AKC Canine Health Foundation Grant Updates:
Research Currently Being Sponsored By
The Vizsla Club of America Welfare Foundation

GRANT PROGRESS REPORT REVIEW Grant: 00748: *SNP Association Mapping for Canine Epilepsy*
Principal Investigator:
Dr. Ned Patterson, DVM PhD

Report to Grant Sponsor from Investigator:

English Springer Spaniels: We now have over 900 samples from English Springer Spaniels with 91 dogs with recurrent seizures with no known other cause. In the past two years we have done extensive seizure surveys, phone interviews, and rechecking all dog's status to be sure dogs included in SNP analysis have the correct diagnosis. Our previous pedigree and segregation analysis indicated that idiopathic epilepsy in this breed was most likely not a simple Mendelian (one gene fully penetrant) trait and that the pedigrees available were of marginal power to detect a genetic marker, so we did not attempt a genetic linkage analysis. Rather, we concentrated on selecting a sample set that is most appropriate for a genetic association study with single nucleotide polymorphism (SNP) markers. To date, we now have run 53 affected and 91 unaffected ESS for genome wide marker analysis with the 20,000 Illumina SNP chip. We continue to have weak association and on 3-4 chromosomes with one that is slightly stronger, but further studies with more dogs and/or more markers are still needed since none of the hits reach statistical significance. The best association was found on one specific chromosome, but this did not reach statistical significance with this number of dogs analyzed. There were 3-4 other chromosomes too with weak association and further studies with more dogs are required to confirm the initial results. With the funds remaining in this grant, we have just completed genome wide SNP analysis for the best 24 affected and 24 normal ESS on the new Illumina 170,000 SNP array and will have the results completely analyzed by in the next few months.

It has become increasingly apparent that IE is polygenic in many breeds. Our, formal agreement with the U of MO and Finland allows us to compare possible chromosomal areas across breeds - ESS and Aussie's (also Vizslas and other breeds) in case there is a shared gene across breeds in which putting the data together might be more efficient in identifying the specific gene. Dr. Patterson attended the LUPA meeting in May 2009 in Sweden and met in person with Dr. Hannes Lohi about all of the Epilepsy projects with an emphasis on the data in ESS, (and Aussies, and Vizslas). In Dr. Lohi's recent presentation to the American Epilepsy Society in December 2009 in Boston (for which Dr. Patterson was a co-author) regarding more than 15 breeds it is clear that are a limited number of breeds where one gene may cause epilepsy, but in most breeds the genetic predisposition is influenced by more that one gene which is very likely to be the case for ESS. Despite the complexity in ESS, we are committed to continue following through on our search for markers and genes in ESS as long as funds and new DNA samples are available. At this point the most important efforts will be to collect more samples of

affected ESS. Currently we have more than enough unaffected ESS.
If we get strong association for ESS in the whole genome analysis with the 170,000 SNP array we would then work on fine mapping and developing a linkage test in this new CHF grant application in the Spring of 2011, similar to what is proposed for specific aim 3. If we do not yet find significant association, we would then work on continued collection of affected dogs and more whole genome analysis with the 170,000 array in this new grant application.
Australian Shepherds: For Australian Shepherds there are over 150 affected Aussies and over 1100 total samples banked between us and our collaborators of the University of Missouri-Columbia. We are continuing to try to collect more Aussie DNA samples to be sent to our lab for this and any needed future analysis, and to verify the diagnosis whenever possible.
With our formal written agreement for the sharing of Australian Shepherd samples and data between the U of MO, Hannes Lohi in Finland, and us, we are attempting to not unnecessarily repeat DNA testing to ensure maximum efficiency and the best use of funds.
38 cases and 39 controls from US Aussies collected by Dr. Johnson's lab have been analyzed in Europe by Dr. Lohi. The results do not reveal any single association peaks, indicating that the epilepsy is either caused by more than one gene or there might be different type of epilepsies present in Aussies.
The best association was found on one specific chromosome, but this did not reach statistical significance with this number of dogs analyzed. There were 3 other chromosomes too with weak association and further studies with more dogs are required to confirm the initial results. In the last 12 months we have collected 19 additional affected Aussies and 21 additional controls and the University of Minnesota.
Dr. Lohi and we have held off additional SNP analysis for Aussies in the last 12 months waiting for the new 170,000 Illumina SNP array which has just become available and has much more power that the previous Illumina (and Affymetrix SNP arrays). We just completed whole genome SNP analysis for 19 affected and 21 unaffected Aussie, and will have the data fully analyzed in the next few months.
Drs Patterson and Lohi meet in Sweden in May of 2009 with an emphasis on the data in Aussies, ESS (and Vizslas). In Dr. Lohi's recent presentation to the American Epilepsy Society in December 2009 in Boston (for which Dr. Patterson was a co-author) regarding more than 15 breeds it is clear that are a limited number of breeds where one gene may cause epilepsy, but in most breeds the genetic predisposition is influenced by more that one gene which is very likely to be the case for Aussies. Despite the complexity in Aussies, we are committed to continue following through on our search for markers and genes in Aussie as long as funds and new DNA samples are available. At this point the most important efforts will be to collect more samples of affected Aussies. Currently we have more than enough unaffected Aussies.
If we get strong association for Aussies in the whole genome analysis with the 170,000 SNP array we would then work on fine mapping and developing a linkage test in this new CHF grant application in the Spring of 2011, similar to what is proposed for specific aim 3. If we do not yet find significant association, we would then work on continued collection of affected dogs and more whole genome analysis with the 170,000 array in this new grant application.

GRANT PROGRESS REPORT REVIEW Grant: 00615B: *Heritable and Sporadic Genetic Lesions in Canine Lymphoma*
Principal Investigator:
Dr. Matthew Breen, PhD

Report to Grant Sponsor from Investigator:

At the end of the second year of this project we have completed genome wide assessment of DNA copy number variation to profile tumor DNA from 252 dogs diagnosed with lymphoma. This is far in excess of our initial goal. We have performed a detailed statistical evaluation of the genome wide data derived both from 'fresh' lymphoma tissue and from archival lymphoma tissues in the form of formalin fixed paraffin embedded (FFPE) specimens. Our analysis has indicated that in many cases we can obtain robust data from FFPE specimens and use this alongside data obtained from fresh cases. While fresh tissue is always preferred, this finding means that we are able to access pathology archives to substantially increase sample numbers and so rapidly verify genomic changes that we have discovered. In essence, we are able to take a step back in time as a means to take a leap forward in discovery.
Ongoing analyses of our genome wide data have identified several high frequency aberrations some of which are associated recurrently with either B cell or T cell canine lymphoma and some of which may be associated with further subtypes. In addition many of the cases used in this study were treated with standard of care chemotherapy and so as we follow the outcome of these patients we will be n a position to determine if any of the recurrent chromosome changes we have identified are associated with prognosis. As we proceed we now are using much higher resolution technology which is allowing us to narrow down the regions of interest and this ultimately will lead to more accurate gene discovery.
When considering the breed specific nature of copy number changes of dog chromosomes 14, 15 and 36, the frequency with which we see aberrations of chromosome 14 has continued to remain evident, while those involving chromosomes 15 and 36 have declined. There are a number of subchromosmal changes along the length of these chromosomes, however, that may be breed specific, as well as numerous other regions of the genome. These regions are now being evaluated at ~100 fold increase in resolution and so as these data become available we will be able to determine if these are significantly associated with breed.

GRANT PROGRESS REPORT REVIEW Grant: 01131: *Genetic Background and the Angiogenic Phenotype in Cancer*
Dr. Jaime F Modiano, VMD PhD

Report to Grant Sponsor from Investigator:

Certain dog breeds are prone to develop certain types of cancer; yet, there has been little progress to define genes or other factors that account for this risk. Our recent work on hemangiosarcoma is the first to clearly demonstrate that a dog's genetic background, defined by "breed," can influence the profile of genes that are expressed by tumors. Among other important implications, this implies that certain breeds are diagnosed with specific cancers more frequently than others because of the behavior of tumors after they

arise, and not simply because they arise more frequently. Specifically, this may apply to the observed predisposition for hemangiosarcoma seen in Golden Retrievers, German Shepherd Dogs, and Portuguese Water Dogs. Here, we have begun to test this premise by evaluating genome-wide gene expression profiles in these three breeds. We also have started complementary experiments to determine if potential treatment targets behave equally in dogs from different breeds. Our preliminary results suggest that differences at the molecular (submicroscopic) level in these tumors will indeed influence their behavior and their response to treatment approaches.

Grant: 01352-A: *Detection of Brucella canis DNA in canine urine, semen and vaginal cells via qPCR Analysis*

Principal Investigator:
Dr. Lin Kauffman, DVM

Report to Grant Sponsor from Investigator:

Brucella canis, a bacteria that causes reproductive disorders in dogs, has been on the increase across the US. This goes hand in hand with increasing costs associated with reproduction losses due to disease and euthanasia of infected animals. The potential for human infection from dogs increases concern over this infectious disease. Currently the only available tests for this disease are not great. The bacteria is hard to culture and serological tests detect the disease 8-12 weeks post-infection but cannot detect early disease. Present laws make canine brucellosis a reportable disease subject to quarantine. In some states this means testing all breeding animals and euthanasia of infected animals. Early detection of disease would shorten the quarantine period and would add a measure of safely for buyers of puppies or breeding stock. The goal of this project is to use Polymerase Chain Reaction (qPCR) assay to detect Brucella sp. in samples of suspect B. canis urine (male), semen and vaginal cells (female) and compare how this test works vs. traditional culture and serology.

To find a more specific and timely diagnostic, this study assessed the ability of qPCR analysis to detect B. canis Omp25 DNA in a variety of samples (blood, urine, vaginal swab) and compared those results against current detection methods for B. canis infection in dogs (serology). qPCR analysis identified the presence of B.canis Omp25 DNA in multiple dogs prior to seroconversion. Non-invasive samples from the genito-urinary tract, including vaginal swabs and urine, were found to be the most sensitive for detection of B. canis Omp25 DNA via qPCR. Use of these samples would make collection of diagnostic samples within the ability of some dog owners and breeders.

The results of this study are very encouraging for use of B. canis Omp25-specific qPCR as a diagnostic screening tool for B. canis. The potential of this assay qPCR for early detection could be very valuable for elimination of B. canis from kennels without having to wait for seroconversion. Additionally, Omp25 qPCR could be a valuable screening tool for B. canis in newly purchased dogs prior to adding these dogs into a new home or kennel. B. canis is a reemerging infectious disease in the canine breeding industry. A better screening and detection method will be very useful to prevent further spread of this insidious disease. B. canis Omp25 qPCR may be this critical diagnostic component to decrease economic effects of canine brucellosis on the canine breeding industry and prevalence of canine brucellosis in the US.

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