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.

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

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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.

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

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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.

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

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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

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