**RNA sequencing of whole blood from dogs with and without spontaneous calcium oxalate kidney stones reveals association with natural killer cell immunity**

Baker, Lauren A.[1], Furrow, Eva[2]

[1] School of Veterinary Medicine, University of Wisconsin-Madison, Madison, WI, USA.
[2]Department of Veterinary Clinical Sciences, College of Veterinary Medicine, University of Minnesota, St. Paul, MN, USA.

**Introduction:** Genetic predisposition plays an important role in the development of calcium oxalate (CaOx) kidney stones. Increasing evidence suggests the pathophysiology underlying the genetic basis of complex traits, including stone disease, lies in transcriptional regulation of core genes that drive disease processes. Genetic drivers of stone disease may be better characterized through RNA sequencing (RNA-Seq), which measures all genes expressed in the tissue at the time of sample collection. Miniature Schnauzer dogs are genetically predisposed to spontaneous CaOx stones and have less genetic variability than humans, which may emphasize differences between cases and controls. In this study, we performed whole blood RNA-Seq to identify RNA biomarkers of kidney stone disease in the Miniature Schnauzer model through differential expression and co-expression network analyses.
**Methods:** Peripheral blood RNA from N=43 dogs (20 cases, 23 controls) was processed and sequenced through the University of Minnesota Genomics Center. Trimmed reads were aligned to the canFam6 reference assembly using HISAT2. Low count genes were removed and differential expression analysis was performed using the DESeq2 R package. Variance stabilized gene counts were used to calculate weighted co-expression networks in the WGCNA R package, which describes correlation patterns between genes across samples. WGCNA groups correlated genes into modules, which may represent biological pathways. The module-eigengene is a measure of combined gene expression within a module. Module-trait relationships were estimated using the correlation between each module eigengene and clinical traits: age, sex, case-control status, stone recurrence, blood ionized calcium, urine calcium:creatinine, and serum triglycerides. Pathway analysis for significant modules was performed using the statistical overrepresentation test (Fisher's exact test) within the PANTHER classification system and the GO Biological Process Complete annotation data set. For all analyses, FDR<0.05 was considered significant.
**Results:** After removing low-count genes, 14,913 genes remained for analysis. No genes reached statistical significance for differential expression between cases and controls. WGCNA identified 20 modules of varying size. A single module containing 172 genes was significantly correlated with case-control status (cor=0.39, *P*= 0.01). This module was also correlated with age (cor=0.37, *P*= 0.01), and blood ionized calcium (iCa) (cor=0.39, *P*= 0.01). Genes in this module were also correlated with CaOx status (cor=0.46, P= 2.2E-10). Pathway analysis indicated this module may be representative of an immune process, specifically positive regulation of natural killer (NK) cell-mediated cytotoxicity (FDR= 0.02).
**Conclusion:** Though no genes met significance thresholds individually, network analysis, which considers gene expression in combination, identified a cluster of genes correlated with case-control status. Top genes were connected to NK cell immunity, indicating that inflammatory processes are upregulated in peripheral blood of CaOx stone forming dogs. It is unclear whether this is a cause or consequence of stone disease. A comparative analysis of RNA from human CaOx stone patients and controls is underway.
**Research Area:** Nephrolithiasis