



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:** 4/30/2018

Progress Report: Mid-Year 2

Report Due: 10/31/2017 **Report Received:** 12/21/2017

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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:

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

Report to Grant Sponsor from Investigator:

The main goal for this project is to identify and characterize a type of kidney disease in Dalmatians. A popular sire died with proteinuria, which means that the kidneys had a disease that resulted in loss of significant amounts of protein in the urine. Since then, many of the progeny from this sire (as well as other Dalmatians) have been diagnosed with kidney disease, but the specific type(s) have not been consistently documented. Thus there is concern among Dalmatian breeders that this proteinuric kidney disease might have a genetic basis and that the Dalmatian breed might be predisposed to the disease.

A previous publication reported that canine Alports syndrome was present in a lineage of Dalmatians from Australia. Canine Alports syndrome would result in proteinuria, so it is possible that American Dalmatians might have a similar hereditary disease. Notably, there are many causes of proteinuric kidney disease in dogs, and according to our records at the International Veterinary Renal Pathology Service (IVRPS) none of the kidney biopsy samples from proteinuric dogs had lesions consistent with canine Alports syndrome. Therefore, our first goal is to diagnose the type of changes (based on evaluation of renal tissue and biofluids, namely serum and urine) that occur in proteinuric Dalmatians. After knowing what disease(s) occur, pedigree analysis could be performed to determine which type of genetic analysis would be the most suitable.

Collection of kidney tissue, serum and urine are all routine steps in the diagnosis of proteinuric kidney disease. As the first phase in this study we proposed to examine "archived" kidney samples from Dalmatians that were autopsied or biopsied prior to the commencement of the study. The archived tissue would be re-examined by the PI of this grant (REC), who has expertise in nephropathology. Samples would be examined in the context of reported clinicopathologic data and pedigree information (if available). This re-examination of tissue would be at no cost to the owner. During the first year of the study, we re-examined all of the Dalmatian kidney samples and associated medical records that had been archived at the Ohio State University since 2006. None of the 10 Dalmatians had clinical tests documenting that they were proteinuric. However, two dogs had significant renal lesions at the time of euthanasia, and they have been included in this retrospective part of our study. Of note, neither dog had renal lesions consistent with canine Alports syndrome. Nineteen Dalmatians have been autopsied at Texas A&M University, 3 of which did not have kidney tissue examined on histopathology. The remaining 16 dogs had a wide variety of lesions. Three dogs did not have significant pathologic renal lesions and also did not have definitive clinicopathologic evidence of renal disease. Two dogs had renal lesions that were secondary to another process (immune-mediated



hemolytic anemia and pancreatitis with cholangitis). Review of special stains of the remaining kidney slides by REC is pending.

In addition to the archived samples from Ohio State and Texas A&M, a flyer to advertise this part of our study has been shared with the Dalmatian Club of America Foundation (DCAF) and various members. We have had limited response, but have identified 2 deceased Dalmatians with clinical evidence of renal disease. Unfortunately, neither dog had tissue harvested after euthanasia. As such, 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 previously deceased relatives. We will not be intrusive in this request; however, many of the owners and breeders are willing to help notify their peers about this study. With their guidance regarding additional interested owners / breeders, we can use our resources and background to request and review medical records. These records might help us determine if certain relatives were potentially affected or if there was no evidence of renal disease during the course of their care.

We have also been prospectively collecting and assessing urine and serum from Dalmatians. This cohort includes non-proteinuric Dalmatians that are related to proteinuric Dalmatians. It also included Dalmatians with documented proteinuria which are not undergoing biopsy at this time. Since the beginning of this study, we have examined urine and serum samples (without associated kidney samples) from 15 pedigreed Dalmatians. We reported the information regarding 4 dogs in our Year 1 Report, and the remaining 11 were collected since then. It is encouraging that we have had increased interest over the past 2 months, much of which can be attributed to improved communications with breeders and owners. Notably the 8 of the 11 dogs do not have evidence of proteinuria based on these analyses. One dog was borderline proteinuric, and the last two dogs are proteinuric. Interestingly, the proteinuric dog is a descendant of the popular sire with proteinuria. Obviously a rigorous pedigree analysis needs to be performed before any conclusions regarding heritability of the renal disease can be made. Samples of whole blood have been submitted from these patients in the event that genetic testing is developed.

The last component of the study is examining prospectively collected kidney tissue (either autopsy or biopsy samples) in conjunction with urine and serum. To date, we have examined kidney tissue, urine and serum from three proteinuric Dalmatians. We have been told that samples from an additional Dalmatian with end stage kidney disease will be submitted in the near future. None of the dogs have had evidence of canine Alports syndrome. Specifically, one dog had immune complex mediated glomerular disease, one dog had evidence of malformed kidneys and the last dog had glomerulosclerosis (scarring of the glomeruli).

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.