67df7653-0cd8-4e8e-a3e1-d5c565b19dce] DISCUSSION/SIGNIFICANCE: As SOC patients with CLE have significant potential for permanent pigmentary alternations, early treatment is imperative. Effective treatments for refractory CLE are elusive. Our study represents the largest single-center cohort of CLE patients treated with anifrolumab and suggests that it is a promising therapeutic option for patients with SOC.

426

A Beat Away from Precision Medicine: Characterizing Human Cardiac Fibroblast Responsiveness to Hemodynamic Unloading in Heart Failure with Reduced Ejection Fraction*

Rachel Biggs, Daniel N. Silverman, Yuhua Zhang, Catalin F. Baicu, Michael R. Zile and Amy D. Bradshaw
Medical University of South Carolina

OBJECTIVES/GOALS: Myocardial interstitial fibrosis leads to high hemodynamic load resulting in heart failure (HFrEF). Previous studies show that treatment with a left ventricular assist device (LVAD) does not reduce fibrosis. We hypothesize that human cardiac fibroblasts are highly activated in HFrEF and remain unresponsive to hemodynamic unloading by LVAD. METHODS/STUDY POPULATION: Forty human subjects with HFrEF undergoing LVAD implantation were enrolled to provide a portion of myocardium routinely removed during LVAD placement. In addition, 7 biopsies previously collected from transplanted hearts with extended LVAD treatment were also evaluated (LVEX). RESULTS/ANTICIPATED RESULTS: Quantification of PSR-stained sections reveals a significant increase in collagen content in the HFrEF tissue (CVF = 2.8) compared to control tissues (CVF = 0.9) that remained elevated in LVEX hearts (CVF = 3.1). HCFs from LV biopsies were isolated and grown to confluence. HCFs from HFrEF patients and control HCFs were plated on substrates with stiffnesses reflective of normal myocardium (2kPa) or HFrEF myocardium (8kPa). Cells were collected at 4- and 7-day time points and levels of collagen I and alpha-smooth muscle actin were quantified by western blot analysis. Control HCFs were responsive to changes in substrate stiffness producing more Col I and a-SMA on 8kPa versus 2kPa, HCFs from HFrEF patients were unresponsive to changes in stiffness exhibiting no significant difference in protein production on 2 vs. 8kPa. DISCUSSION/SIGNIFICANCE: Our data suggests that HCFs isolated from the failing myocardium do not respond to changes in mechanical load and might contribute to persistent increases in fibrosis. These findings bring us one step closer to elucidating mechanisms behind fibrosis in HFrEF which could lead to targeted therapies to improve patient outcomes from LVAD support.

427

Defining the Impact of the Fecal Microbiome and Secretome on Multiple System Atrophy and α-Synuclein Aggregation

Michelle K. Bland[1], Wolfgang Singer[2] and Marina R. S. Walther-António[3]
[1]Mayo Clinic Graduate School of Biomedical Sciences, Rochester, MN; [2]Mayo Clinic Department of Neurology, Rochester, MN and [3]Mayo Clinic Department of Surgery, Department of Obstetrics and Gynecology, Microbiomics Program, Center for Individualized Medicine, Rochester, MN

OBJECTIVES/GOALS: Aim 1: We will determine whether temporal changes in the fecal microbiome signature correlate with a clinical multiple system atrophy (MSA) phenotype. Aim 2: We will evaluate whether secretomes cultured from fecal samples from MSA patients enhance intracellular and extracellular α-synuclein (αSyn) aggregation using in vitro functional assays. METHODS/STUDY POPULATION: Aim 1: Gut microbiome profiling will be performed by 16S rRNA gene sequencing, tandem mass spectrometry for expression proteomics, and targeted metabolomics in fecal samples from 30 MSA cases matched to 30 healthy controls, a Parkinson's disease comparison group, and household controls. Aim 2: Microbial species will be isolated using dilution-to-extinction on MSA fecal samples and then will be cultured to obtain secretomes. To assess the effect of MSA fecal secretomes on αSyn aggregation, culture media from microbial isolates will be used in fluorescence resonance energy transfer (FRET) assays and luciferase reporter assays, both modified to measure αSyn aggregation. Positive tests will undergo expanded metagenomic characterization of the microbes and secretome to identify potential causative agent(s). RESULTS/ANTICIPATED RESULTS: Based on cross-sectional metagenomic studies on MSA, MSA cases are expected to have genus reductions in Blautia and Dorea (acetate production); Paraprevotella (succinic and acetic acid production); and Ruminococcus, Coprococcus, and Faecalibacterium (butyrate production). Increases in genus Bacteroides (clinical pathogen) and Akkermansia (mucin degradation) and pro-inflammatory families Clostridiaceae and Rikenellaceae are also expected. MSA is predicted to be associated with reduced levels of short chain fatty acids and increased lipopolysaccharide. These microbial proteins and metabolites are anticipated to modulate intracellular and extracellular αSyn aggregation in vitro. Microbe isolation and secretome culturing methods are expected to identify additional drivers of αSyn aggregation. DISCUSSION/SIGNIFICANCE: This study's novel use of longitudinal sampling, household controls, and secretome culturing aim to develop a more comprehensive understanding of the complex interactions between the gut microbiome and MSA. The success of this work offers the potential for new insights into the impact of the gut microbiome and secretome on MSA and αSyn aggregation.

428

Promoting Infant Gut Barrier Development Through Culturally Relevant Adoption of Fruit and Vegetable Intake.

Brian D. Piccolo[1], David Keith Williams[1,2], Andrew P. Neilson[3], Jerry Simecka[4] and Mario G Ferruzzi[1,5]
[1]Arkansas Children's Nutrition Center; [2]Department of Biostatistics, University of Arkansas for Medical Sciences; [3]Plants for Human Health Institute, North Carolina State University; [4]Department of Pharmaceutical Sciences, University of North Texas Health Science Center and [5]Department of Pediatrics, University of Arkansas for Medical Sciences

OBJECTIVES/GOALS: To determine in vitro mechanisms by which fruits and vegetables (FV) contribute to colon barrier development in Latin American infants. We hypothesize that simulated colonic fermentation of FVs will stimulatein vitro cell barrier function by activating the hypoxia-inducible factor (HIF) pathway in colonocytes. METHODS/STUDY POPULATION: FVs consumed by US-based Latin American infants 6-12 months old (identified from NHANES-What We Eat in America Surveys) will be combined with human breast-milk samples from women self-identified as Hispanic or non-Hispanic, and then subjected to in vitro digestion and

https://doi.org/10.1017/cts.2024.370

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.

429

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

Taylor S Hulahan[1], Yeonhee Park[2], Laura Spruill[1], Hari Nakshatri[3], Marvella Ford[1] and Peggi M Angel[1]
[1]Medical University of South Carolina; [2]University of Wisconsin-Madison and [3]University of Indiana

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.

430

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

Jeewoo Kim[1], Ariel Williams[2], Hannah Noh[1], Megan M. Shuey[3,4], Todd L. Edwards[3], Digna R. Velez Edwards[3] and Jacklyn N. Hellwege[3]
[1]Vanderbilt University; [2]National Human Genome Research Institute; [3]Vanderbilt University Medical Center and [4]BWHS, Black Women's Health Study eMERGE, Electronic Medical Records and Genomics Network

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

https://doi.org/10.1017/cts.2024.370