



RESEARCH PROGRESS REPORT SUMMARY

Grant 02263-MOU: Characterization of Kidney Disease in Dalmatians

Principal Investigator: Rachel Cianciolo, VMD, PhD

Research Institution: The Ohio State University

Grant Amount: \$31,434.00

Start Date: 5/1/2016 **End Date:** 4/30/2020

Progress Report: End-Year 3

Report Due: 4/30/2019 **Report Received:** 5/20/2019

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Original Project Description:

Chronic kidney disease is a significant progressive problem in dogs. Two different hereditary diseases of the urinary system are being studied in Dalmatian dogs: urinary stone formation (urolithiasis) and glomerular disease. These diseases cause distinct clinical signs: urolithiasis leads to urinary tract obstruction while glomerular disease results in protein loss into the urine (proteinuria). The genetic cause of urolithiasis is known while the genetic cause of glomerular disease has not yet been identified. Although one specific type of glomerular disease has been reported in the literature, preliminary investigations indicate that there may be multiple causes of proteinuria in Dalmatians. Evaluation of kidney tissue by the International Veterinary Renal Pathology Service has revealed diverse types of glomerular diseases in Dalmatians, at least 4 of which might be hereditary. Therefore, the most common disease type is unknown and must be identified and characterized. A detailed review of autopsy and biopsy sample archives previously obtained from Dalmatians with proteinuria will be performed. Next, prospective examination of select kidney samples using advanced techniques (electron microscopy and immunofluorescence) will ensure an accurate diagnosis of the glomerular disease. Ultimately, genetic analyses could be performed on related dogs that demonstrate similar glomerular lesions to identify candidate genes.

Funding for the research is provided through the efforts and generosity of the Dalmatian Club of America and Dalmatian Club of America Foundation. The AKC Canine Health Foundation supports the funding of this effort and will oversee grant administration and scientific progress reports.



Publications:

There have not yet been any publications. Submission of publications will require collection and characterization of more samples.

Presentations:

“Study Update: Characterization of Renal Disease in Dalmatians” Jessica Hokamp, Rachel Cianciolo and Mary Nabity. Betty Garvin Memorial Speaker Series at the Dalmatian National Specialty Show. May 14, 2019.

Report to Grant Sponsor from Investigator:

Our combined retrospective and prospective study was designed to identify and characterize kidney disease in Dalmatians. A popular sire had clinical evidence of protein loss into the urine (proteinuria). This is a symptom of kidney disease and is one of the criteria for the diagnosis of Chronic Kidney Disease (CKD) in dogs. Some of the descendants from this popular sire (and other Dalmatians) have been diagnosed with proteinuric CKD. There was concern among Dalmatian breeders that the disease might be hereditary, based on the dogs that were related to the popular sire. However, there are many possible causes of proteinuria—some of which are hereditary—and the specific type(s) that cause proteinuria in Dalmatians are not known.

Unfortunately, there is limited information in the peer-reviewed literature regarding hereditary kidney disease in Dalmatians. A group of related Dalmatians in Australia were reported to have lesions similar to those seen in canine Alport syndrome. Because this hereditary disease causes proteinuria, it was possible that American Dalmatians might have a similar genetic disease. Canine Alport syndrome is due to a mutation in a gene that is important in the glomerulus, which is the part of the kidney that filters the blood. The lesion that results from the mutation can only be identified via transmission electron microscopy. In our study proposal, we provided preliminary data from samples that had been previously submitted to the International Veterinary Renal Pathology Service (IVRPS). None of the cases had the diagnostic lesion of Alport syndrome in the glomeruli. Instead there was a variety of glomerular diseases in the Dalmatians that were diagnosed by the IVRPS. Therefore, our first goal is to correctly diagnose the renal diseases that cause proteinuria in Dalmatians. These diagnoses must be based on comprehensive evaluation of renal tissue, serum and urine. After the correct diagnoses are made, pedigree analysis would help determine if the disease is heritable. Additionally, pedigree analysis can also help us determine which type of genetic assay would be appropriate.

Routine diagnosis of canine proteinuric kidney disease often entails collection and examination of kidney tissue, serum and urine. First, we proposed to re-examine kidney samples from Dalmatians that had already been autopsied or biopsied prior to the onset of the study. These archived tissue samples



could be re-examined by a veterinary pathologist with expertise in nephropathology (REC) to correctly categorize the type of disease. Clinicopathologic data and pedigree information would also be gathered. Unfortunately, it appears that many of the diagnoses of renal disease in Dalmatians in the pedigree of the popular sire were based only on bloodwork. A definitive pathologic diagnosis based on evaluation of tissue was not obtained in most cases.

To attempt to gather more cases, we have re-examined all of the kidney samples and associated medical records from Dalmatians that were autopsied at the Ohio State University (OSU) and Texas A&M University. Cases with renal lesions have been retrospectively enrolled in our study as part of Objective 1. Although we have attempted to collect pedigree data from these dogs, many of them were euthanized >10 years ago and the pedigrees are unavailable. Additionally, we were contacted by one owner whose Dalmatian was autopsied at the University of Florida and the samples from that dog have also been collected for this portion of Objective 1. We have also contacted veterinarians (and / or owners and breeders) of Dalmatians within the affected pedigrees, when we have the consent and guidance of owners and breeders. Many owners and breeders have volunteered to help communicate the goals of this study to other Dalmatian owners. This has allowed us to identify 2 Dalmatians that were euthanized because of progressive renal disease. Autopsy samples of kidney tissue were not evaluated; however, we can still collect the data and document these Dalmatians as affected relatives. In addition to retrospective data collection, we have also prospectively enrolled Dalmatians into our study by collecting urine, serum, blood and sometimes kidney samples. Biofluid samples are urine, serum and blood. These types of samples have come from proteinuric Dalmatians and sometimes their non-proteinuric relatives. Thirty dogs had urine samples submitted to us for evaluation of proteinuria, 19 of which did not have concurrently submitted kidney tissue samples. Nine dogs did not have evidence of proteinuria. Six dogs were borderline proteinuric, and the remaining Dalmatians were proteinuric. DNA has been harvested from 27 of the patients' whole blood samples and these DNA samples have been appropriately stored in the event that genetic analysis is pursued.

One major component of this study is the prospective examination of appropriately collected kidney tissue (either autopsy or biopsy samples) together with biofluid samples. To date, we have examined kidney tissue, urine and serum from 11 Dalmatians. Importantly, none of the 11 dogs had the diagnostic lesion of canine Alport syndrome. Specifically, three dogs have had glomerular disease secondary to immune-mediated disease, five dogs had glomerulosclerosis (scarring of the glomeruli), and three dogs had abnormal kidney development. This is in agreement with the types of renal diseases the IVRPS had previously diagnosed in Dalmatians prior to the onset of the study. As such, a single type of glomerular disease has not yet been identified in our cohort proteinuric Dalmatians. We are still actively recruiting cases. We know that many owners are reluctant to submit biopsy tissues for evaluation, and we are always sensitive to this issue. We try to offer a step-wise approach starting with analysis of serum and urine. Then the decision regarding biopsy can be made in light of those results. We continually work with clinicians to ensure that samples taken at autopsy are appropriately stored so that our advanced diagnostic techniques can still be performed.



The study is advertised by the Dalmatian Club of American Foundation. A lecture regarding the project was given by our colleague, Dr Jessica Hokamp, and it seemed to be well-received. The IVRPS sends flyers and emails to all veterinarians that submit samples, to make them aware of the study. The Clinical Trials Office at OSU has also created a brief communication, which is on their website: <https://vet.osu.edu/vmc/cto/clinical-trials/characterization-kidney-disease-dalmatians>

As stated previously, if we identify a specific type of proteinuric kidney disease that appears to be inherited, then we will select the best candidate Dalmatians for genetic sequencing. This last step will be done in conjunction with analysis of the pedigree and the guidance of the geneticist for our study. The largest pedigree, which contains the previously mentioned popular sire, has more detail regarding the presence / absence of renal disease. If renal disease is present, then the definitive diagnosis is also denoted on the pedigree. This type of informed approach will ensure that we are examining DNA from Dalmatians that are affected by a similar disease process.