

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.

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Drs. Steven Friedenberg and Leigh Anne Clark hope to ultimately be able to predict, prevent and treat Addison's Disease

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ADDISON'S DISEASE - GENETIC STUDY IN PWDs

Dr. Steven Friedenberg (University of Minnesota) and Dr. Leigh Anne Clark (Clemson University) have been partnered with The Foundation since May on research using the latest genetic tools to better understand Addison's disease in the Portuguese Water Dog. Dr. Friedenberg attended the PWDCA National Specialty in Virginia Beach to update attendees on Addison's Disease, where he described his holistic approach of studying genetics, immunology and the environment. Some of the cutting edge genetics techniques in his use include SNP genotyping arrays, whole genome sequencing, and direct sequencing of key immune system genes. Long term research goals include:

- Predicting which dogs will develop Addison's disease
- Developing new ways of preventing and treating Addison's disease
- Understanding the environmental triggers.

The research team is still seeking both Addisonian dogs and unaffected dogs that are older than 9 years. All that is required is a simple blood draw that can be done at your local vet and completion of an online survey providing details about your PWD. Although researchers have started the genome evaluation between affected and unaffected dogs, they are still hoping to add many more dogs to their population. Bolstering population size (especially for unaffected dogs) will be key in identifying Addisonian variants and understanding the genetics of the disease.

Also, for owners of Addisonian dogs, the researchers are interested to know if your dog was altered (spayed/neutered) before or after their presentation/diagnosis of Addison's disease. Owners may reach out to this email (pwdstudy@umn.edu) to enroll their dog or to update the team about alter-diagnosis information (i.e. "My dog, ___, was diagnosed with Addison's at age ___, after/before being spayed/neutered at age ___").

In addition to the study on genetics of Addison's in the PWD, Dr. Friedenberg is conducting a second study that he would like to open to all PWD owners in the US and Canada. This second study aims to understand the environmental exposures that may trigger Addison's disease. Owners of healthy and affected dogs of any age can participate. All that's required for the second study is a 10-minute survey that you can complete from your computer or mobile device.

For more information, please go to www.pwdfoundation.org or the study website at z.umn.edu/addisons.

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FINAL REPORTS

Scientific and Clinical Assessment of Fecal Microbiota Transplant in Obese Dogs: SLIM Study*Jenessa Winston, DVM, PhD, The Ohio State University*

Obesity is a growing epidemic in companion animals. Obesity results from a prolonged positive energy balance leading to excessive fat accumulation, which promotes dysregulation of metabolic, hormonal, and inflammatory responses. Ultimately these changes lead to physical impairment, comorbidities, and reduced quality of life. Evidence is mounting that the intestinal microbiota (collection of microorganisms that live in the intestines) contributes to obesity, and rational manipulation of this ecosystem may confer a health benefit.

This study's goals were to provide a comprehensive scientific and clinical assessment of the efficacy of fecal microbiota transplantation (FMT) as an adjunctive therapy for canine obesity management. The investigators hypothesize that FMT (the transfer of feces from a healthy, lean donor dog into an obese dog) will amplify weight loss in obese dogs compared to the use of standard dietary obesity management. A randomized, placebo controlled clinical trial in client-owned obese dogs consisting of three groups: diet alone, diet + FMT, diet + placebo will provide data on weight loss and characterize the intestinal microbiota and metabolic function. Success of this study will benefit obese dogs by providing a microbial intervention to augment current strategies for canine obesity management aimed at promoting weight loss, normalizing metabolic status, and improving quality of life.

To date, researchers have screened 31 dogs for the SLIM study. Of these, 23 have met the inclusion criteria and were enrolled. Of these enrolled dogs, six dogs have completed at least 12 weeks of the 24-week clinical trial. The study is averaging 1-2 dogs screening for the study per week. Our patients' owners are thrilled with how their pets are progressing in their weight loss goals.

Efficacy of Cannabidiol (CBD) for the Treatment of Canine Epilepsy*Stephanie McGrath, DVM, MS Colorado State University*

Epilepsy is the most common neurologic condition in dogs. Approximately 20-30% of dogs receiving standard therapy remain uncontrolled for their seizures. Additionally, the side effects of the antiepileptic drugs (AED) are often unacceptable. Thus, there is a need for an AED that is efficacious with minimal side effects. Cannabidiol (CBD), a prominent non-psychoactive component of the Cannabis sativa plant, has been shown to have anti-convulsant properties. While CBD offers promise as a treatment for canine epilepsy, controlled studies are needed to prove its effectiveness. In this randomized, double-blinded, placebo-controlled, crossover clinical trial, client-owned dogs with uncontrolled epilepsy are enrolled following a full seizure evaluation, including bloodwork and magnetic resonance imaging. The canine patients received either a placebo or CBD in addition to their standard AED protocol and then the opposite drug in this crossover designed study. Seizure frequency and medication side effects were monitored by owners using a seizure log and questionnaire. Regular CBD plasma concentrations, routine bloodwork and serial physical examinations were monitored by the investigator. The primary goal of the study has been to determine the efficacy of CBD in the treatment of canine epilepsy. If CBD is effective in decreasing seizure frequency, it has the potential to improve the quality and length of life for dogs with uncontrolled epilepsy, and add a much-needed tool for veterinarians in the treatment of canine epilepsy.

The research team began enrolling patients for the study at the end of January 2018. They anticipated enrolling 20 patients per year; and have completed the anticipated enrollment of 60 dogs. Additionally, the team has been working with outside clinics in Colorado to help in the enrollment process of the study. Three local specialty clinics with board-certified neurologists are conducting the study with CSU's oversight at their hospitals and general veterinarians are helping with the recheck appointments and blood draws. The study has continued to gain media attention, which has helped generate interest in the study from pet owners and veterinarians. No severe adverse effects have been noted. Unfortunately, with the onset of the COVID-19 pandemic, this study was delayed for about 6 months. However, with a no-cost extension, we were recently able to complete enrollment and data collection for all 60 dogs. The study has officially ceased enrollment and the research team is in the process of analyzing the data.

PROGRESS REPORTS

Developing a Next Generation Sequencing Diagnostic Platform for Tick-Borne Diseases

Pedro Diniz, DVM, PhD, Western University of Health Sciences

Diagnostic tests based on the detection of DNA from harmful organisms in clinical samples have revolutionized veterinary medicine in the last decades. Currently, diagnostic panels for several vector-borne diseases (VBDs) are available through universities and private labs in the USA and abroad. However, the vast majority of results from sick dogs are negative, which frustrates veterinarians and dog owners trying to reach a definitive diagnosis. These panels are based on the detection of previously known DNA sequences of each microorganism, which limits their ability to detect novel organisms. This study proposes the adaptation of high-throughput next generation sequencing (NGS) to the detection of tick-borne bacteria in dog blood to overcome the limitations of the current diagnostics. The team's previous results showed the potential of NGS in detecting known and potentially novel tick-borne bacteria. Adaptations and optimizations of the technique are required for adequate efficiency and accuracy in detecting tick-borne bacteria.

Researchers are investigating methods to concentrate the harmful bacteria in clinical samples, precisely detect them using NGS, and better characterize these bacteria using genetic sequences. Most recent efforts have focused on finding new and better "DNA fingerprints" of these harmful bacteria, so they can precisely detect them from blood samples of naturally-infected dogs. Since the last report, the team has identified three candidates for the best "DNA fingerprints," and are performing extensive benchtop and genetic testing to identify the best candidate. Collectively, efforts may yield a novel diagnostic platform for the broad detection of tick- and blood-borne organisms in dogs.

Investigating Cancers and Exposure to Environmental Chemicals

Lauren A. Trepanier, DVM, PhD, University of Wisconsin

Researchers at the University of Wisconsin, Madison studied how dogs break down environmental carcinogens. The team focused on an important family of enzymes called glutathione S-transferase enzyme or GSTs. When dogs or humans are exposed

to toxic chemicals in the environment, they use GST enzymes to break down and neutralize those chemicals. When GST enzymes are not working, toxic chemicals sometimes lead to cancers.

The team tested how well four major GST dog enzymes neutralize several common environmental chemicals, including those found in tobacco smoke and yard products. They wanted to get a clearer picture of whether individual dogs with low-acting GSTs may be sensitive to specific chemicals and, as a result, more susceptible to developing environmentally associated cancers. The team used several methods to screen canine GST activity in known cancer-causing toxins. However, they did not find that any of the four GST enzymes studied recognized any of the chemicals, a failure they attribute to their methodology. Their next step is to use a different system to achieve their goals. More sensitive tests are needed. However, the team did find an appropriate positive control substance that will be invaluable as they move forward.

Researchers hope redefining their tools will provide valuable information on canine GST activity and environmental toxins. This much-needed data may provide a foundation for more targeted research on environmental exposures and cancer in dogs. The researchers' ultimate aim, as they continue their work, is to provide veterinarians with evidence-based prevention strategies to share with their clients, so pet owners have the tools they need to minimize certain cancer risks in their dogs.

Analysis of the Health, Behavioral, and Longevity Data Collected in the 9/11 Medical Surveillance Longitudinal Study

Cynthia Otto, DVM, PhD, University of Pennsylvania

Following the attacks of September 11, 2001 on the World Trade Center and Pentagon, the AKC CHF awarded funds to the only lifetime longitudinal study tracking the medical and behavioral impacts of a major national disaster on the health and behavior of search & rescue (SAR) dogs. With 15 years of data, including annual radiographs, bloodwork, and handler surveys (health, performance, and behavior), the opportunity for in-depth analysis and discovery of new best practices and protocols for SAR dogs has never been greater. Data collected from deployed dogs was compared to data

collected from control SAR dogs that underwent similar training and careers, but did not deploy to 9/11. It is hoped critical information gleaned from this study will have major implications applicable to the development, training, and care of our nation's SAR dogs, other working canines, and even companion dogs. Importantly, following characterization of trait heritability, this data could be critical to a focused breeding program. The complete analysis of the occupational hazards of SAR dogs will shape preventive practices to allow these dogs to safely and effectively fulfill their mission of saving human lives.

Data collected over the 15 years of the 9/11 study represents a massive amount of never before available information on the short and long-term impacts of a search & rescue deployment on the health and behavior of the search dog. The data analyzed in this project cover three areas: behavior, occupational hazards, and longevity. With the ever changing and improving methods for data collection, the research team has spent most of the time tracking, organizing, validating and preparing the 15 years of data to be analyzed. The data for remaining analysis is in a format in which it can be analyzed and available for cross referencing several important questions regarding behavior, health and longevity that we have proposed. We have valuable information about behaviors that are associated with deployment status, neuter status, certification type and retirement status. At least one CBARQ from each of the 150 dogs was included in the analysis, with a median of 6 completed CBARQs per dog and a range of 1-13. The German Shepherd was the most common breed (37%) followed by Labrador (27%) and Golden Retriever (9%). Dogs still in their working careers accounted for 461 CBARQs (67%), 197 CBARQs (29%) were from retired dogs, and retirement status was missing for 29 CBARQs (4%). The categories of Trainability, Attention Seeking and Energy all decrease with age, independent of deployment status. Touch Sensitivity was not influenced by age. Excitability differed by deployment status. Deployed dogs started high and gradually decreased with age, Control dogs started low, increased to a peak and then decreased with age. No dog was retired in year 1, by year 10 all participating dogs were retired.

ADDITIONAL PROGRESS REPORTS

Embracing Polygenicity of Common Complex Disease in Dogs: Genome-wide Association of Cruciate Ligament Rupture

Peter Muir, BVSc, PhD, University of Wisconsin, Madison

Cruciate ligament rupture (CR) is a common disabling, degenerative condition of the knee. It places a large financial burden on the American public.

Inflammation of the stifle and fraying of cruciate ligament fibers, particularly in the cranial cruciate ligament, eventually leads to ligament rupture with associated stifle instability in affected dogs. CR is a moderately heritable, complex disease with genetic and environmental risk. CR is common in certain breeds, such as the Labrador Retriever, and rare in other breeds. There is a critical gap in knowledge regarding the genetic contribution to CR, as the number of genes influencing disease risk has never been studied in detail. The researcher's main goal has been to comprehensively analyze the genetic features of the disease across the genome and use this knowledge to develop a genetic test for CR disease risk using genomic prediction. The team aims to advance genetic testing using genomic prediction in the Labrador Retriever, the most common purebred dog breed. The rationale for this work is that detailed knowledge of the genetic features of CR will advance development of a genetic test for CR risk using genomic prediction. This work will fundamentally advance knowledge of the genetic architecture of CR, a very common canine disease. Consequently, such knowledge will provide an invaluable guide to future research into other canine complex diseases. CR genetic testing would enable early identification of at-risk dogs for precision medical care, and selective breeding to reduce the disease burden.

The team has met its Labrador Retriever recruitment for this study over the past six months, and will continue to complete DNA isolation and marker genotyping of the remaining dogs needed for the study as long as the genotyping service remains open under the Covid-19 pandemic. Public engagement with the project continues to be good. Over the next 6 months, researchers will focus on finalizing the marker data set for the study for which

they do not yet have SNP genotypes. They will then undertake the computing needed to robustly estimate the heritability of cruciate ligament rupture in the Labrador Retriever and further advance prediction of risk of disease using the genomic marker data. A final data set is expected to include more than 1,000 Labrador Retriever dogs as promised under the award.

Precision Medicine for Canine Lymphoma

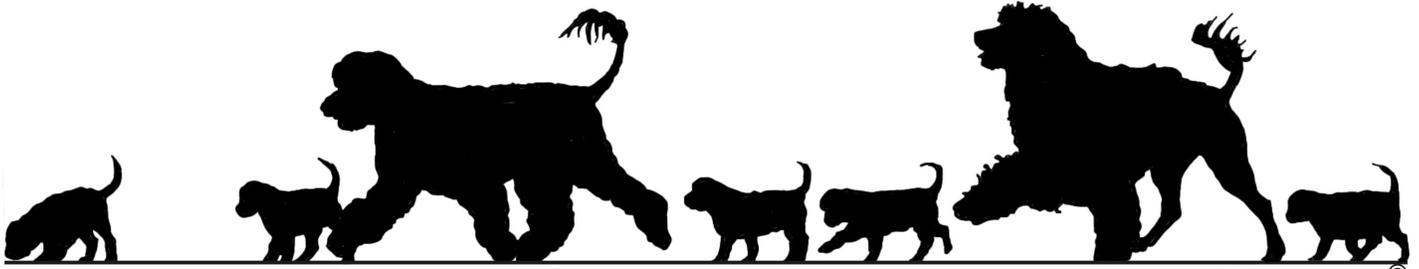
Nicola Mason, BVetMed, PhD, University of Pennsylvania

The clinical response of dogs with lymphoma to multi-agent chemotherapy is highly variable. Although up to 85% of dogs respond initially, some relapse within weeks, while others enjoy remission times of two years. This heterogeneity in clinical response is in part explained by the recognition that "lymphoma" is not a single disease entity, but consists of different subtypes that can be characterized on a molecular level by mutations in specific genes. As in human medicine, it follows that different lymphoma subtypes, driven by different molecular mechanisms, may respond better to therapies that are specifically selected to inhibit the driver mechanisms within that patient's tumor. Recent work using sophisticated genetic sequencing tools (next-generation sequencing (NGS)) has begun to shed light on the different molecular subtypes of canine B cell lymphoma, and specific therapies aimed at targeting patient-specific driver genes and pathways are being developed. To enable targeted therapies to move into the clinic, a personalized diagnostic tool must be developed that can rapidly and cost-effectively determine the mutational profile of a patient's cancer allowing selection of the most effective drug for that patient. The investigators aim to develop a NGS diagnostic test that can be employed on standard biopsy samples to identify molecular drivers of a patient's lymphoma (personalized diagnostics), enabling the most appropriate targeted therapy to be selected for that patient. In addition, they aim to determine whether specific mutational profiles within canine lymphoma identified by their NGS panel are predictive of clinical outcome.

Researchers have successfully developed a canine cancer gene panel that they have called the Canine Oncopanel, using cutting-edge, next-generation sequencing technology (NGS). The Canine Oncopanel allows sequencing of 283 cancer-related genes and detection of mutations within these genes that may drive the tumor cells to proliferate and survive. The canine oncopanel sequences a total target region that equates to ~3% of the canine genome. Analyzing the genomic composition of this broad target region allows evaluation of common genomic alterations that can lead to the development of cancer. The Canine Oncopanel is suitable to map mutation profiles and identify driver mechanisms in both common and rare canine cancers to provide a better understanding of the tumor genome and its biology.

Furthermore, as in human cancer genetics, targeted sequencing panels like the canine oncopanel are being used to provide an estimate of total mutational burden of the tumor, which has relevance in predicting response to certain immunotherapies. In addition, through identification of clinically-actionable biomarkers, the Canine Oncopanel serves as a genomic diagnostic tool that may guide selection of appropriate targeted therapies and possibly predict treatment response and patient outcome. To date, the team has analyzed 60 canine DLBCL (cDLBCL) cases using the validated Canine Oncopanel. Putative driver genes were found in 51 cases, which included driver genes previously identified by whole exome sequencing and RNA sequencing in cDLBCL or human DLBCL (hDLBCL), as well as novel drivers found in other cancers.

Clinical follow-up data were collected retrospectively and analyzed in the context of mutational profiles. Mutations in genes including TRAF3 and TP53 appeared to occur more frequently in cases with shorter overall survival times, suggesting that these mutations might delineate a more aggressive subgroup of cDLBCL and serve as prognostic markers. However, this hypothesis will need to be tested in a prospective clinical trial.



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PWD Club of Northern California in memory of "Beej" Cutwater Day Dreamin' Boy TD AX MXJ MJB MXF T2B2 CWD who was loved by George Ziembra

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Marian A. Ormont in honor of Lorenzo

In loving memory of Finne, loved by Dr. Patti Cox, from her many friends

Vicki & Ken Goldberg, in thanks to all the fabulous breeders and health testing of PWDs

Jere McInerney in memory of Charlotte, my first PWD

Robert & Susan Dick, in memory of PWDs Lisbon, Lolita, Polo and Chukker and in honor of PWD Adele

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PWD Club of Northern California in memory of "Dax" GCH CH Covey Run's Promise Her Anything RN AWD, loved and missed by Marsha Litke

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Buzz and Ingrid Fermin in tribute of "Syd" who left us too early

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Rachel Eken in honor of the PWD PSG

Ian Michael Koturbash in honor of the PWD PSG

Kathleen Souza in honor of the PWD PSG

Kathleen Souza in memory of Sealsle Jorge O Rei Do Pirata, loved and missed by Holly, Scott, and Jake Taylor

Judy Cheguis in memory of Kalista's Knee Jerk Reaction "Krickie" – Therapist, Super Sniffer and Companion, loved by Deb and Gregg Schofield

Kathy Souza in memory of "Beej" Cutwater Day Dreamin' Boy TD AX MXJ MJB MXF T2B2 CWDX loved and missed by George Ziemba

Janice Reilly in honor of the PWD PSG

Linda K. & Krista K. Hunt, Kalista in congratulations on your new title to "Lyra" Kalista's Vega A Lyrae RI SWN SBE SIE SENE SEA TKI for her SIE and SENE

Fabiana Bazzani

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Amanda Ford and Pamela Coffeen in memory of JoJo Lookout Wind and Rain and Fire VCD1 RA CD TD CGC AXJ XF AXP WWD GROM MAC-3 C-ATCH2 CT-ATCH3, loved and missed by Valerie and JP Tangen

Dorothy Finkelson, in memory of Neptune, Ocea & Moby and in honor of Hydro, Sprite & Splash, the best grandpups ever!

Peggy Mesna

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Julie Conger in honor of the PWD PSG

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Barbara & Don Niemann in memory of "Hook" Jewel de Aqua Captain Hook of Newport loved and missed by Denise Gilbert

Barbara & Don Niemann in memory of "Beej" Cutwater Day Dreamin' Boy loved and missed by George Ziemba

Alan Feinberg

Claire P. von Meysenbug in memory of Cody

Kimberly & Jerome Wolcoveick in memory of Alice Vicha and all the Norvic PWDs

Linda Hunt, Kalista, in memory of Joe Kremer, reunited with Pedro, Tina & Buster, Loved and missed by Carol and Rosa.

Linda K. & Krista K. Hunt, Kalista in congratulations on your new titles to "Trip" Kalista's Asked 'N Answered BN RN WWD for his BN & RN

Lita Cohen Rudy in memory of Bodhi, a wonderful and loving pet, missed by Ylisa Kunze

Mary Lou McCutcheon

Elana Winsberg & Michael Barber in memory of Marisol

Nancy Vener

Marilyn Rimmer, in memory of Linwood Kulp

Linda Shultz

Deck Hand up to \$49

Linda K. & Krista K. Hunt, Kalista in memory of "Mira" Kalista Miranda Writes A New Chapter RN CGC, loved and missed by Julie Carver and family

Linda K. & Krista K. Hunt, Kalista in memory of "Otis" Kalista's Little Tenderness, loved and missed by the Van Ert family

Linda K. & Krista K. Hunt, Kalista in congratulations on your new title to "Yikes!" Kalista's Already AWOL NF

Linda K. & Krista K. Hunt, Kalista in congratulations on your new title to "Roma" Kalista Bonny Billow Diver CGC TKI for her TKI

Linda K. & Krista K. Hunt, Kalista in congratulations on your new title to "Lyra" Kalista's Vega a Lyrae RI SWN SIA SEA SBE TKI for her SBE & SEA

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

Peter & Anne Paige

Ann J. Bowley & Carol Mattingley

Linda K. & Krista K. Hunt, Kalista in congratulations on your new title to "Freya" Kalista's You Betcha NA NAJ for her NA

Linda K. & Krista K. Hunt, Kalista in memory of "Krickie" Kalista's Knee Jerk Reaction RN SCA SIA SEN TKA THDD CGCA loved and missed by Deb Scofield

Linda K. & Krista K. Hunt, Kalista in memory of "Mocha" Kalista's Lil' Sip Of Mocha BN RA MX MXJ MJS OF THDX JWD TKI loved and missed by Karen Derr and Family

Linda K. & Krista K. Hunt, Kalista in memory of "Breaker" HIT Kalista's Icebreaker UD RAE AX OAJ TKP WWD GROM loved & missed by Nicole Bearman

Melinda Harvey, in memory of "Zoey" Redwood's Sea Flower, loved and missed by Robert and Doreen Madden

Linda K. & Krista K. Hunt, Kalista in congratulations on your new title to "Yikes!" Kalista's Already AWOL NA NAJ OF for her NAJ

Leigh Pelc in memory of "Barley," Watersong Home Brew, loved by Suzanne Bohan

Linda K. & Krista K. Hunt, Kalista in memory of "Polo" Kalista's Pursuit Of Happiness WWD, loved and missed by Sandi Klanderma

Linda K. & Krista K. Hunt, Kalista in congratulations on your new title to "Beckon" Kalista Zummon With A Wave BN RN NA NAJ NF TKA for his OA

Deck Hand up to \$49

Linda K. & Krista K. Hunt, Kalista in memory of "Loey" loved and missed by Fay Gallus & Richard Sweet

Linda K. & Krista K. Hunt, Kalista in congratulations on your new title to "Freya" Kalista's You Betcha NA OAJ for her OAJ

Linda K. & Krista K. Hunt, Kalista in congratulations on your new title to "Yikes!" Kalista's Already AWOL OA OAJ OF for her OA

Linda K. & Krista K. Hunt, Kalista in congratulations on your new title to "Tally" GCHB CH Kalista's What A Catch RN TKI NA OAJ NF for her OAJ!

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

Linda K. & Krista K. Hunt, Kalista in congratulations on your new title "Sprite" Kalista's Serendipity RN NA NAJ SWN SCE SCM SIE SEE CGC TKI for her SCM

John Haeger, in memory of "Rudi" Crews'nPort's Rudolph's Glo CD

Gail Browne-McDonald

Rita Araujo, in memory of Patricia Cofield

Linda K. & Krista K. Hunt, Kalista in congratulations on your new title to "Scuppers" Kalista's By The Book TKI RN for his RN

Linda K. & Krista K. Hunt, Kalista in congratulations on your new title to "Freya" Kalista's You Betcha OA OAJ for her OA

Linda K. & Krista K. Hunt, Kalista in congratulations on your new title to "Lyra" Kalista's Vega A Lyrae RI SWA SENE SIE SBE SEE TKI for her SEE

Linda K. & Krista K. Hunt, Kalista in congratulations on your new title to Kalista's Little Dob'll Do Ya RA OA OAJ NJP NF AWD "Fiddle" for his NJP

Southern California Portuguese Water Dog Club in memory of "Snorkel" AGCH PACH4 Portcolborne's Snorkel MXP12 MXPC PDSP MJP23 MJPB3 PJSP PAX4 MFPG TQXP T2BP6 SWA SCE SEM SBE SHDE CGC TKI CWD who was loved by Lara Schindler

Mary Koehn

Dr. Helene Bass-Wichelhaus in memory of Bodhi, loved and missed by Ylisa Kunze

Linda K. & Krista K. Hunt, Kalista in memory of "Rosa" Kalista's Nickel For A Kiss, loved and missed by Carol Kremer

Carol Mattingley & Ann Bowley in memory of Mike Mobley

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