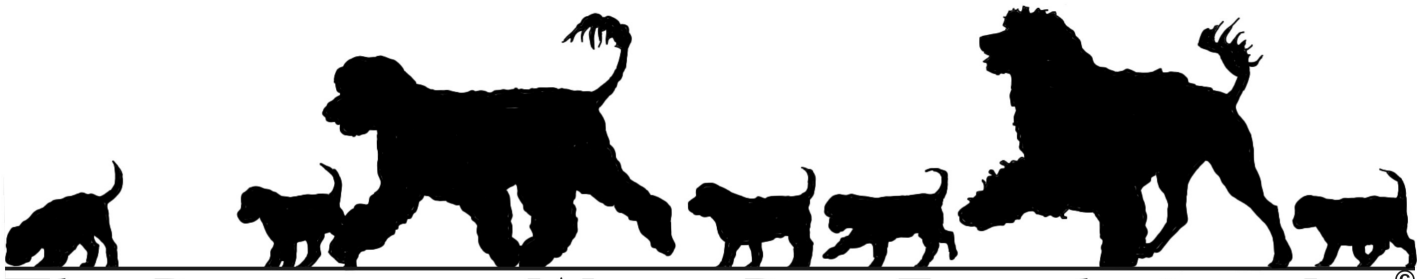

The Portuguese Water Dog Foundation, Inc.©

The Portuguese Water Dog Foundation, Inc.
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Parker Ford, PA 19457-0203
Tel 610-707-2589

The Portuguese Water Dog Foundation, Inc. needs your help and support to fund research to improve the quality of life and health of our Portuguese Water Dogs. Your tax-deductible donation, in any amount, would be greatly appreciated. In addition to personal donations, a donation may be made in memory or honor of a friend or loved one, whether human or canine. Donors' names will be kept anonymous upon request.

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Honor Roll of Donors

Thank you to all of you who support the efforts of The Foundation. We appreciate every dollar you donate. And we still have much to accomplish. This list includes people who contributed between January and June 2020.

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Jean B. Courtney

Nutmeg PWD Club in honor and appreciation of all those PWD lovers that purchased the Nutmeg 2020 calendar

Peter & Anne Paige

Mary Kanter in loving memory of her sister, Marge Schreiber

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Captain $500-$999

Friends in loving memory of Theresa Zorad, Aqua Dulce Kennels, a true patron of the breed. She will be dearly missed

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Dick & Chris Bishop in memory of our two sweet PWD boys, Sienna Coach and Ebo Ref, that we lost way too early.

Colorado PWD Club in memory of "Popeye" GCHS UCH IntlCH Odyseas Reel Sailors Eat Spinach UD RA NAP NJP CWDX NW3-ELT2 THD TKA CGC AOM GROM MAC-5 loved and missed by Fred & Susan Forman

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Colorado PWD Club in memory of "Keilani" CH Del Sur's Alohi Keilani CGCA

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John & Susan Cucura

John Eadie in memory of Mary Eadie

Marjory Hein in loving memory of "Hershey" Rustyco Hot Hershey Kisses RN

Annette Claire Konga

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Angela & Anthony Rogerson in memory of Even Keel's Poppy – we miss you.

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Delmarva PWD Club in honor of Frank Cardillino for judging the Delmarva PWD Club Water Trial

Jane Freeman in honor of the PWD PSG

Nancy Gills in honor of Mollie Tobin's Birthday

Vicki & Ken Goldberg in honor of all the essential workers during the pandemic

Jennifer Grosshandler in honor of our beloved "Flash" who will live on through the Grosshandler family forever.

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Melinda Reid Hatton in memory of "Layla" and "Lilly"

Linda K. & Krista K. Hunt, Kalista in memory of "Justine" Kalista's Justine Ormus NA OAJ JWD loved and missed by Krista & Joseph Altendahl

Katrina Jackson in honor of the PWD PSG

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Corine Knudsen in memory of "Rita" CH Newel De Agua Senhorita NAP NJP – 5/10/2006 – 8/7/2020

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Lou Ann & Bob Lindquist in honor of Chloe & Bernie

Thomas & Linda Majcher in memory of Sagmar's Lochness Monster ('Bama)

Jason & Shelley Mart in memory of "Iggy" and "Ziva" – joyful learners, companions & people trainers

Kristine Martinsek in memory of my sweet & loving Lake Breeze Oreo

Jenny & Mike Mobley in honor of the PWD PSG

Nutmeg PWD Club in memory of Sandra Nightingale

Nutmeg PWD Club in memory of James Nightingale

Nutmeg PWD Club in memory of Karen Riley and her dedication to perfecting the Nutmeg calendar

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Southern California PWD Club in memory of Bobbe Kurtz founding member of the SCPWDC

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Bob & Charlene Wolfe in loving memory of our dear friend, Kitty Braund (October 14, 1920 – July 4, 2020)

Sailor $50-$99

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Julie Asbed in honor of the PWD PSG

Rita Araujo in honor of the PWD PSG

Randi Astrom in memory of our boy "Henry" who passed away from Hemangiosarcoma

Jim & Kimberly Beach in honor of the PWD PSG

Leanne Bertino in memory of "Ziva" and "Iggy"

Suzanne Bohan in memory of "Soli" Aspencove Good Day Sunshine RN CWDX

Kimberly & Michael Bonner

Michael & Susan Burke

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Barb Crowther in honor of the PWD PSG

Bill & Judy DeSana in memory of "Murphy"

Anne Duffy in honor of the PWD PSG

Angelika Ebenig in honor of the PWD PSG

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Michael & Lynne Flater

Anne Herberholz in honor of the PWD PSG

Nancy Hollmeier in memory of Cyd, Az & Jane

Jayne Hopkins in honor of the PWD PSG

Linda K. & Krista K. Hunt, Kalista in congratulations on your new title to "Beckon" Kalista Zummon With A Wave RN TKI for his TKN and TKI!

Jayne Kenyon in loving memory of E. Niles Kenyon

Diane & Bill Keppen

Keystone PWD Club in memory of Marge Schreiber

George & Catherine Knopp

Peter & Dorothy Kowey

Helen Krepin in memory of my very good boy "Shadow" Moussaillon Shad Eau CKC rescue. I pray he has all the fun in heaven he was denied here on earth.

Bill Kulhanek in memory of Dewey, Bethany & Gidget

Amy Lane in honor of the PWD PSG

Diogo Lopes in honor of the PWD PSG

Patti McKnight in honor of the PWD PSG

Gale Meadow in honor of the PWD PSG

Sandy Myers in honor of the PWD PSG

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Christine & Christopher Peper in memory of "Jazz"

Nancy Perry in memory of "Nakita"

Willa Price in memory of the Responte's beloved "Sam"

Susan & Ron Pross in memory of Marge Schreiber

Elaine Rasmussen in honor of the PWD PSG

Janice Reilly in honor of the PWD PSG

Wyman & Joyce Robb in honor of Bob & Dottie Roth and Annie, Sardi & Brit

Martha Ruskai in honor of the PWD PSG

Kenneth J. Ryan

Mary Salvary in honor of the PWD PSG

Marion Schaufler in honor of the PWD PSG

Kathy Souza in honor of the PWD PSG

Kathy Souza in memory of "Hershey" Rustyco Hot Hershey Kisses RN loved and missed by Marj Hein

Janet Warnsdorfer in memory of "Izzy" Galaxy's Three Sheets to the Wind loved and missed by Gwenn Sawchuk

Kristin Winoker

Kim & Jerry Wolcoveick in memory of Alice Vicha & all the Norvic PWDs

Deck Hand up to $49

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Diane Duciewicz in memory of Kramer

John Haeger in memory of "Rudi" Crews'n Ports Rudolph's Glo

Linda K. & Krista K. Hunt, Kalista in memory of "Ziva" CH Bayswater's Ziva La Diva UDX SWN WWD loved and missed by Judy Bruch

Linda K. & Krista K. Hunt, Kalista in memory of "Rogue" MACH7 PACH RiverRun Can't Catch This Wave UD PUDX GN RE MXB3 MJC2 MXP4 MXPB MJP4 MJPB PAX AWD loved and missed by Paula Kerezsi, Tracy Kittrell & Beckon

Linda K. & Krista K. Hunt, Kalista in congratulations on your new title to "Beckon" Kalista Zummon With A Wave RN for his RN!

Linda K. & Krista K. Hunt, Kalista in memory of "Iggy" Kalista's It's All About Me VCD1 MX MXJ MJB WWD GROM loved and missed by Judy Bruch

Linda K. & Krista K. Hunt, Kalista in memory of "Iko" Kalista's Iko Indigo NA NAJ WWD loved and missed by Cary & Sandi Manson

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Trudi Olson in memory of "Westley" Waterbaby Silver Dollar AX AXJ XF T2B CWDX, my favorite PWD friend and snuggle buddy

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Additional Progress Reports

Identifying the Disease-Defining Autoantibodies in Canine Addison's Disease
Steven Friedenberg, DVM, PhD, University of Minnesota

The goal of this project is to identify autoantibodies that are present in the blood of dogs who are newly diagnosed with Addison's disease in three breeds: Standard Poodles, Portuguese Water Dogs, and English Cocker Spaniels. To accomplish these goals, we have been focusing on (1) collecting blood samples from dogs across all three target breeds, and (2) employing methods that allow us to detect these autoantibodies.

In terms of collecting blood samples, during the first two years of this project we have collected all the samples required from Standard Poodles and Portuguese Water Dogs. Currently, we are focusing our efforts on increasing the number of newly diagnosed English Cocker Spaniels we have enrolled in the study. We are also continuing to actively recruit newly diagnosed dogs across all three breeds through many online resources.

Over the past year, we have started to use these samples to detect the presence of autoantibodies in newly diagnosed dogs. Given some of our early results, we focused our early efforts on a type of experiment called a Western blot. To date, we have performed over 20 Western blots per breed to test for anti-adrenocortical autoantibodies in all three dog breeds. These results strongly suggest that there are autoantibodies that are consistently present against one of several adrenal proteins in newly diagnosed dogs. This is an exciting finding!

Currently, we are focusing our efforts on narrowing which adrenal protein(s) are the most likely target of these autoantibodies. Once we have done this, we will proceed to the next phase of our work, which is to synthesize the relevant adrenal protein(s) in cell culture and test individual dogs for the presence of anti-adrenocortical autoantibodies.

Addison's Disease and Symmetrical Lupoid Onychodystrophy in Bearded Collies Provide Common Ground for Identifying Susceptibility Loci Underlying Canine Autoimmune Disorders
Anita Oberbauer, PhD, University of California, Davis

Hypoadrenocorticism or Addison's disease (AD) consists of a life-threatening clinical condition that afflicts multiple purebred and mixed breed dogs. The condition results from autoimmune destruction of the adrenal glands leading to life-long cortisol deficiency. Similarly, another autoimmune condition causing pain and suffering to dogs is Symmetrical Lupoid Onychodystrophy (SLO).

For the study of AD and SLO we are investigating the Bearded Collie breed due to the relatively high prevalence of both conditions in this breed and a genomic structure favorable for identifying DNA variations. All SLO, AD and control Bearded Collie samples proposed in the grant for genotyping and genome-wide association (GWA) analyses have been collected and processed. After removing closely related individuals from the dataset, GWA analysis for SLO revealed genome-wide significant peaks on CFAs 12 and 17; the region of association on CFA12 harbors the DLA class II genes for which we have already determined an association (Gershony et al. 2019). The region on CFA 17 was more strongly associated with phenotype when only dogs that carried DLA class II risk haplotypes for SLO were considered.

Promising candidate genes were identified in both regions of association, and WGS data for SLO and healthy controls is currently under analysis for identification of potential causative mutations. A similar approach was used for AD. Initial GWAS done on 103 unrelated dogs (41 cases, 62 controls) showed a single genome-wide significant peak; additional data analysis revealed two other regions of association on two different chromosomes all of which contain potential candidate genes involved in immune function and regulation. Dogs carrying multiple risk genotypes across these regions are at greater risk of AD.

Two manuscripts have now been published as a result of this study and a third manuscript will be submitted for publication by the end of March. Samples from 21 Bearded Collies (6 AD, 6 SLO and 9 controls) have been submitted for WGS and variant data obtained.Analysis of whole genome sequencing (WGS) is underway to identify mutations that contribute to disease development in these dogs. Regions of association identified in GWAS will be prioritized, followed by exploration of the entire genome. Four new DNA samples (2 AD and 2 controls) have been collected and soon will be submitted for WGS.

The remaining control sample needed to meet the numbers stated in the grant is actively being recruited with assistance of the Bearded Collie Foundation for Health (BeaCon) and regional US Bearded Collie breed clubs.

Additional information about newly funded research grants and progress reports on previous grants are available at our website:

www.PWDFoundation.org