

α Syn aggregation in vitro and in vivo. From these studies, lipopolysaccharide and bacterial amyloid protein are expected to increase α Syn oligomerization, while short-chain fatty acids, such as butyrate, are expected to interfere with and decrease oligomerization. As a complementary systemic approach, this study's agnostic methods involving MSA stool culture combined with the proposed dilution-to-extinction method are expected to identify additional MSA stool secretome components modulating α Syn oligomerization that might otherwise be missed in earlier reductionist approaches. DISCUSSION/SIGNIFICANCE OF IMPACT: Completion of this reverse-translational work will aid in discovering MSA stool secretome components modulating α Syn oligomerization. Identification of specific factors contributing to pathologic α Syn behavior might set the stage for patient screenings for identified stool markers and could lead to microbiome-based interventions for MSA.

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The effect of short- and long-term diets on mechanisms of healthy brain aging: A protocol

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OBJECTIVES/GOALS: The second highest fear of the aging population is cognitive decline. Diet is associated with brain aging; therefore, the objective is to determine the effects of a Western diet (WD) on cognitive decline and the efficacy of a Mediterranean diet (MeDi) fecal microbiota transplant (FMT) in WD-induced cognitive deficit progression in aged rats. METHODS/STUDY POPULATION: For Study 1, 12-month-old Fischer344 rats (NIA Aging Colony) will be randomly assigned to a WD, MeDi, or control (positive control) for 6 or 12 months. Microbiota composition, blood pressure, and body composition (DXA Scan) will be longitudinally assessed. Groups will undergo a battery of neurobehavioral assessments to measure cognitive performance. At the end of the study, mitochondria bioenergetic assays in isolated cerebral microvessels will be used to determine changes in cerebrovascular function. For Study 2, 18-month-old Fischer344 rats (NIA Aging Colony) will be randomly assigned to a WD, MeDi, or control for 6 months. At month 4, the WD+ MeDi-FMT group will receive once weekly MeDi-FMT for two months. Assessments will be performed as described in Study 1. RESULTS/ANTICIPATED RESULTS: It is anticipated that the WD-related gut dysbiosis will increase blood pressure, fat-free mass, neurovascular dysfunction, and induce cognitive impairment relative to a MeDi. When using a MeDi-FMT as an intervention, it is anticipated that there will be measurable improvements in cognitive function relative to a WD through the regulation of gut dysbiosis, blood pressure, fat-free mass, and neurovascular dysfunction. DISCUSSION/SIGNIFICANCE OF IMPACT: These results are expected to have an important positive impact because they will provide insights into the WD-induced gut dysbiosis-associated cognitive impairments, and evaluate the roles and mechanisms of MeDi-FMT in the therapeutic intervention of aged rats.

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Bacterial dysbiosis and its association with pancreatic cancer progression and poor survival

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OBJECTIVES/GOALS: Bacterial dysbiosis has emerged as an accomplice in the progression of many cancers. The pancreas microbiome changes in pancreatic cancer patients. The mechanisms via which components of the microbiome regulate tumor growth is unclear. We seek to determine if bacterial dysbiosis influences cancer cell behavior thereby promoting tumor progression. METHODS/STUDY POPULATION: We performed immunohistochemistry for lipopolysaccharide and observed bacteria preferentially located in close proximity to cancer cells. We utilized an in vitro cell culture system and in vivo mouse models, in the presence and absence of gut bacteria, to assess the effect of bacteria and bacterial metabolites on pro-tumorigenic signaling and transcriptional changes in the cancer cell. We analyzed cancer cells and epithelial cells using RNA sequencing, flow cytometry, and enzyme-linked immunosorbent assay. We also used targeted metabolomics to identify bacterial and cancer cell produced metabolites. RESULTS/ANTICIPATED RESULTS: We found microbial dysbiosis can induce proliferation, an inflammatory response and an increase in tryptophan metabolism via the kynurenine pathway in the pancreatic cancer cell. Along with upregulated expression of IDO1 in vivo, we observe an increase in nicotinic adenine mononucleotide. Also, we observe an increase in nicotinic acid in vitro and nicotinic adenine dinucleotide within the cancer cell compartment in the presence of bacteria and bacteria conditioned media. Due to the critical role in many vital pathways of cell survival, NAD⁺ production is thought to play a significant role in cancer progression. Nicotinic acid can stimulate NAD production to protect cells from cell death. DISCUSSION/SIGNIFICANCE OF IMPACT: Pancreatic cancer is associated with a distinct tumor microbiome and ablation slows disease progression. Our data delineate mechanisms via which microbes modulate the pancreatic cancer cell and provide insight into therapeutic strategies for gut microbial modulation in treating pancreatic cancer.

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Effects of extracellular matrix on pacemaking cardiomyocyte function

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OBJECTIVES/GOALS: The extracellular matrix (ECM) of the sinoatrial node (SAN) is critical for maintaining automaticity in hiPSC-derived pacemaking cardiomyocytes (PCMs) under cyclic strain.

We aim to determine the ECM ligands responsible for cell-ECM mediated mechanotransduction and the resulting phenotype in PCMs. **METHODS/STUDY POPULATION:** hiPSCs are differentiated to PCM and replated on substrate with 5 or 15 kPa PDMS that are coated with 5 or 25 $\mu\text{g}/\text{cm}^2$ of either collagen I or fibronectin at sub-confluent density to restrict junction engagement to only costameres. Then, PCM are subjected to 10% cyclic mechanical strain at 1 Hz for 48 hours, with static culture as control. PCMs from all conditions are subsequently fixed and stained for cardiomyocyte-specific troponin T (TnT), pacemaking HCN4 channel, and pro-pacemaking transcription factors (Shox2, Isl1, Tbx3, Tbx18). Additionally, PCM cell size will also be assessed. **RESULTS/ANTICIPATED RESULTS:** Considering the amount of hypertrophy and myofilament in CMs correlates with mechanical strain, we expect a reduced degree of mechanotransduction in hiPSC-PCM on collagen I with a stiffness 15 kPa to induce smaller cell size with fewer myofilament and an upregulation of HCN4 and pro-pacemaking transcription factors than those on 5 kPa and those on fibronectin of either 5 or 15 kPa after cyclic strain. This is because COL1 is reported to have a lower signaling threshold but a limited sensitivity to force which contributes to the diminished mechanotransduction signaling. **DISCUSSION/SIGNIFICANCE OF IMPACT:** Effects of the microenvironment on hiPSC-PCMs via costamere mechanotransduction may provide insights for engineering biopacemakers with a suitable ECM, to potentially preserve automaticity in hiPSC-PCMs and sustain long-term pacemaking function, making biopacemakers a step closer to reality.

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Shared mood and anxiety symptom variance as a prospective predictor of PTSD symptoms

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OBJECTIVES/GOALS: Mood and anxiety disorders are a risk factor (Ozer et al., 2003) for posttraumatic stress disorder (PTSD) following trauma exposure. As such, a latent internalizing dimension may also be a risk factor. We examine how the shared variance between mood and anxiety symptoms (as in HiTOP; Kotov et al., 2021) impacts development of posttraumatic stress (PTS) symptoms, and symptom clusters. **METHODS/STUDY POPULATION:** Using data from a prior study of individuals who arrived at emergency rooms and were assessed at later time points (AURORA study; McLean et al., 2020), our sample included 1866 participants (1208 females, $M_{age} = 38.49$ years) with available data for the proposed analyses. A latent factor (INTtotal) was operationalized as the shared variance between mood and anxiety symptoms (PROMIS Anxiety and Depression; Cella et al., 2010) as well as PTS symptoms (PCL-5, Weathers et al., 2013). We computed a second internalizing factor excluding PTS symptoms (INTma) to isolate the contribution of baseline affect and anxiety from PTS at baseline. We examined how baseline PTS symptoms, INTtotal, and INTma compare as prospective predictors for PTS symptoms at later time points and how these variables predict individual PTS symptoms. **RESULTS/ANTICIPATED RESULTS:** Baseline INTma, INTtotal, and PTS symptoms were significant prospective predictors of PTS symptoms across all time points (all with $t > 10$, $p < .005$). When focusing on INTma relative to DSM-5 PTSD criterion (American Psychiatric Association, 2013), INTma significantly predicted later symptoms at six months

posttrauma pertaining to Criterion D ($t = 18.88$, $p < .005$), negative alterations in cognition and mood, Criterion E ($t = 15.44$, $p < .005$) arousal and reactivity, and Criterion B, intrusion ($t = 15.44$, $p < .005$). INTma significantly predicted symptoms in Criterion C, avoidance, though to a lesser degree ($t = 12.87$, $p < .005$). **DISCUSSION/SIGNIFICANCE OF IMPACT:** These findings bolster the utility of examining PTSD risk factors through a transdiagnostic lens. INTma was predictive of later PTS symptoms, independent of baseline PTS. Our analyses reveal clinical implications for the assessment of PTSD, and the tailoring of treatment for patients high in internalizing following trauma exposure.

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Endoscopic third ventriculostomy versus ventriculoperitoneal shunting for treatment of hydrocephalus in patients with myelomeningocele

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OBJECTIVES/GOALS: Optimal hydrocephalus treatment with permanent cerebrospinal fluid (CSF) diversion in myelomeningocele (MMC) patients is not well understood, especially how treatment response varies with time of MMC repair. We evaluate two treatment methods in this population—endoscopic third ventriculostomy (ETV) and ventriculoperitoneal shunting (VPS). **METHODS/STUDY POPULATION:** We retrospectively identified patients from St. Louis Children's Hospital who were diagnosed with MMC prenatally and underwent either prenatal or postnatal repair and subsequently underwent permanent treatment for hydrocephalus (VPS or ETV +/- choroid plexus cauterization) between 2018 and 2024. The primary outcome was failure (defined as need for revision) of procedure and time to failure. All revisions were shunt insertions/revisions. Differences in preoperative and 6-month postoperative head circumference (HC) and WHO standard HC z-score were examined. Differences in preoperative and 6-month follow-up fronto-occipital horn ratios (FOHR), a validated age-independent measure of CSF within the brain, on CT and MRI were additionally examined. **RESULTS/ANTICIPATED RESULTS:** Eighty-three MMC patients were identified. 46 (55%) underwent CSF diversion: 37 (80.4%, 9 pre- and 28 postnatal) VPS and 9 (19.6%, 5 pre- and 4 postnatal) ETV +/- choroid plexus cauterization. Six (16%) VPS patients required revision vs. 3 (33%) ETV patients ($\Delta 17\%$; 95% CI -9 – 50). Mean time to failure was longer after VPS vs. ETV (516, SD 470 vs. 34, SD 7 days) [$\Delta 482$; 95% CI 163 – 800]. The decrease between pre- and postoperative FOHR was greater after VPS vs. ETV (6 mo: -0.14, SD 0.10 vs. -0.03, SD 0.07) [$\Delta 0.11$; 95% CI 0.04 – 0.18]. Differences in pre- and postoperative HC were similar (VPS 5.67, SD 2.91 vs. ETV 4.04, SD 1.66 cm) [$\Delta 1.63$; 95% CI -0.71 – 3.98]. Greater, but not significant, z-score decreases were seen after VPS vs. ETV (-1.04, SD 2.22 vs. -0.13, SD 1.11) [$\Delta -0.91$; 95% CI -2.68 – 0.86]. Similar trends were observed in pre- and postnatal MMC repair groups. **DISCUSSION/SIGNIFICANCE OF IMPACT:** Failure rates in MMC-associated hydrocephalus patients were greater in the ETV group, but no definitive conclusion can be made due to imprecision. Those treated with ETV have less time to failure and smaller FOHR decreases than those treated with VPS, indicating less CSF drainage. Due to the need for more subjects, future research should be multicenter.