anaerobic colonic fermentation using human feces. FV fermenta will be incubated with Caco2 monolayers to measure in vitro cell permeability and protein levels of cellular tight junction, metabolic, and HIF signaling enzymes. To examine their effects in vivo, FVs identified to modulate in vitro barrier function, will be fed (5% freeze dried powder) to wild-type mice and the above parameters will be examined. If in vivo effects are found, intestinal specific HIF knockout mice will be used to examine the role of HIF signaling in mediating these effects. RESULTS/ANTICIPATED RESULTS: We expect that fermenta derived from human milk and FVs will reduce in vitro gut permeability in Caco2 monolayers by increasing gene and protein expression of the HIF signaling complex relative to fermenta of human milk alone. This will be reflected with higher cellular transepithelial resistance and greater expression levels of tight junction proteins. We expect FV powder consumption will similarly increase in vivo gut permeability and expression of related genes in mice as compared to mice fed diets without FVs. As we expect an increase in HIF signaling in the colon, we expect that FV powder consumption will not enhance in vivo gut permeability in mice colons with an intestinal specific knockout of HIF. DISCUSSION/SIGNIFICANCE: Data from this study will provide mechanistic evidence to help clinicians promote relevant FVs recommendations for Latin American infants and families. Due to the link between gut permeability and obesity, our next step will be to conduct a dietary intervention in this population.

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Spatial Investigation of the Extracellular Matrix Metastatic Niche in Invasive Breast Cancer by Mass Spectrometry Imaging*

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OBJECTIVES/GOALS: Metastasis to regional areas decreases invasive breast cancer (IBC) survival rate by 13%. Despite the clinical importance of lymph node involvement, the role of extracellular matrix (ECM) remodeling in metastases is unknown. We hypothesize that the spatial dysregulation of the collagen proteome facilitates pro-tumorigenic immune infiltration. METHODS/STUDY POPULATION: Lymph node metastases were compared to patient-matched primary tumor and normal lymph nodes using tissue microarrays (TMA) from 31 generational South Carolina women with IBC (black women, BW n=10, white women, WW n=21) and lumpectomies from 5 triple-negative breast cancer (TNBC) patients (BW n=3; WW n=2) by ECM-targeted mass spectrometry imaging. RESULTS/ANTICIPATED RESULTS: Between metastatic and normal lymph nodes, 10% of peptides, primarily from fibrillar collagens, were significantly different by area under the receiver operating curve (AUROC>70%; p-value< 0.01) within the TMAs. In a subsequent preliminary study of the TNBC metastatic niche, a segmentation analysis of 152 putatively identified peptides and 117,909 pixels revealed 10 uniquely localized proteomic groups. 12 peptides were found to have significantly decreased relative peak intensities in lymph node metastases compared to the primary tumor and normal lymph nodes by a one-way ANOVA test (p< 0.05). 7 peptides could discriminate between metastatic and normal lymph nodes, while 22 peptides could discriminate between metastatic lymph nodes and the primary tumor (AUROC>0.70; p-value < 0.05). DISCUSSION/SIGNIFICANCE: Our preliminary interrogation highlights emerging differences between lymph node metastases, the primary tumor, and normal lymph nodes. Future work is needed to connect these discrete ECM proteomes to immune infiltration alterations, which could contribute to disparate patient outcomes.

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Genome-wide meta-analysis identifies novel risk loci for uterine fibroids across multiple ancestry groups*

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OBJECTIVES/GOALS: Uterine fibroids are benign tumors of the uterus with a high disease prevalence and burden, yet there are few multi-ancestry genetic studies. This is the largest and most diverse fibroid GWAS to-date. Our goal is to identify novel genetic variants and gene expression pathways associated with fibroids and characterize their biological relevance. METHODS/STUDY POPULATION: We performed a cross-ancestry meta-analysis of GWAS summary statistics from eight datasets. The total sample size was 74,294 cases and 465,810 controls with participants of European (80% of sample), African (4%), East Asian, and Central South Asian (16%) ancestry. We mapped variants to genes with OpenTarget Genetics and used Functional Mapping and Annotation to conduct tissue expression gene-set enrichment and identify lead variants. We used S-PrediXcan to estimate genetically predicted gene expression (GPGE) associated with fibroid risk. This was with models that predicted gene expression across 49 different tissue types. Ingenuity Pathway Analysis compiled significant GPGE genes and their weights with a scientific literature database to identify overlapping pathways. RESULTS/ANTICIPATED RESULTS: We identified 370 independent significant variants. Among these, we identified variants mapped to three novel genes (PAX2, VIP, FOXO3) and eight genes not previously validated (TEKT1, SLC16A11, RPEL1, RASL11B, ASGR1, SLC12A7, TTC28, POLR2A). Many loci have roles in cell cycle regulation or are associated with fibroid risk factors like blood pressure, BMI, and vitamin D levels. Loci were significantly enriched in DNA damage and cell cycle pathways. Of 588 significant predicted expression gene-tissue pairs, 173 unique genes were novel fibroid associations. These genes are also associated with cancers, estradiol, and endometriosis. Top enriched pathways included p53 signaling, HOTAIR, BRCA1DNA damage response, and pulmonary fibrosis signaling. In uterine tissue there were 15 novel GPGE associations. DISCUSSION/SIGNIFICANCE: Using this large and diverse data, we identified novel loci associated with fibroids that are enriched in hormone-response, DNA damage, and cell-cycle pathways. GPGE loci were in tumorigenesis and fibrosis pathways. These novel genetic loci and uterine gene expression

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findings may provide translational opportunities for novel fibroid treatments.

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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.

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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.

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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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