Tibetan Terrier
Health & Welfare
Foundation

Project Title: Identification of Mutations Responsible for Progressive Retinal Atrophy in Tibetan Terriers by Whole-Genome Sequencing

Project Abstract: In the past we have been able to identify causal mutations in important diseases of Tibetan Terriers including primary lens luxation, adult-onset neuronal ceroid lipofuscinosis, and degenerative myelopathy. In addition, we have found that a mutation already known to cause older-adult-onset progressive retinal atrophy (PRA) in setters is also associated with adult onset PRA in Tibetan Terriers.

We have recently had some success in identifying mutations responsible for inherited canine diseases of other breeds by computer analysis of whole genome sequences, generated by Next Generation sequencing with DNA from a single affected dog. So far we have identified four disease-causing gene modifications in this way. In each case we were able to verify the association between the mutation and the disease by analyzing DNA from additional normal and affected dogs. We have used this approach to investigate 16 other inherited canine diseases but have not yet been successful in identifying causal mutations in these dogs. We are continuing our analysis of these 16 whole genome sequences, and expect to identify additional causal mutations in them; however, our current success rate is 20% and limitations to the current technology make it unlikely that our success rate will exceed 50% in the near future. The rapidly improving technology, including improved computer software for analyzing whole genome sequences, make it likely that much higher success rates will be achievable further into the future. Thus, we plan to continue using this strategy to investigate additional heritable canine diseases.

We are interested in applying the whole-genome sequencing strategy to identify additional causes of progressive retinal atrophy (PRA) in Tibetan Terriers. In this application, we are requesting $20,000 from the Tibetan Terrier Health & Welfare Foundation to initiate this research. As soon as this grant is approved we will begin to generate 35-fold coverage whole-genome sequences from a Tibetan Terrier with earlier onset form of PRA (diagnosed when the dog was 3 yrs old) and a later onset form of PRA (diagnosed when the dog was 9 yrs old). The DNA samples from both of these dogs tested "homozygous normal" for the above-mentioned mutation associated with PRA in setters.

The first step in generating the whole-genome sequences will be to submit DNA from the targeted Tibetan Terrier to a company (Global Biologics) that specializes in DNA library preparation so that they can construct paired end libraries with average lengths of 300 bp and 400 bp. Next we will submit the DNA libraries to the University of Missouri DNA core facility for sequencing in two lanes flow cell lanes of sequence in their Illumina Hi/Seq 2500 DNA sequencer. With this instrument, the sequence reads from two flow cell lanes have typically provided 30 to 40 fold average genome coverages.

The reads will then be processed with quality control software that trims the adapters and eliminates random sequence variants likely to arise from miscalls and many of the PCR duplicates that can confound downstream analysis. When this step is completed, we will use NextGENe software to align the reads to the canine genome reference sequence (build 3.1). The approximately one million sequence differences between the reference sequence and the aligned reads (called sequence variants) will be filtered to produce at least two mutation reports from each alignment: one report will list all

gene-associated sequence variants that occur only in the affected Tibetan Terrier (absent from the whole-genome sequences generated so far from dogs of other breeds) and another report listing sequence variants associated with all genes known to cause retinal disease in people (listed in NetRet, https://sph.uth.edu/retnet/sym-dis.htm) plus genes such as *MDM1* known to cause retinal disease in animal models but not yet associated with human retinal disease.

The sequence variants identified in the two mutation reports will be subjectively prioritized for their potential to be the cause of the Tibetan Terrier PRA. For this we will search the scientific literature with OMIM (http://www.ncbi.nlm.nih.gov/omim) and PubMed (http://www.ncbi.nlm.nih.gov/pubmed/) to determine what is known about the biology and molecular genetics of the genes in the mutation reports. In addition, we will determine whether or not the amino acid sequence alterations predicted by the nucleotide sequence variants in the mutation reports involve amino acid sequences that are conserved in corresponding proteins from related species with a blastp (http://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastp&BLAST_PROGRAMS=blastp&PAGE_TYPE=BlastSearch&SHOW_DEFAULTS=on&LINK_LOC=blasthome) computer search. Also, online programs such as SIFT (http://sift.jcvi.org/) and PolyPhen 2 (http://genetics.bwh.harvard.edu/pph2/) which predict whether or not an amino acid substitution affects protein function will be used in our subjective prioritization of candidate sequence variants.

The highest priority candidate sequence variants will be evaluated by genotyping samples selected from our collection of over 909 individual Tibetan Terriers. We anticipate that the causal sequence variant will be homozygous in many samples from Tibetan Terriers with PRA but absent or heterozygous in DNA from older Tibetan Terriers with normal retinas. The nature of the sequence variant will determine the genotyping method. In the past, we have often used PCR-restriction-fragment-length polymorphism, TaqMan allelic discrimination, or automated Sanger sequencing for this purpose. We were aware the Dr. Simon Peterson-Jones (Michigan State University) has previously investigate Tibetan Terrier PRA and have contacted him by email to see if he would like to collaborate on this project by sharing some of the samples he has collected. Dr. Peterson-Jones indicated that he will contact us after he has had time to inventory his Tibetan Terrier samples. This collaboration may provide us with access to additional samples to establish associations between clinical PRA and candidate mutations.

As suggested in the second paragraph of this abstract, we believe there is a 20%-to-50% likelihood that we will be successful in our attempt to discover the causes of PRA and in the whole genome sequences of the two affected Tibetan Terriers. If we are able to identify the mutations responsible for PRA in the targeted Tibetan Terrier and if these mutations explain all or almost all of the Tibetan Terrier PRA not explained by the mutation first identified in setters, we will offer DNA tests at a reasonable price to help Tibetan Terrier breeders eliminate PRA in future generations. Also, we will publish our findings in the scientific literature so that other laboratories can offer similar DNA tests. If we discover the mutations responsible for PRA in the targeted Tibetan Terriers but these mutations do not explain all or almost all of the PRA in Tibetan Terriers, we will still offer reasonably priced DNA tests and publish or results. In addition, we will apply for more funds from the TTHWF to identify the remaining cause (or causes) of Tibetan Terrier PRA. Furthermore, we believe that there is at least a 50% likelihood that we will be unable to identify both of the PRA-causing mutations within the 4-to-6 month timeframe of the proposed project. Identification of the causal mutation may require a mapping experiment or the generation of additional whole-genome sequences. In that case we will apply for additional funds from the TTHWF when we submit the final report for the project in this proposal.

Duration of Project: Four to six months

Proposed start date – 1/2/13
Proposed end date – 4/30/13 to 6/30/13

Director and Staff:

Director – Gary S Johnson, DVM, PhD (0.04 FTE for 5 months)
Co-Investigator, Robert Schnabel, PhD (0.01 for 5 months FTE)
Douglas Gilliam, graduate student (0.30 FTE for 5 months)

Final report to TTHWF: A Word file containing a detailed summary of our results and their interpretation will be submitted to Brenda Brown, President of the TTHWA. In addition, we will work with Ms Brown (or another individual designated by the TTHWF) to prepare brief summary suitable for distribution to Tibetan Terrier breeders and owners.

Total amount of Project Funding Requested: $20,000

Itemized Budget

DNA library construction (4 DNA libraries x $250/library)	$ 1,000
Illumina HiSeq2500 sequencing (4 lane x $2,750)	$11,000
Graduate student salary and benefits (0.4FTE for 5 months)	$ 4,000
Primers, restriction enzymes, and other genotyping reagent	$ 4,000
Total requested	$20,000

Person Responsible for Financial Oversight of the Project:

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