Database, featuring high-resolution multi-contrast MRIs, and a comprehensive clinical, behavioral, and demographic dataset, we are developing a hierarchical learning-based software tool to compute maps correlating brain structure-function and individual cognitive function. Our MRI analysis employs a three-compartment model (NNLS>0.96). Functional scores are defined by individualized accuracy during the modified information processing speed task (e.g., m-SDMT). We utilize a Bayesian classifier with explicit Pearson's correlation for tissue classification (BF10>100) to compute an index of the likelihood of correlation with cognitive impairment throughout brain tissue. RESULTS/ANTICIPATED RESULTS: This approach allows us to reveal subtle cognitive changes and their potential links to myelin integrity, offering vital insights into disease progression and management. The m-SDMT strongly correlates with the standard SDMT ($r=0.79$, $p<0.001$), confirming reliability as a cognitive assessment tool in clinical and research contexts. Analysis of the COMS dataset emphasized insights into the role of fine myelin structure in MS patients' cognitive functionality. Our findings heightened the pivotal significance of myelin integrity in preserving cognitive abilities and identify disruptions in myelin synthesis and homeostasis as primary contributors to cognitive decline. This discovery stresses the critical role that specialized brain pathways, influenced by myelin integrity, play in the pathology of MS. DISCUSSION/SIGNIFICANCE: This development bridges advanced neuroimaging techniques with practical clinical applications, emphasizing the nuanced role of myelin integrity in MS-related cognitive deficits. Our findings advocate for a multidisciplinary approach to MS management, demanding collaborative workforce development and education in translational science.

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Deciphering the Immune Landscape in Benign Breast Disease: Implications for Risk Stratification and Breast Cancer Prevention

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OBJECTIVES/GOALS: The objective of our research is to define unique molecular and immune markers in benign breast tissue to better identify women at risk of node-positive breast cancer (BC). The goal of the work is to improve individualized risk assessment, to guide targeted prevention and screening recommendations, and to reduce disease incidence and mortality. METHODS/STUDY POPULATION: From the Mayo Clinic's Benign Breast Disease (BBD) cohort, we matched women who developed node-positive breast cancer after a BBD biopsy (cases; n=42) with women who remained cancer-free (controls; n=37), considering patient age and biopsy date. We used NanoString gene expression profiling to identify differentially expressed genes (DEGs) between cases and controls. We optimized a multiplex immunofluorescence (mIF) approach to simultaneously detect multiple markers within single FFPE tissue slides to correlate cells expressing DEGs in relation to innate and adaptive immune effectors. We used tissue segmentation, cell phenotyping, and spatial relationships to define molecular and immune differences between cases and controls. RESULTS/ANTICIPATED RESULTS: We discovered higher gene expression levels of IRF8 (interferon regulatory factor 8, a factor involved in immune cell differentiation) in controls as compared to cases ($p = 0.0024$) and found that IRF8 expression is also associated with longer cancer onset times among cases ($p = 0.0012$). Our pilot mIF experiments revealed higher frequencies of CD4+, CD8+, CD68+, CD20+ and CD11c+ cells in controls compared to cases. We predict that higher IRF8 expression and increased frequencies of immune cells in BBD biopsies indicate a proactive immune environment that may act to prevent cancer development. Furthermore, we predict that our analyses of the spatial localization of these markers by mIF may offer further predictive insight. DISCUSSION/SIGNIFICANCE: Deciphering the relationship between immune alterations in BBD and risk of node positive BC has the potential to improve individualized risk prediction. These insights will foster improved surveillance and informed screening and prevention, ultimately reducing BC incidence and mortality.

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Anifrolumab for the treatment of refractory cutaneous lupus erythematosus in patients: interim analysis of real-world outcomes

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OBJECTIVES/GOALS: * Patients with skin of color (SOC) are disproportionately affected by systemic lupus erythematosus (SLE) and cutaneous lupus erythematosus (CLE). In this study, we aim to address this disparity and characterize the real-world efficacy and tolerability of anifrolumab in CLE patients using validated disease activity instruments. METHODS/STUDY POPULATION: This single-center, prospective observational cohort study includes SLE patients with severe or refractory CLE who have received ≥ 1 dose of anifrolumab. Cutaneous disease activity is assessed periodically at 2, 6, 9, 12, and 18 months using the Cutaneous Lupus Disease Area and Severity Index (CLASI). Adverse events and concurrent treatments are also routinely evaluated. To date, 22 patients have been enrolled, with 6-month follow-up data available for 15. At the time of anifrolumab initiation, 95% of participants had discoid LE (DLE), 60% had mucosal DLE, and 13% had subacute CLE. Nine patients identified as SOC, two as White, and four did not report race/ethnicity. RESULTS/ANTICIPATED RESULTS: A Friedman test showed statistically significant changes over time in CLASI activity score (CLASI-A) ($\chi^2(2) = 20$, $p<0.0001$) (Figure 1) and CLASI damage score (CLASI-D) ($\chi^2(2) = 9.5789$, $p=0.0083$) (Figure. To estimate effect sizes, we employed linear mixed models, which demonstrated statistically significant reductions in the CLASI-A score from baseline by an average of 14 points at 2 months ($p<0.001$) and 18 points at 6 months ($p<0.001$); notably, a reduction in CLASI-A of 4 is considered clinically meaningful. At 2 months, 20% of patients experienced a 50% or more reduction in CLASI, which increased to 60% of patients at 6 months. Patients on systemic corticosteroids could taper off. Adverse events were minimal and did not lead to treatment discontinuation. Fig. 1:[blob:https://acts.slayte.com/045319b4-7272-4351-a771-78ba9ee57f5c] Fig. 2:[blob:https://acts.slayte.com/

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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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