Introduction:
Osteoarthritis (OA) is a debilitating and progressive disease which results in the erosion and loss of articular cartilage. Because early diagnosis and treatment can slow down the progression of OA, and improve the quality of life in affected individuals, the earliest stages of OA need to be fully characterized. We previously reported gene expression changes occurring at 2 weeks after induction of OA in dogs’ knees (Stoker, ORS 2005). In the present study, the canine Pond-Nuki anterior cruciate ligament transection (ACL-X) model was used to study the regional changes in gene expression at 4 weeks after induction of OA. Cartilage tissue was harvested from defined regions on the femoral condyles (FC) and tibial plateau (TP) to determine the site-specific effects of ACL-X on articular cartilage (AC) of the knee during development of OA.

Methods
Pond-Nuki Model: The ACL in one knee (stifle) of three adult mongrel dogs (mean weight 26.2 kg) was completely transected (ACL-X) through a lateral parapatellar arthrotomy. After recovery from surgery, the dogs were allowed unrestricted use of the limb. The contralateral knee of each dog was used as the nonoperated control. After four weeks, the dogs were euthanatized and evaluated.

Tissue harvest: Separate full-thickness cartilage samples were collected from the cranial medial femoral condyle (CrMF), cranial lateral femoral condyle (CrLF), cranial lateral tibial plateau (CrLT), cranial medial tibial plateau (CrMT), caudal lateral tibial plateau (CaLT), cranial medial tibial plateau (CaMT), cranial lateral femoral condyle (CaLF), cranial lateral tibial plateau (CaLT), and caudal lateral tibial plateau (CaLT) of both knees and used for gene expression and biochemical evaluation.

RNA extraction: Total RNA was extracted using the Trispin method, and stored at -80°C until used for gene expression analysis.

Real time RT-PCR: Real time RT-PCR analysis was performed on total RNA (1µg) to determine the relative gene expression levels of collagen 1 and 2 (COL 1, 2), aggrecan (Agg), tissue inhibitors of metalloproteinases 1 and 2 (TIMP-1, -2), matrix metalloproteinases 1, 2, 3, 9, and 13 (MMP-1, -2, -3, -9, -13), aggreganases 1 and 2 (ADAMTS 4, 5), inducible nitric oxide synthase (INOS), and cyclooxygenase 2 (COX-2) compared to the housekeeping gene GAPDH using the QuantiTect SYBR Green PCR master mix.

Biochemical analysis: Total sulfated GAG content was determined using the dimethylmethylene blue (DMMB) assay. Total collagen content was determined by measuring hydroxyproline (HP) content.

Statistical Analysis: Relative expression levels for the genes studied were determined using QGene. Significant differences in gene expression levels between groups were determined using the relative expression statistical tool, REST-XL. Biochemical data were analyzed by ANOVA with significance at p<0.05.

Results
Biochemical and Histological: The CaMT from the ACL-X knee had significantly lower GAG content compared to contralateral limb, and the CaMF and CrMF had near significant reductions in GAG content in the ACL-X compared to the contralateral control.

Gene Expression (Figure 1): Significant differences in gene expression between ACL-X and control knees were observed in every region analyzed, and each region exhibited a unique gene expression profile. Aggrecan and TIMP-2 were the only genes analyzed that were downregulated in cartilage from the ACL-X knee compared to the control knee.

Figure 1: Differential gene expression in ACL-X compared to control articular cartilage by region. Normal font indicates significant increase in gene expression in the ACL-X tissues compared to normal tissues, while italics indicate significant decrease in ACL-X tissue gene expression. Genes in blue were differentially regulated at 2 weeks in that region. Darker shading in a region indicates that a larger number of genes showed differential expression in that region.

Discussion
The regional biochemical and gene expression data from this study correlate well to the patterns observed two weeks post-ACL-X (Stoker, ORS 2005). The major differences in gene expression in the articular cartilage at week 4 compared to week 2 involved synthetic (Col 1, Col 2), degradative (MMP13, ADAMTS 5), and anti-degradative (TIMPS 1 and 2) processes. The changes seen in the regulation of the genes at 4 weeks suggest that cells in the articular cartilage are further progressing in phenotype toward degradation and that differential gene expression noted at 2 weeks was indicative of these changes. In addition, COX-2 was no longer differentially expressed in the ACL-X cartilage compared to contralateral controls indicating that the upregulation of this gene seen at 2 weeks may have been associated with surgery-induced inflammatory changes. However, this early, apparently transient change in gene expression associated with inflammation may still be an important marker for early OA and should be considered further. Ongoing studies in our laboratory include sham operated and alternative insult controls in order to help determine the significance of gene expression changes associated with inflammation. Biochemical data appear to validate the molecular data in that the decreases in GAG content in the CaMT and medial femoral condyle correspond to regions exhibiting increased ADAMTS 5 and decreased Aggrecan gene expression in the ACL-X knee. Taken together, these data indicate that the canine ACL-X model is useful for investigating changes in articular cartilage gene expression that may serve as markers for early changes in OA. Current work in our laboratory is aimed at further assessing the usefulness of gene expression changes in articular cartilage and whole blood, as well as synovial fluid and serum protein biomarkers for predicting and diagnosing OA through correlation to biochemical, histologic, and clinical measures of disease.