findings may provide translational opportunities for novel fibroid treatments.

431

## Defective uromodulin polymerization and peptide excretion in a natural canine model of kidney stones

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OBJECTIVES/GOALS: Using a natural canine model of kidney stone disease, we previously identified a pathogenic variant in the uromodulin gene (UMOD) that imparts a dramatic risk for calcium oxalate (CaOx) stones. This study was designed to characterize the effects of the pathogenic variant on uromodulin processing, specifically polymerization and peptide excretion. METHODS/STUDY POPULATION: Uromodulin polymerization status and peptides were measured in random urine samples from CaOx stone-forming dogs with the pathogenic UMOD variant and breed-, sex-, and age-matched healthy control dogs. Polymerization status was determined using an ultracentrifugation protocol and Western blotting in 6 CaOx cases and 3 controls; relative abundance of the polymerizing and nonpolymerizing forms was evaluated. Uromodulin peptide abundances were measured by LC-MS/MS with 4 dogs per group; results were summed to determine total uromodulin peptide excretion for each dog, and individual peptide abundances were calculated as a percentage of the total. Polymerization status and peptides were compared between groups. RESULTS/ANTICIPATED RESULTS: Dogs with the pathogenic UMOD variant had abnormalities in both uromodulin polymerization and peptide processing. The polymerization data showed that the polymerizing form of uromodulin was abundant in all healthy controls but absent or severely reduced in most dogs with the variant. In contrast, nonpolymerizing uromodulin was detected in all dogs with no observed difference between those with and without the variant. The peptidomics data showed that stone-forming dogs with the pathogenic UMOD variant lacked a peptide cleavage site, resulting in the loss of two common peptides that terminate at that site and the presence of longer peptides that span the site. DISCUSSION/SIGNIFICANCE: These findings implicate uromodulin polymerization and peptide processing defects in kidney stone risk. Future studies will define the mechanisms through which these defects affect stone formation, ultimately informing development of novel preventative therapies.

432

## Ligament Engagement and In-Situ Force During Multiplanar Loading of the Medial Knee Ligaments

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OBJECTIVES/GOALS: Load sharing across the arc of knee flexion of the medial knee ligaments (MKLs) is not well understood. The goal of this research is to characterize ligament engagement and in-situ force within the deep and superficial medial collateral ligament (dMCL, sMCL) and the posterior oblique ligament (POL) in response to externally applied multiplanar loads. METHODS/STUDY POPULATION: Ten human cadaveric knees, 5 male and 5 female, age 32±7 (25-42) [mean±SD (range min-max)] years, were mounted to a force sensor and a 6-degree-of-freedom robotic arm. Knee kinematics, before and after serial dissection of the sMCL, dMCL, and POL, were recorded from 0-30 degrees during applied isolated external rotation, valgus angulation, and anterior tibial moments, and the force (Newtons, N) borne by each structure was measured via the principle of superposition. Loads in the dMCL, sMCL, and POL will be compared across each knee and at each flexion angle with paired t-tests and repeated-measures analysis of variance with Tukey post hoc testing. Ten knees will provide >99% power to detect differences of 5N ± 3% at p=0.05, which is considered the threshold for clinically meaningful force differences. RESULTS/ANTICIPATED RESULTS: Our anticipated results include characterization of the means and standard deviations of the in-situ forces within the dMCL, sMCL, and POL in response to externally applied valgus angulation, tibial external rotation, and anterior-directed tibial loading at 0, 15, and 30 degrees of knee flexion. Our statistical analysis will determine if there are clinically meaningful differences (5N ± 3%) in the loads within each ligament at different knee flexion angles and will also provide data regarding differential relative ligament engagement for each applied force scenario, which is an indication of the percentage of contribution that each structure contributes to knee stability during application of forces and torques to the knee. DISCUSSION/SIGNIFICANCE: Data on ligament engagement and in-situ forces will help clinicians better diagnose potentially injured ligaments when they observe pathological knee laxity in an injured patient. Our results will also inform future computer modeling studies on injury mechanisms, individual anatomical variability, and surgical planning.

434

## Investigating the metabolic-inflammatory mechanisms of cachexia symptoms in head and neck cancer patient plasma via multiomics integration of the metabolome, lipidome, and inflammation cytokines

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OBJECTIVES/GOALS: Cachexia is the involuntary and irreversible loss of muscle and fat and is a major cause of morbidity and mortality in head and neck cancer (HNC). It remains a poorly understood disease diagnosed by weight loss and a confluence of symptoms. We explored the metabolic and inflammatory mechanisms of cachexia symptoms via an multiomics network algorithm. METHODS/STUDY POPULATION: Prior to chemoradiotherapy, HNC subjects completed questionnaires and donated blood for untargeted (metabolites) and targeted (lipids and cytokines) assays. Metabolites and lipids were measured by liquid chromatography mass spectrometry. Cytokines were measured by multiplex assays. We plotted a multiomics network graph by estimating partial least squares correlations amongst metabolites, lipids, cytokines, and common cachexia symptoms—max percent weight loss over 1 year, baseline BMI, fatigue, performance, albumin, hemoglobin, and white blood cell count. To interpret the network, an algorithm identified highly correlated clusters of metabolites-lipids-cytokines-symptoms representing possible biological relatedness, which were functionally annotated via metabolic enrichment analysis. RESULTS/ANTICIPATED RESULTS: In 123 subjects (59 years of age, 72% male, 84% white, avg weight loss of 13%), we analyzed 186 metabolites, 54 lipids, 7 cytokines and 7 cachexia symptoms. We required a correlation


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