GRANT PROGRESS REPORT REVIEW

Grant: 01485: *Study of PLE/PLN (Protein-losing Enteropathy/Nephropathy) in Soft-coated Wheaten Terriers*

Principal Investigator: Dr. Meryl P. Littman, VMD

Research Institution: University of Pennsylvania

Grant Amount: $50,000.00

Start Date: 1/1/2011 **End Date:** 6/30/2012

Progress Report: 12 month

Report Due: 12/31/2011 **Report Received:** 12/30/2011

Recommended for Approval:

(Content of this report is not confidential. A grant sponsor's CHF Health Liaison may request the confidential scientific report submitted by the investigator by contacting the CHF office. The below Report to Grant Sponsors from Investigator can be used in communications with your club members.)

Original Project Description:

In 1997 the Soft-coated Wheaten Terrier (SCWT) Club of America helped us start an Open Registry which lists dogs affected with familial diseases common to this breed such as inflammatory bowel disease (IBD), protein-losing enteropathy (PLE), protein-losing nephropathy (PLN), combination PLE/PLN, Addison's disease (AD), and juvenile renal disease/renal dysplasia (JRD). The 2009 update lists almost 1000 affected dogs, with the vast majority affected with PLN, PLE, or PLE/PLN, in that order. These protein-losing diseases have had a devastating impact on the SCWT breed because: 1) there are no predictive tests, just annual screening tests, 2) there is no age limit, so dogs might be used for breeding before they show illness, and 3) the mode of inheritance is unknown and appears complex. The PennVet SCWT DNA Bank contains more than 500 blood or tissue samples from affected dogs as well as geriatric (14 years or older) non-affected Wheatens. Most affected samples are from confirmed PLE and/or PLN cases, with diagnosis documented by blood, urine, and histopathology test results.

The proposed study will utilize SNP chip and genome-wide association analysis to identify chromosomal regions that are associated with these serious diseases. Further testing (fine-mapping) of regions of interest may then reveal specific mutations, deletions, or insertions, which could be used to identify carriers of disease-predisposing alleles. We also hope to learn more about the pathogenesis of these diseases (immune-dysregulation vs. structural/functional abnormalities) which may help Wheatens, other breeds of dogs, and humans with these diseases.

Grant Objectives:
Objective 1: To perform whole genome genotyping and association analysis using DNA from SCWT dogs affected with protein-losing enteropathy (PLE), protein-losing nephropathy (PLN), or with both PLE/PLN, and from geriatric SCWT control dogs

Objective 2: To examine mapped predisposing loci for candidate genes known to be involved in similar human diseases and perform fine-mapping studies

Objective 3: To examine the renal lesions of SCWT PLN more extensively, by electron microscopy (EM), immunofluorescence (IF), and thin section light microscopy (LM).

Publications:
Wiley CA, Littman MP, Raducha MG, Henthorn PS. Genome-wide Association Study of Protein-losing Nephropathy in Soft-coated Wheaten Terriers. 5th Tufts Canine and Feline Breeding and Genetics Conference. 2011.

Report to Grant Sponsor from Investigator:
Protein-losing nephropathy (PLN) affects 5-15% of Soft-coated Wheaten Terriers (SCWT). The SCWT Open Registry, which lists hundreds of SCWT with PLN diagnosed since 1997, shows no limitation for age of onset nor evidence of predictive biologic markers. The mode of inheritance appears complex.

Samples from the PennVet SCWT DNA Bank were used in a genome-wide association study (GWAS) using 177,000 single nucleotide polymorphisms (SNPs), of which 81,097 SNPs were informative. Disease status was defined by blood, urine, and histopathologic criteria. Because the average age of onset for PLN is 7.1 years, control dogs were unaffected SCWT aged 14-18 years. The GWAS showed strongest support for association of PLN to a locus on chromosome 1 that contains two significant candidate genes encoding the podocyte slit diaphragm proteins nephrin and Neph3 (filtrin). DNA sequencing of the genes encoding these proteins identified a novel canine SNP in the nephrin gene (NPHS1) changing a glycine to arginine in the nephrin protein that is associated with PLN-affected SCWT. The gene encoding canine Neph3 (KIRREL2) contains a novel SNP responsible for a proline to arginine substitution in the Neph3 protein, also associated with PLN.

DNA samples of 753 dogs representing 114 other breeds were assayed for the NPHS1 SNP using an MspA1I restriction enzyme digest. The KIRREL2 SNP in 190 dogs of other breeds was analyzed through sequencing. One bloodhound was heterozygous at only the KIRREL2 SNP. Only 1 dog, an Airedale Terrier, was heterozygous for both polymorphisms. The only dog homozygous at both SNPs was also an Airedale and had been diagnosed with PLN. Eight additional PLN-affected dogs of other breeds lacked the novel alleles.

Both nephrin and Neph3 are found in the podocyte slit diaphragm, a fundamental component of the glomerular filtration barrier in the kidney. Mutations in nephrin have been associated with PLN in humans. While no mutations in Neph3 have been identified, decreased glomerular expression of Neph3 has been observed in humans with PLN. The amino acid changes caused by these SNPs may have pathologic effects on the glomerular filtration barrier and warrant further investigation. In addition, these studies indicate that the availability of DNA-based tests for these SNPs could lead to a decrease in the incidence of PLN in the SCWT breed through selective breeding.

GRANT PROGRESS REPORT REVIEW

Grant: 00945: *Mucosal Gene Expression Profiles in Canine Inflammatory Bowel Disease*

Principal Investigator: Dr. Albert E. Jergens, DVM, PhD

Research Institution: Iowa State University

Grant Amount: $60,000.00

Start Date: 6/1/2008 **End Date:** 12/31/2011

Progress Report: 36 month

Report Due: 6/30/2011 **Report Received:** 10/3/2011

Recommended for Approval: Approved

(Content of this report is not confidential. A grant sponsor's CHF Health Liaison may request the confidential scientific report submitted by the investigator by contacting the CHF office. The below Report to Grant Sponsors from Investigator can be used in communications with your club members.)

Original Project Description:

Background: Canine inflammatory bowel disease (IBD) is a chronic intestinal disorder likely resulting from the interaction between genes and environmental factors. While it is generally accepted that luminal bacteria play a critical role in provoking gut inflammation, genetic factors may also contribute to the bacterial-driven inflammatory response. Several susceptibility genes, such as NOD2/CARD15, have recently been identified in humans with IBD and provide a basis for the development of aberrant immune responses to bacteria in certain individuals. It is reasonable to hypothesize that susceptibility genes also affect clinical disease in dogs with IBD by negatively affecting the interaction with intestinal bacteria and/or their products. Genetic factors are thought to contribute to the pathogenesis of canine IBD as in humans. A role for luminal bacteria is suggested by observations that antibiotics reduce clinical signs, and by reports of increased bacterial numbers in intestinal biopsy specimens obtained from dogs with IBD. Given the recognized breed predispositions, genetic susceptibility to IBD is also likely, although studies are lacking.

Objective: The researchers are utilizing unique molecular biology tools to: (1) identify key genetic factors contributing to disease expression, (2) characterize gene expression profiles which may predict responsiveness to specific therapies, and (3) provide the framework upon which to facilitate identification of IBD susceptibility genes that predispose specific canine breeds to clinical disease.

Grant Objectives:
Hypothesis: Gene expression profiles in intestinal tissue samples of dogs with IBD will provide comprehensive insight into altered gene expression patterns contributing to gut inflammation.

Objective 1: To investigate global gene expression patterns of inflamed intestinal tissues and normal control intestinal tissue using RNA microarrays. The differentially expressed transcripts will identify patterns associated with inflammation and host immune responses.

Objective 2: To utilize quantitative RT-PCR to confirm microarray data and validate unique gene expression signatures in dogs with IBD.

Objective 3: Evaluate the clinical, microbiologic, and anti-inflammatory effects of FOS administration in dogs with IBD. (Appended Objective)

Publications:
- Suchodolski, Js, Xenoulis, Pg, Paddock, Cg, Steiner, Jm and Jergens, Ae (2010) Molecular analysis of the bacterial microbiota in duodenal biopsies from dogs with idiopathic inflammatory bowel disease. Veterinary Microbiology. 142, 394-400.
http://www.sciencedirect.com/science/article/B6TD6-4XNF6FB-1/2/0563bf86e9fc8c4da7851cf9654ac8dd

Report to Grant Sponsor from Investigator:
The objective of this study is to characterize mucosal gene expression patterns mediating intestinal inflammation in dogs with chronic enteropathy (CE). Eighteen dogs diagnosed with CE and 6 healthy control (HC) dogs were evaluated. Mucosal biopsies of the small intestine were obtained endoscopically from all dogs. Disease severity in CE dogs was defined and stratified based on clinical (CIBDAI scores) and histologic findings. Total RNA extracted from intestinal biopsies was analyzed using Affymetrix GeneChip Canine Genome 2.0 arrays comprising 43,000 probe sets. Gene expression profiles were compared between HC and CE dogs. Quantitative RT-PCR was used to confirm the microarray data. Results indicate that a total of 1875 transcripts were differentially expressed between CE and HC dogs. 1582 (85%) transcripts in CE dogs were down regulated which have been implicated in the pathogenesis of human inflammatory bowel disease (IBD). Another 293 transcripts were up-regulated and included genes related to extracellular matrix degradation, inflammation, iron transport, and immunity. CE dogs with protein-losing enteropathy (PLE) showed the greatest number of differentially expressed marker genes as compared to less severely diseased dogs. Gene expression patterns were confirmed with qRT-PCR studies. The coordinated activity of multiple genes regulating mucosal inflammation is markedly altered in dogs with CE and varies by disease phenotype. Moreover, the differential expression of select genes suggests that the molecular pathogenesis of canine CE bears resemblance to chronic intestinal inflammation in human IBD.