Saluki Health Research, Inc. has financially supported the following AKC/CHF Grants and has received the following progress reports.

**Grant 01147: Identifying Mutations in Genes Associated with Canine Hemangiosarcoma**, Dr. Chieko Azuma, DVM PhD, Tufts University. Grant amount: $75,000.00. Start date: 1/1/2009. End date: 12/31/2009.

Canine hemangiosarcoma is a devastating disease often striking suddenly. With the new SNP arrays, we have mapped six disease associated loci in Golden Retrievers. This project aims to identify the actual mutations within the six regions of DNA. Introduction: Hemangiosarcoma (HSA), a malignant tumor of blood vessels, is a significant health concern in dogs. The disease is frequent in several breeds including Golden Retrievers (15%), German Shepherd dogs (10%), and Labrador Retrievers suggesting that genetic risk factors exist. We have identified six regions in the canine genome associated with HSA in Golden Retrievers. Subsequently, when we examined the DNA of four other breeds (Labrador Retriever, German Shepherd Dogs, Boxers, Leonberger), we found that the all of the regions of interest in Golden Retrievers were shared with at least one other breed (CHF grant 593). Most associated regions of DNA contain excellent candidate genes with known function as well as some novel candidates. Some of the candidate genes for susceptibility of HSA include tumor suppressor genes and angiogenesis factors that are likely to be relevant to formation of the tumors.

*Objectives:* The goal of this project is to identify mutations associated with canine HSA. Based on our preliminary data, we hypothesize that some of the susceptibility is due to germ-line mutations in the regulatory regions rather than coding regions of many of these genes and multiple genes are responsible for the increased risks for developing HSA. Sample population: All sample submissions have been carefully reviewed to verify the diagnosis of HSA based on the histopathology. The breed clubs including Golden Retriever, Labrador Retriever, German Shepherd Dog, Boxer, Bullmastiff, Leonberger and Briard have been actively participating and organizing sample collection efforts. Blood samples from affected and control dogs from other breeds have also been collected. We are also actively collecting tumor tissues to be able to study the effects of the disease genes in the tumors.

*Plans:* We are continuing the search for the actual mutations and have started the work to look at the functional consequences of the mutations using expression analysis. We are continuing to encourage participation of veterinarians, dog owners and breeders. We will reach out to a larger set of breeds to allow for the assessment of the risk alleles in many breeds. Once we know the pattern of mutations across breeds, it will be possible to develop appropriate genetic tests for HSA. By understanding how the mutations cause disease and which pathways are affected, it will be possible to develop better prevention and treatment strategies for HSA.

**Grant 00613: The Prognostic Significance of Chromosome Aneuploidy in Canine Lymphoma**, Dr. Matthew Breen, PhD, North Carolina State University. Grant amount: $113,929.00. Start date: 8/1/2008. End date: 7/31/2010.

Lymphoma is the most common life-threatening cancer in dogs, accounting for up to 24 percent of all canine malignancies. A large proportion of canine lymphomas are responsive to chemotherapy, increasing both the length and quality of an affected dog's life. However, there is considerable difference in the response to therapy working and overall survival time. This shows that there is a need to develop more improved forms of classification. In human lymphoma, the use of cytogenetics has been used to show the presence of frequent chromosome abnormalities that have both diagnostic and predictive importance. In previous studies the researchers have identified frequent chromosome abnormalities in canine lymphoma, including copy number changes (aneuploidy) of dog chromosomes 6, 15, 16, and 18.

*Objective:* In this project the researchers will use molecular cytogenetics to study a collection of lymphoma specimens, taken from dogs that were all treated with the same chemotherapy procedure as part of a clinical trial. This approach will allow us to determine if these frequent copy number abnormalities are able to predict response. This project hopes to increase the sophistication of diagnosis and life expectancy for canine lymphoma.

During the first year of this two year project, we have shown that pooling DNA from overlapping BAC clones results in a more robust fluorescent signal in interphase analysis than using a single BAC clone and provides a higher signal to noise ratio. We have generated the DNA used for the probes being used for this project en masse. Cells have been isolated from 200 of our 315 archival patient samples and prepared for multicolour FISH analysis. Thus far we have performed FISH analysis of the first 150 archival cases and have acquired images for each these cases. Data for the copy number of each of the four loci has been assessed and statistical evaluation has indicates that at least one of the four loci may be associated with disease free interval. Over the next year we will

continue processing cases as we aim towards development of a prognostic test for dogs diagnosed with lymphoma.

**Grant 00947A: Heritable and Sporadic Genetic Lesions in Canine Osteosarcoma**, Dr. Matthew Breen, PhD, North Carolina State University. Grant amount: $147,912.00. Start date: 8/1/2008. End date: 7/31/2010.

Osteosarcoma (OSA), bone cancer, is the most common primary malignant bone tumor, occurring spontaneously in both humans and dogs. In humans, around 900-1000 cases of OSA are diagnosed per year while in dogs more than 8000 cases are reported per year making the disease incidence much larger in dogs than humans. Previous research focusing on human and dog OSA have discovered that these tumors contain a high degree of genetic abnormalities. Several studies on human OSA have indicated that some genetic abnormalities in humans are correlated with a poor prognosis. Currently, only a little is known about how genes influence the risk and progression of bone cancer in dogs. In order to assess the degree of genetic abnormalities in dogs, we have recruited a population of 122 cases of canine osteosarcoma. Using genome-wide array based comparative genomic hybridization (aCGH) and multicolour fluorescence in situ hybridization (FISH) analysis, we have identified numerous recurrent DNA copy number changes in canine osteosarcoma. In this process, we have identified genetics that appear to be different between dog breeds, and have also identified similarities between genetic abnormalities seen in human and dog bone cancer. Our population comprises patients from four breeds; Golden Retrievers, Great Pyrenees, Greyhounds, and Rottweilers. Data thus far indicate that there are significant differences in genetic changes in osteosarcoma between these four breeds. Our discovery of genetic abnormalities that appear to be shared between human and dog OSA highlight the dog as an appropriate model system for the disease, allowing our research to aid both human and canine cancer patients. As this project continues we aim to evaluate how the genetic abnormalities we have identified may be influencing the disease risk and also progression in dogs. This will serve as an aid in the development of diagnostic and prognostic tools for dogs affected with bone cancer.

**Grant 00757A: Hereditary Mutations in Genes Associated with Osteosarcoma in Large Dog Breeds**, Dr. Kerstin Lindblad-Toh, PhD, Broad Institute. Grant amount: $91,481.04. Start date: 4/1/2007. End date: 9/30/2009 .

Osteosarcoma (OSA), or bone cancer, affects 8,000-10,000 dogs in the United States annually. Large and giant breeds are at a much higher risk for this disease, suggesting that inherited risk factors are involved. The purpose of this study is to identify the mutations causing the increased risk for bone cancer in Rottweilers and Greyhounds. To do this, we have proposed to compare the genotypes of dogs diagnosed with OSA to healthy older dogs using a statistical analysis.

To date, we have collected ~500 blood samples from dogs diagnosed with OSA and ~1500 healthy dogs over 8 years old. Of these, we have collected 171 blood samples from Greyhounds diagnosed with OSA and 276 healthy Greyhounds over 8 years old. We have localized genetic risk factors that are associated with OSA to three chromosomal regions in Greyhounds and are currently narrowing in on the precise mutations that cause the disease. The biological effects of the mutations will be studied to better understand the cause and progression of the disease. This work should allow the development of specific genetic tests for carriers of OSA and suggest improved treatments for OSA.

In our completed CHF study "Mapping Genes Associated with OSA in Large Breed Dogs," we have identified genomic regions associated with OSA in Rottweilers and Greyhounds using genome-wide association with the newly developed ~27,000 SNP array. Results of genome-wide scans show that three regions are associated with OSA from the genome-wide screen in Rottweilers and three different and non-overlapping regions are associated with OSA in Greyhounds. In this study, we have proposed to conduct further fine-mapping of these candidate regions using additional Rottweiler samples paired with Mastiff-type breeds (Golden Retrievers and Leonbergers) and, likewise, additional Greyhound samples paired with Long-limbed Hound type breeds (Irish Wolfhounds and Great Danes). We have now performed fine-mapping in nine breeds. All six loci are supported, and several candidate genes have been interrogated for mutations. Since no coding candidate mutations have been identified so far, we believe that regional resequencing will be necessary and the methodology to do this has been developed. We have also continued to fine-map in larger sample numbers to identify the most highly associated regions in preparation for regional resequencing to identify mutations. Regional resequencing is now ongoing to identify mutations.

**SHR has a new website. Please visit us at www.salukihealthresearch.com**