



RESEARCH PROGRESS REPORT SUMMARY

Grant 02263-MOU: Characterization of Kidney Disease in Dalmatians

Principal Investigator: Rachel Cianciolo, VMD, PhD

Research Institution: Ohio State University

Grant Amount: \$31,434.00

Start Date: 5/1/2016 **End Date:** 4/30/2019

Progress Report: End-Year 2

Report Due: 4/30/2018 **Report Received:** 5/1/2018

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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 the Dalmation Club of America Foundation. The AKC Canine Health Foundation supports the funding of this effort and will oversee administration of funds and scientific progress reports.

Publications: None at this time.



Presentations: None at this time.

Report to Grant Sponsor from Investigator:

We proposed a combined retrospective and prospective study to identify and characterize kidney disease in Dalmatians. Previously, a popular sire was diagnosed with proteinuria, which is a kidney disease wherein significant amounts of protein is lost via the urine. Since then, some of this sire's progeny (as well as other Dalmatians) have also been diagnosed with kidney disease, but the specific type(s) are unknown. There was concern among Dalmatian breeders that the disease might be hereditary. One previous publication reported that canine Alports syndrome (a genetic disease) was present in a lineage of Dalmatians from Australia. Because this hereditary disease causes proteinuria, it was possible that American Dalmatians might have a similar genetic defect. Canine Alports syndrome has a very specific lesion that can be identified via transmission electron microscopy. None of the samples from the database of the International Veterinary Renal Pathology Service (IVRPS) had that specific diagnostic lesion. Moreover, there are a wide variety of diseases that can cause the clinical signs of proteinuria in dogs. Therefore, our first goal is to correctly diagnose the type of changes that occur in proteinuric Dalmatians. These diagnoses must be based on comprehensive evaluation of renal tissue and biofluids, namely serum and urine. After knowing what disease(s) occur, pedigree analysis can be performed to determine if the disease is heritable. If heritability is proven, then the pedigree analysis could help guide the decision regarding which genetic assay would be appropriate. Collection of kidney tissue, serum and urine are all routine diagnostic steps for the work up of proteinuric kidney disease in dogs. First, we proposed to re-examine kidney samples from Dalmatians that were autopsied or biopsied prior to the commencement of the study. Given the reports of proteinuric Dalmatians related to the popular sire, we had hoped that many of them would have been biopsied or autopsied. These archived tissue samples could be re-examined by a veterinary pathologist with expertise in nephropathology (REC) together with the reported clinicopathologic data and pedigree information (if available). This re-examination of tissue would be at no cost to the owner. Unfortunately, although we have been contacted by 2 owners with Dalmatians that were euthanized because of progressive renal disease, samples of kidney tissue were never evaluated. We have requested that medical records be sent to us so we can utilize any clinicopathologic data available. Additionally, we have re-examined all of the kidney samples and associated medical records from Dalmatians that were autopsied at the Ohio State University (OSU) since 2006. Although none of the 10 Dalmatians were diagnosed with proteinuria, 2 dogs had significant renal lesions at the time of euthanasia. These Dalmatians have been included in this retrospective part of our study. There are also archived renal tissues from 16 Dalmatians that were autopsied at Texas A&M University. These samples are being sent to OSU for re-examination and possible inclusion into this retrospective portion of the study.

Because archival tissue from pedigreed Dalmatians has been limited, we plan to redirect our efforts towards contacting veterinarians (and / or owners and breeders) of Dalmatians within the pedigrees of affected dogs in order to gather more clinical data from related Dalmatians. This will be done with the



consent and guidance of owners and breeders, many of whom have already volunteered to help notify their peers about this study. These records might help us document affected relatives. We can also identify relatives with no clinical evidence of renal disease, and therefore rule them out as “affected dogs” in the pedigree. A summer research student will be helping with this part of the data collection. In addition to retrospective data collection, we are also prospectively collecting biofluids and kidney tissue samples from Dalmatians. Biofluid samples have come from proteinuric Dalmatians and sometimes their non-proteinuric Dalmatians relatives. In total, we have examined urine and serum samples (without associated kidney samples) from 15 pedigreed Dalmatians. The results from all 15 dogs were reported in previous Progress Reports. Notably, 8 dogs do not have evidence of proteinuria. One dog was borderline proteinuric, and the remaining Dalmatians are proteinuric. Interestingly, one of the proteinuric dogs is a descendant of the popular sire with proteinuria. DNA has been harvested from samples of the patients’ whole blood and 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 five proteinuric Dalmatians. We have been told by two additional owners (who have submitted biofluids) that they plan to submit tissue samples from their Dalmatians with proteinuric renal disease after euthanasia. Importantly, none of the five dogs (from which we already have tissue) had the diagnostic lesion of canine Alports syndrome. Specifically, two dogs have had immune complex mediated glomerular disease, one dog had glomerulosclerosis (scarring of the glomeruli), one dog had evidence of malformed kidneys and the analysis of the last dog is pending. As such, a single type of glomerular disease has not yet been identified in proteinuric Dalmatians. We would like to emphasize that we are actively trying to recruit cases and samples into the study but owners are reluctant to submit biopsy tissues for evaluation. We have been working hard with clinicians to ensure that samples taken at autopsy are appropriately stored so that our advanced diagnostic techniques can still be performed. We are actively advertising our study with the Dalmatian Club of American Foundation and we send flyers and emails to all veterinarians that contact the IVRPS regarding renal biopsy. 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, the last part of our study is dependent on what is identified in these first steps. If we identify a specific type of proteinuric kidney disease that appears to be inherited, then we will select the best candidates 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. This type of informed approach will ensure that we are examining DNA from Dalmatians that are affected by a similar disease process.



Please note this study has requested a one-year extension due to having approximately half of the estimated cases with most of them having been submitted during year 2 and anticipating recruiting the remaining cases during a 3rd year.