# Sixteenth

POSTDOCTORAL

# DATA & DINE

SYMPOSIUM

**May 24, 2023 | 4–7 PM**

**Connolly Ballroom**

**Alumni Hall**

UPPDA
University of Pittsburgh Postdoctoral Association

**May 24, 2023**

Dear Colleagues,

Welcome to the **Sixteenth Postdoctoral Data & Dine Symposium**. This year's event is dedicated to the postdoctoral fellows at the University of Pittsburgh, a highly talented and innovative pool of early stage investigators whose passion and dedication to science fuels new discoveries every day.

The goals of this annual symposium continue to be to provide a forum of support and networking for postdoctoral fellows across the University, as well as to serve as a showcase of the significant scientific accomplishments of our postdoctoral community.

We are delighted to continue our tradition of giving awards for best poster presentations. Thanks to the generous support of our academic community, the University of Pittsburgh Postdoctoral Association will be recognizing ten postdoctoral fellows with professional development awards.

This event would not have been possible without the help of the Office of Academic Career Development, and financial support from the Office of the Senior Vice Chancellor for the Health Sciences, the Office of the Provost, and academic sponsors. Special thanks are also extended to the poster judges, and other volunteers contributing to tonight's success.

And finally, we thank fellows, faculty, and administrators for sharing this wonderful evening with us!

*--2023 UPPDA Executive Board*

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# AGENDA
## May 24, 2023 | 4:00-7:00 PM
## CONNOLLY BALLROOM, ALUMNI HALL

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|---|---|
| **2:00-4:00 PM** | **Poster Set-Up** |
| **3:30-4:00PM** | **Registration** |
| **4:00-4:45 PM** | **Poster Session 1** |
| **4:45-5:30 PM** | **Poster Session 2** |
| **5:30-7:00 PM** | **Networking & Awards Reception** |

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# POSTDOCTORAL ALUMNI AWARD WINNER

## Dr. Gau

David Gau is currently a lecturer in the Bioengineering Department at Pitt funded by an NCI K99/R00 award and working understanding the role of Profilin-1 in clear cell renal cell carcinoma. He obtained his PhD in Bioengineering in 2017 at Pitt. David has a long-standing history of representing various trainees at the University including serving as the President of UPPDA from 2019-2022. During his tenure, David transformed the shared governance system at Pitt by successfully advocating for postdoc representation in Pitt's shared governance system. This led to greater understanding about the postdoctoral population at the University and created more communication between postdocs and the senior administration at Pitt. David also laid the foundation for improving benefits available to postdocs and standardizing postdoc salary to NIH standards. As a lecturer, David continues to advocate for postdocs as a member of the ad hoc dependent care committee.

**ABOUT THE POSTDOCTORAL ALUMNI AWARD** The University of Pittsburgh Postdoctoral Association offers an annual Postdoctoral Alumni Award to an individual who has demonstrated a profound, sustained, or leadership contribution to the Postdoctoral Association. Nominations are welcomed from postdoctoral professionals, faculty members, and administration.

**Past Alumni Award Recipients**

2019 – Kimberly Payne, PhD
2018 – Chelsea Stillman, PhD
Collin Diedrich, PhD
2017 – Karen Carney, PhD
2016 – John Merriam, PhD
2014 – Catherine Haggerty, PhD
2013 – Timothy Maul, PhD
2012 – Aaron Bell, PhD
2011 – Natacha De Genna, PhD
2010 – Lei Hong, PhD
2009 – David Robinson, PhD
2008 – Richard Bodnar, PhD
2007 – Steven Wendell, PhD
2006 – Elsa Strotmeyer, PhD
2005 – Stuart Olmsted, PhD

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# POSTDOCTORAL ADVOCATE AWARD WINNER

## Dr. Godley

Amanda Godley joined the School of Education at the University of Pittsburgh in 2002. In addition to being Vice Provost for Graduate Studies, she is a Professor in the Department of Teaching, Learning and Leading in the School of Education and holds secondary appointments in the Department of Linguistics and the Gender, Sexuality and Women's Studies Program. She is also a faculty fellow in the Center for Urban Education and the Honors College, and a center associate at the Learning Research and Development Center (LRDC).

Godley's research is focused on improving instructional quality and equity at the college and high school levels and relates to educational excellence, diversity and innovation. She pursues three strands of research: high-quality classroom discussions, dialect diversity and inclusion in the classroom, and peer review and revision of writing, particularly in urban schools. Her most recent line of research draws from advances in Natural Language Processing and Machine Learning to develop computer-based methods and apps for supporting writing development and high-quality classroom talk in high school and college settings. Godley's research has been funded by the American Educational Research Association, the Institute of Education Sciences, the National Science Foundation, and the Spencer Foundation.

Dr. Godley is the author of the book *Critical Language Pedagogy: Interrogating Language, Dialects and Power in Teacher Education* (2018, Peter Lang). Her research has appeared in peer-reviewed journals such as *Educational Researcher, Reading Research Quarterly, Research in the Teaching of English, Linguistics and Education,* and *Urban Education*. In addition to her scholarship, Godley has led numerous educational workshops for school districts, national professional development organizations, and universities.

**ABOUT THE POSTDOCTORAL ADVOCATE AWARD** The University of Pittsburgh Postdoctoral Association offers an annual Postdoctoral Advocate Award to an individual who has demonstrated a profound, sustained, or leadership contribution to the Postdoctoral Association. Nominations are welcomed from postdoctoral professionals, faculty members, and administration.

**Past Advocate Award Recipients**

2019 – Nathan Urban, PhD
2018 – Thomas Smithgall, PhD
2017 – Arvind Suresh, MS
2016 – Jennifer Woodward, PhD
2015 – Ora Weisz, PhD
2014 – Alan Sved, PhD
2013 – Charles Nieman, PhD, DSO, ARO
2012 – Darlene Zellers, PhD
2011 – Christopher Martin, PhD
2010 – Patricia Beeson, PhD
2009 – Bruce Freeman, PhD
2008 – Andrew Blair, PhD
2007 – Joan Lakoski, PhD
2006 – Elizabeth Baranger, PhD
2005 – Arthur Levine, MD

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# POSTDOCTORAL MENTOR AWARD WINNER

## Dr. Scott

Dr. Iain Scott received his PhD in 2006 from the University of St. Andrews, then moved to the National Institutes of Health in Bethesda, MD for his postdoctoral work. Dr. Scott joined the University of Pittsburgh School of Medicine as an Assistant Professor in August 2014, and was promoted to Associate Professor of Medicine with Tenure in June 2020. His group currently studies the biological mechanisms that regulate fuel substrate metabolism in cardiovascular disease. Dr. Scott was appointed as Director of the Postdoctoral Training Program in the Department of Medicine at the University of Pittsburgh in 2021. In this role, he helps to coordinate mentorship and career development activities for over 150 postdoctoral fellows across the department.

**ABOUT THE POSTDOCTORAL MENTOR AWARD** The University of Pittsburgh Postdoctoral Association Mentor Award criterion is modeled from the National Postdoctoral Association (NPA) Mentor Award, which recognizes a faculty member who has been engaged in exceptional mentoring of postdoctoral fellows and postdoctoral scholars. Nominations are welcomed from postdoctoral professionals, faculty members, and administration.

**Past Mentor Award Recipients**

2019 – Ronald Buckanovich, PhD
2018 – Tina Goldstein, PhD
2017 – Brooke Molina, PhD
2016 – Melissa Bilec, PhD
2015 – Jennifer Silk, PhD

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# POSTER PRESENTATION AWARD GUIDELINES

Posters will be judged from 4 PM to 5:30 PM.
Presenters with even numbered posters will be judged from 4 PM to 4:45 PM.
Presenters with odd numbered posters will be judged from 4:45 PM to 5:30 PM.
*Presenters are to be present at their posters at their assigned times.*

Ten poster presenters will each receive a $750 Professional Development Award. These awards will provide funds for professional development, including participation in a scientific conference, and are to be used by recipients between July 2023 and May 2024. The award categories include:

- *Basic Biomedical and Pharmacological Sciences*
- *Behavioral Sciences*
- *Cellular and Molecular Biology*
- *Clinical and Surgical Sciences*
- *Engineering, Physical, and Computational Sciences*

All posters have been assigned one of these categories for judging purposes. One or more posters in each category will be recognized.

Posters will be judged by a group of faculty and postdoctoral fellows knowledgeable in the above fields. The judges will not be identified during the poster sessions. Scoring will be based upon creativity, style, content, impact, presentation, and overall impressions.

Award winners will be announced during the reception following the poster sessions.

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## POSTER PRESENTATION AWARD SPONSORS

### GOLD SPONSORS
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**UPMC Hillman Cancer Center**

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*Poster 68*

**The Influence of sex on the correlation between astrocyte reactivity and hippocampal volume in Alzheimer's disease**

**Abbas, Sarah[1]; Bellaver, Bruna; Ferreira, Pamela; Ferrari-Souza, João Pedro; Povala, Guilherme; Lussier, Firoza; Zalzale, Hussein; Aguzzoli, Cristiano; Soares, Carolina; Cabrera, Arlec; Lemaire III, Peter; Rohden, Francieli; Leffa, Douglas; Tissot, Cécile; Therriault, Joseph; Servaes, Stijn; Stevenson, Jenna; Rahmouni, Nesrine; Benedet, Andrea L.; Ashton, Nicholas; Karikari, Thomas; Zetterberg, Henrik; Blennow, Kaj; Zimmer, Eduardo; Rosa-Neto, Pedro; Pascoal, Tharick**

**[1]Pascoal Lab, Department of Psychiatry, University of Pittsburgh School of Medicine**

**Aims:** Growing evidence suggests an increased prevalence of Alzheimer's disease (AD) in females compared to males. Elucidating how disease biomarkers correlate in females and males is critical to understanding the basis of sex differences in AD. Our aim was to evaluate sex-related differences in the association of plasma Glial fibrillary acidic protein (GFAP) levels, a biomarker of astrocyte reactivity, with hippocampal volume, a biomarker of downstream neurodegeneration in Alzheimer's disease (AD) pathophysiology.

**Methods:** We cross-sectionally assessed participants from TRIAD cohorts. Unpaired t-test compare the difference in plasma GFAP levels between females and males. We performed linear regression with an interaction term for sex to compare the association of plasma GFAP with neurodegeneration measured with hippocampal volume (HCV) between cognitively impaired females and males.

**Results:** We assessed 308 participants (MCI = 63 , AD =45 CN=200, mean age=69.8 (8) ). Females showed significantly higher GFAP levels than males . We found a significant interaction term and strong correlation between plasma GFAP and neurodegeneration (HCV atrophy) (R-squared= 0.13; P=0.003) only in females. No association was found between GFAP and AD biomarkers in females or males without cognitive impairment.

**Conclusion:** Our results suggest astrocyte reactivity is highly associated with neurodegeneration, a process known to play a key role in AD pathophysiology , in females than males. This may have important implications for elucidating the basis of the higher prevalence of AD in females. Yet, these results were generated with a limited number of subjects and using a cross-sectional design. Therefore, replication in larger, independent longitudinal datasets is needed to better understand the association of GFAP levels, sex, and AD pathophysiology.

*Poster 69*

**Characterizing the phenotypic abnormalities of a novel mouse model of Snyder Robinson-syndrome: for therapeutic development**

**Akinyele, Oluwaseun[1]; Munir, Anushe[1,2]; Johnson, Marie[1]; Perez, Megan S.[1,2]; Gao, Yuan[3]; Foley, Jackson R.[4]; Wu, Yijen[5]; Murray-Stewart, Tracy[4]; Casero, Jr., Robert A.[4]; Bayir, Hulya[3]; Kemaladewi, Dwi U.[1,2]**

**[1]Div. of Genetic and Genomic Medicine, Dept. of Pediatrics, University of Pittsburgh School of Medicine;**
**[2]Dept. of Human Genetics, School of Public Health, University of Pittsburgh;**
**[3]Children's Neuroscience Institute, Dept. of Pediatrics, University of Pittsburgh School of Medicine;**
**[4]Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins School of Medicine;**
**[5]Dept. of Developmental Biology, University of Pittsburgh School of Medicine**

Polyamines (putrescine, spermidine, and spermine) are essential for normal cellular functions and are subject to strict metabolic regulation. Mutations in the gene encoding spermine synthase (SMS) lead to the accumulation of spermidine in an X-linked recessive disorder known as Snyder-Robinson syndrome (SRS), which manifests with a spectrum of symptoms including intellectual disability, developmental delay, thin habitus, and low muscle tone. The development of therapeutic interventions for SRS would require a suitable disease-specific animal model that recapitulates many of the abnormalities observed in affected individuals.

Here, we study the molecular, behavioral, and neuroanatomical features of a mouse model with a missense mutation in the *Sms* gene that results in a glycine-to-serine substitution at position 56 (G56S) of the SMS protein. The G56S mice exhibit a complete loss of SMS protein and elevated spermidine/spermine ratio in the skeletal muscles and the brain, as well as increased anxiety, impaired learning, and decreased explorative behavior. Furthermore, these mice fail to gain weight over time and exhibit abnormalities in brain structure and bone density. Transcriptomic analysis of the cerebral cortex revealed downregulation of genes associated with ribosomal protein synthesis and mitochondrial oxidative phosphorylation, which were functionally recapitulated in fibroblasts. Collectively, our findings establish the first in-depth characterization of an SRS preclinical mouse model that identifies cellular processes that could be targeted for future therapeutic development. Current experiments focus on developing an effective gene therapy approach for SRS via viral-mediated expression of WT *Sms* transgene. We anticipate that successful completion of this study will open the door to a gene therapy approach for SRS and other polyamine-related genetic diseases.

*Poster 1*

**Effect of plasma-derived exosomes resuscitation in a murine model of hemorrhagic shock**

**Alsaadi, Nijmeh[1,2]; Mulla, Joud[1]; Secunda, Zachary[1,2]; Curtis, Justin[1]; Bowman, Annabelle[1]; Neal, Matthew D.[1, 2]**

**[1]Department of Surgery, University of Pittsburgh;**
**[2]Pittsburgh Trauma and Transfusion Medicine Research Center, University of Pittsburgh**

**Introduction:** Hemorrhagic shock remains the second-leading cause of early trauma-related mortality. Plasma products transfusion has been shown to increase survival. Exosomes, a type of extracellular vesicles, are being investigated as treatment options for other pathologies. We hypothesized that resuscitation using plasma-derived exosomes (PDEx) would be comparable to plasma resuscitation in a murine hemorrhagic shock model.
**Methods:** C57BL/6J mice were subjected to a three-hour fixed-pressure hemorrhagic shock model where 50% of the total blood volume is withdrawn to achieve a MAP of 25mmHg. At 90 min, resuscitation using 200μL of lactated Ringer's (LR), murine plasma, or $1x10^{10}$ murine PDEx was administered (n=4/gp). At 180 min, blood was collected to measure TNF- α, IL-6, syndecan 1 (Sdc 1), prothrombin time (PT), activated partial thromboplastin time (aPTT), tissue factor (TF) activity, thrombin-antithrombin complex (TAT), and ALT concentration.
**Results:** Shock increased TNF-a, IL-6, PT, aPTT, ALT, and Sdc 1 levels, with the highest readings being in the no treatment (NoTx) group, followed by the LR group. Interestingly, the PDEx group showed lower post-shock IL-6 and ALT concentrations than the plasma group (4519 vs. 35895pg/mL, $p<0.0247$, and 1498 vs. 3770IU/L, $p<0.0042$, respectively). Alternatively, shock reduced TF activity and TAT concentrations in the NoTx and LR groups to similar levels, without significant changes in those of the plasma and PDEx groups compared to sham.
**Conclusions:** PDEx resuscitation proved comparable to plasma, with lower IL-6 and ALT levels, implying greater organ-protective and anti-inflammatory effects. Our data suggest that PDEx may be a future therapeutic option for hemorrhagic shock.

*Poster 2*

**Cardioprotective efficacy of ultrasound-targeted nitrofatty acid microbubbles (NFABs) in rat myocardial ischemia-reperfusion injury model**

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Over 1 million Americans suffer from acute myocardial infarction (AMI) annually. Although mortality from AMI has decreased, post-AMI congestive heart failure is increasing due to microvascular obstruction (MVO). MVO comprises mechanical obstruction, along with oxidate stress and inflammation. Currently available treatments for MVO are not consistently effective. Hence, we have been developing ultrasound (US)-targeted microbubble cavitation (UTMC) as a potential treatment for MVO. Nitro-fatty acids (NFA) are pleiotropic signaling molecules with broad anti-inflammatory actions, potentially beneficial for the treatment of MVO. NFA are amphipathic thus can seamlessly integrate into the phospholipid shell of MBs. Thus, we have constructed microbubbles with NFA (NFABs) to treat ischemia-reperfusion injury (IRI) with UTMC in the rat myocardial model. Left anterior descending (LAD) coronary artery was ligated for 30 min, allowing for IRI. After 15 min of reperfusion, UTMC+NFABs therapy was administered. Echocardiography measurements were recorded at baseline, during ligation and post-treatment. Left ventricular fractional shortening (%) and ejection fraction (%) were calculated and NFA concentration in cardiac tissue was determined. UTMC with NFABs exhibited promising efficacy in improving fractional shortening and ejection fraction post IRI, and in targeted myocardial delivery of NFA. Studies assessing inflammatory burden, oxidative stress and cytoprotective biomarkers are underway.

*Poster 3*

**Obesity-induced platelet mitofusin-1 expression causes vascular dysfunction**

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The growing epidemic of obesity is strongly linked to cardiovascular disease (CVD). Obesity is known to cause endothelial dysfunction, which then drives vascular injury and subsequent CVD. However, the mechanisms by which obesity instigates endothelial dysfunction remain unclear. Platelets are metabolically active and release vaso-modulatory molecules from their storage granules, and these functions have not been considered in the context of obesity-induced vascular injury. We previously found that mice fed a high fat diet (HFD, 60 kcal% fat) for 10 weeks show weight gain and marked upregulation of platelet mitofusin-1 (MFN1), a small GTP-ase that regulates mitochondrial function, compared with mice on a low fat diet (LFD, 10 kcal% fat). HFD-fed mice showed increased plasma levels of thrombospondin-1 (TSP1), a platelet-derived glycoprotein that mediates endothelial dysfunction. Correspondingly, we observed that platelets from obese humans display a similar upregulation of MFN1, which positively correlates with BMI and waist circumference. Based on these findings **we hypothesized that obesity/weight gain increases platelet MFN1 levels, stimulating the release of TSP1 from platelets to propagate vascular dysfunction.** To test this, we created a murine model with platelet-specific deletion of MFN1 (pltMFN1KO mice). We found that pltMFN1KO mice on HFD have reduced plasma TSP1 levels compared to WT, supporting a causative link between platelet MFN1 levels and TSP1 secretion. Additionally, measurement of endothelial-dependent vascular relaxation by myography showed that HFD caused impaired vascular relaxation (indicative of endothelial dysfunction) in WT mice, while this effect was attenuated in pltMFN1KO mice on HFD. Collectively, these findings reveal a novel platelet-centric paradigm for obesity-associated vasculopathy and provide new therapeutic targets for obesity-induced CVD.

*Poster 45*

**The moderating role of reward activation on the link between rumination and withdrawn/depressive symptoms in autistic adolescents**

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Parallel lines of work have revealed that autistic and depressed individuals demonstrate reward activation differences vs. neurotypical individuals. As depressive symptomology has been linked to reward function, though not in autistic individuals, we were interested in what individual differences might impact these associations. Rumination, a response style characterized by repetitive focus on negative mood/thoughts, has been theorized to potentiate depressed symptoms. Autistic individuals have a propensity to perseverate more than neurotypical individuals, and this propensity is thought to relate to increased rumination. We were interested in whether rumination, reward function, or their relationship could explain depressed symptoms in autistic adolescents.

Twenty-five autistic (M(SD) age=14.95(2.47) years) and 23 neurotypical adolescents (M(SD) age=15.5(2.76) years) completed a coin toss task during functional MRI. Participants were instructed to predict heads/tails within win or loss blocks. A dorsal striatum region of interest (ROI) anatomical mask was used to examine group differences during loss using AFNI 3dttest++ at $p<.005$, empirical contiguity threshold=16 voxels. ROI activation was used as the moderator between rumination (Response to Stress Questionnaire) and withdrawn/depressed symptoms (Child Behavior Checklist).

Autistic adolescents demonstrated diminished activation in an 18-voxel sub-region the dorsal striatum vs. neurotypical adolescents. Within the autistic group, reward activation moderated the relationship between rumination and depressed symptoms ($B=-.16$, $p<.05$, $\Delta R^2=.23$). At lower levels of reward activation, there was a positive relationship between rumination and depressed symptoms ($b=2.94$, $p=.04$), and at higher levels of activation there was a negative relationship ($p=.03$).

Autistic vs. neurotypical adolescents demonstrated decreased reward reactivity to loss. Individual differences in reward activation impact the relationship between rumination and depressed symptoms in autism.

*Poster 70*

**Blood flow regulates *acvrl1* transcription via ligand-dependent Alk1 activity**

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Hereditary Hemorrhagic Telangiectasia (HHT) is an autosomal dominant disease characterized by the development of arteriovenous malformations (AVMs) that can result in fatal complications. HHT is caused by mutations in *ACVRL1*/ALK1 or endoglin (*ENG*); overexpression of *Acvrl1* prevents AVM development in *Eng* null mice, suggesting that enhancing *ACVRL1* expression may be a promising approach to development of targeted therapies for HHT. Therefore, we seek to understand the molecular mechanism of *acvrl1* regulation. We previously demonstrated in zebrafish embryos that *acvrl1* is expressed in arterial endothelial cells proximal to the heart and that expression requires blood flow. Here, we document the time course of this response, demonstrating that *acvrl1* is decreased within 1 hr after stopping heartbeat and re-expressed within 1 hr after restarting flow. Using a transgenic *acvrl1:egfp* reporter line, we find that flow-mediated *acvrl1* regulation is at the level of transcription. Based on these results, we hypothesized that blood flow may be required for distributing the circulating Alk1 ligand, Bmp10, and that Bmp10/Alk1 activity may regulate *acvrl1* expression by a positive feedback mechanism. In support of this hypothesis, we find that *acvrl1* expression is significantly decreased in *bmp10/bmp10-like* double mutants, and that *acvrl1* recovery after restarting flow largely depends on Bmp10. Moreover, we find that rhBMP10 microinjection into the vasculature in the absence of flow maintains *acvrl1* expression at the level of transcription. These data suggest that bmp10 acts downstream of blood flow to maintain *acvrl1* expression and that ALK1 activating therapeutics may have dual functionality by increasing both ALK1 signaling flux and *ACVRL1* expression.

*Poster 46*

**Gender differences in neural mechanism underlying vulnerability to depression and mania**

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**Introduction:** There are significant gender differences in the epidemiology of depression and mania. Despite this, few studies have systematically looked at gender differences in neural mechanisms underlying vulnerability to depression.
**Methods:** 80 women and 35 men (age21.615+/-1.90) were scanned using 3TfMRI during an implicit emotional-faces task. We examined neural activation to all emotional faces versus implicit baseline, using an anatomical region-of-interest mask comprising neural regions supporting emotion processing and attention to salient stimuli. Extracted parameter estimates (FWE<0.05, k>15) along with age and IQ and their gender interactions, were used in two separate penalized regression models with the dependent variable (depressive domain and manic domain score of MOODS-SR-lifetime). GLM regression included the non-zero variables identified above. We tested the model in two independent sample of 75 women and 33 men (age21.610+/-2.092) and 62 women and 31 males (age23.703+/-2.987).

**Results:** 9 clusters and IQ and their gender interactions and 17 clusters and IQ and their gender interactions were non-zero variables with depressive and manic domain scores as the dependent variable, respectively. Poisson regression yielded significant relationships between non-zero variables and depression and mania risk. Dummy variables (women=0, men=1) were used for gender. Further regression analysis then determined whether these findings were replicated in the two test samples. A positive correlation was found between right fusiform activity and depression (beta=0.610) and mania (beta=0.690) risk and between left precuneus*gender (beta=0.743) and mania risk. There was a negative correlation between right fusiform activity*gender (beta=-0.609) and risk of depression. All these findings were replicated in the two test samples(qs<0.05,FDR).
**Conclusion:** We show that robust patterns of higher activity in right fusiform area were differentially associated with increased risk of depression and mania for women and men. These findings may suggest increased attention to facial emotional expression processing associated with fusiform and higher risk of depression in women; and attention to self-reflection, rumination, visuospatial processing associated with precuneus and risk of mania in men.

*Poster 4*

**Targeting polo-like kinase 1 pathway in the treatment of uterine serous cancer**

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**Background:** Uterine serous cancer (USC) is the most lethal subtype of endometrial cancer with a high rate of chemotherapy resistance. There is an urgent need to develop novel treatment approaches against USC. Polo-like kinase-1 (PLK-1) is a kinase that plays important role in mitosis and DNA damage repair (DDR). The therapeutic role of PLK-1 in USC is unknown.

**Hypothesis:** We hypothesize that targeting PLK-1 will synergize with paclitaxel or WEE-1 inhibition in USC models with disruption of DDR pathways and mitosis.

**Design:** To test this hypothesis, we proposed two specific aims: 1; to determine the effects of targeting PLK-1 alone or in combination with paclitaxel and WEE1 inhibition on DNA damage response (DDR) and G2/M cell cycle checkpoint, 2; to evaluate the impact of targeting PLK1 alone or in combination on mitotic slipping and transition from metaphase to anaphase.

**Analysis:** We showed these combination treatments inhibited DDR by depleting RAD51, H2Ax, and ultimately APC/CDH1 leading to the accumulation of DNA damage and over-exhaustion of the G2-M checkpoint recovery. Moreover, PLK1 inhibition arrested mitosis and prevented mitotic slippage evidenced by phosphorylation of Histone 3 and a decrease in the expression CDK1-Cyclin B complex which are the most important mitotic regulators. Our preliminary results enabled us to proceed with in vivo experiments in mice.

**Impact:** We demonstrated the synergistic effect of dual targeting PLK-1 and WEE-1/Paclitaxel on USC cell lines for the first time. Targeting PLK-1 constitutes a potent and novel modality in the treatment of USC.

*Poster 5*

**Hepatocyte specific deletion of epidermal growth factor alters lipid metabolism and fibrosis signaling in a murine fast-food diet model of nonalcoholic fatty liver disease**

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Nonalcoholic fatty liver disease (NAFLD) is the most prevalent liver disorder that is linked to an increased risk of developing liver fibrosis and hepatocellular carcinoma. Epidermal growth factor receptor (EGFR) is mostly known to regulate hepatocyte proliferation and regeneration in liver. In our previous study, we found that the EGFR inhibition utilizing Canertinib, reduced steatosis, liver injury, and fibrosis, in a murine fast-food diet (FFD) model, indicating a potential role for EGFR in regulating NAFLD. To establish this novel role of EGFR, we investigated the effect of hepatocyte-specific EGFR deletion in a murine NAFLD model. 8-10 weeks old $EGFR^{flox/flox}$ mice were injected adeno associated virus 8 (AAV-8) expressing Cre recombinase with a thyroxin binding globulin (TBG) promoter (hepatocyte-specific promoter) to knockout EGFR in hepatocytes ($EGFR^{\Delta hep}$). $EGFR^{\Delta hep}$ or wild-type (WT) mice were fed normal chow diet or FFD for 2 months. FFD-fed $EGFR^{\Delta hep}$ mice displayed significant reduction in serum triglyceride levels with histologically evident lower fat accumulation, specifically in the periportal areas of liver compared to WT mice. At transcriptional level, EGFR deletion significantly reduced expression of SREBF1 (a major transcriptional regulator of fatty acid synthesis) and its downstream fatty acid synthase gene. However, these effects were not observed at protein level. $EGFR^{\Delta hep}$ mice showed lower protein expression of PPARγ, another important transcriptional regulator of lipid metabolism. Further, our transcriptomic analysis via RNA sequencing and subsequent Ingenuity Pathway Analysis revealed significant alteration of hepatic fibrosis/stellate cell activation pathways along with inhibition of TGFβ1 signaling (a key driver of liver fibrosis) in $EGFR^{\Delta hep}$ mice. However, the overall effect of hepatocyte-specific EGFR deletion on steatosis and gene signatures associated with NAFLD was much weaker compared to systemic pharmacological inhibition observed in our previous study. Lastly, deletion of EGFR enhanced expression and phosphorylation of the other ErbB family members (HER2 and HER3), indicating a potential compensatory mechanism for the loss of EGFR signaling in hepatocytes. In conclusion, hepatocyte-specific deletion of EGFR alters lipid metabolism and fibrosis signaling in a murine FFD model of NAFLD and is much less effective compared to EGFR pharmacological inhibition.

*Poster 6*

**Surgical incision engages endogenous kappa opioid receptor (KOR) activity in spinal KOR-expressing neurons to keep chronic postsurgical pain in remission**

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Chronic postsurgical pain (CPSP) develops in millions of patients that undergo surgeries. Prolonged opioid therapy is contraindicated, and other therapeutic approaches lack sufficient analgesic efficacy. We employed a latent sensitization (LS) model of CPSP to test two hypotheses: 1) whether peripheral KORs suppress LS; 2) whether chemogenetic inhibition of spinal KOR expressing interneurons (KOR-INs) prevent LS. LS is a silent, long-lasting sensitization of nociceptive neurons that is tonically masked by compensatory activity of inhibitory G-protein coupled receptors (Taylor and Corder, 2014), including kappa opioid receptor (KOR) (Basu et al., 2021). To test Hypothesis 1 with a conditional deletion approach, we crossed $Pirt^{cre}$ mice with $Oprk1^{lox/lox}$ mice to create $Oprk1^{DRG-/-}$ conditional knockout mice. We performed plantar incision in $Oprk1^{lox/lox}$ controls and cKO mice. 21 days later, we injected either long-acting (LY2456302, 10µg, i.t.) or short-acting (BT-3761, 30mg/kg, i.p.) KOR antagonists, and measured mechanical and heat hypersensitivity. The results indicate that both agents reinstated mechanical and heat hypersensitivity in both controls and cKO mice. To test Hypothesis 2 with a chemogenetics approach, $Oprk1^{Cre}$ mice received intraparenchymal injections of AAV8-hSyn-$hM4D_{Gi}$, or AAV8-hSyn-mCherry (control) into lumbar enlargement of dorsal horn. Three weeks after AAV injection, clozapine N-oxide (3mg/kg, i.p.) was injected 15 minutes before LY2456302 administration. Chemogenetic inhibition of KOR-INs prevented LY2456302-induced mechanical and heat reinstatement. For the first time we report that surgical incision engages endogenous KOR activity in the spinal KOR-expressing INs to keep CPSP in remission. Therapeutic goals to treat CPSP include prevention of the acute-to-chronic pain transition either by: ***A)*** maintaining tonic KOR analgesic activity thus restricting LS to the remission stage, or ***B)*** by improving the drugs or factors that inhibit LS at the maintenance stage, thereby arresting LS.

*Poster 71*

**Treg poorly migrate to inflamed allografts in absence of effector T cells**

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**Purpose:** $CD4^+$ regulatory T cells (Treg) maintain transplantation tolerance through suppression in both secondary lymphoid organs and allografts. However, the signals and the role of effector T cells (Teff) in Treg migration to allograft remain undefined.

**Methods:** We adoptively transferred activated Treg alone or with Teff into splenectomized B6.LTβR$^{-/-}$ (H2b) mice, which don't mount an immune response, and were transplanted with allogeneic Balb/c islets (H2d). Graft-infiltrating cells were enumerated by flow cytometry and visualized in situ using intravital microscopy.

**Results:** When transferred alone, Treg minimally migrated to inflamed allografts, despite being readily found in elevated numbers in the circulation, lungs, and liver. In contrast, the presence of Teff promoted Treg migration to inflamed allografts by 30-folds. This was specific to the allograft since the presence of Teff did not affect Treg migration to lungs and liver. Intravital imaging of allografts 15-20hrs after cell transfer revealed that Treg localized to the same area as Teff. We then investigated whether Teff potentiate Treg migration through chemokine receptor signaling in Treg. Pre-treatment of Treg with pertussis toxin (PTx) before cell transfer with Teff drastically reduced their migration to allografts by 22-folds. Consequently, and unlike untreated Treg, PTx-treated Treg were unable to suppress Teff responses and prevent graft rejection in splenectomized B6.LTβR$^{-/-}$ recipients. Finally, we investigated whether Teff potentiate Treg migration to allografts through interferon-λ (IFNλ) secretion. Interestingly, we found that Treg equally migrated to allografts when transferred with either WT Teff or IFNλ$^{-/-}$ Teff or when transferred into WT or IFNλ$^{-/-}$ mice.

**Conclusions:** Overall, our findings demonstrate that Treg minimally migrate to inflamed allografts in absence of a parallel effector T cell response. In turn, these data establish an unexpected role for effector T cells in potentiating chemokine receptor dependent Treg migration to allografts, which has implications for Treg therapy.

*Poster 113*

# Reactive astrocytes trigger amyloid β-dependent tau phosphorylation in preclinical Alzheimer's disease

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A significant percentage of Aβ-positive cognitively unimpaired (CU) individuals do not develop downstream tau pathology and consequent cognitive decline. Experimental studies suggest that reactive astrocytes are necessary to unleashing Aβ effects on tau hyperphosphorylation. Here we investigated whether astrocyte reactivity is key to determining the association of Aβ burden with tau phosphorylation in preclinical Alzheimer's disease (AD). We assessed 1,016 CU individuals from three cohorts (TRIAD, Pittsburgh, MYHAT) with Aβ, plasma p-tau and GFAP measures. Individuals were classified as positive (Ast+) or negative (Ast-) for astrocyte reactivity based on plasma GFAP. Lowess method and linear regressions accounting for age and sex were used to model the trajectories of plasma p-tau as a function of Aβ. Cohen's *d* was used to estimate effect sizes among groups. We observed that plasma p-tau181 levels increased as a function of Aβ only in Ast+ individuals from Pittsburgh ($\beta = 0.35$, $p = 0.003$), MYHAT ($\beta = 0.20$, $p = 0.026$) and TRIAD ($\beta = 0.57$, $p < 0.0001$) cohorts. A significant interaction between Aβ burden and astrocyte reactivity status on plasma p-tau181 levels was observed in the Pittsburgh ($\beta = 0.29$, $p = 0.022$), MYHAT ($\beta = 0.19$, $p = 0.038$) and TRIAD ($\beta = 0.46$, $p = 0.004$) cohorts. Cohen's d analysis revealed that individuals Aβ+/Ast+ have a large magnitude of effect on tau phosphorylation (Pittsburgh = 0.67; MYHAT = 0.69; TRIAD = 0.98), whereas only Aβ+ presented a negligible effect size. Similar results were observed for other plasma p-tau231 and 217.Voxel-wise analysis confirmed that Aβ levels in brain regions presenting early Aβ accumulation in AD associated with plasma p-tau181 only in Ast+. Tau-PET deposition occurred as a function of Aβ burden only in CU Ast+, affecting 100% and 62% of Braak I and II regions. We observed first biomarker evidence that astrocyte reactivity plays a key role in the association of Aβ with tau pathology in preclinical AD, which might have implications for the biological definition of preclinical AD and selecting individuals for early preventive clinical trials.

*Poster 114*

**Intestinal epithelial cGAS deficiency is associated with decreased intestinal autophagy and increased intestinal epithelial tumorigenesis**

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**Background:** Cyclic-GMP-AMP Synthase (cGAS) is a cytoplasmic PRR involved in many inflammatory and autoimmune disease processes. Our previously published data suggest that cGAS plays a key role in maintaining the intestinal epithelial homeostasis during human IBD and murine colitis by regulating a balance between autophagy and cell death. Autophagy has been shown to play a crucial role in tumorigenesis. We hypothesize that intestinal epithelial cGAS deficiency leads to increased intestinal tumorigenesis via diminished autophagy.

**Methods:** We subjected cGAS KO, WT, cGAS villin-cre, and cGAS floxed mice to the standard AOM/DSS colitis associated colon cancer model. Mice were euthanized after three cycles of DSS. We measured colonic epithelial tumor count and tumor size (<2mm vs. >2mm). We stained the slides with H&E and a blinded pathologist evaluated them for inflammation and dysplasia. Furthermore, we assessed protein levels of cGAS and LC3 via WB analysis.

**Results:** Our published data showed that cGAS KO mice had exacerbated intestinal inflammation and reduced epithelial autophagy. Our preliminary data suggests that there is an increase in cGAS protein levels in tumors of WT mice compared to peritumoral tissue. AOM/DSS treatment resulted in a significantly higher number and larger colonic tumors in cGAS KO mice compared to WT mice. A blinded pathologist found that cGAS KO mice had high grade dysplasia/intramucosal carcinomas compared to WT mice who only had low grade dysplasia, when H&E staining was used. In addition, we found that LC3 protein levels were lower in cGAS KO tumors than WT tumors. LC3 is a marker for autophagy. In order to more comprehensively study the role of intestinal epithelial cGAS in on CAC, we subjected cGAS vc and floxed mice to AOM/DSS. Our data showed that cGAS vc mice had an increased number of colonic tumors compared to cGAS floxed mice.

**Conclusion:** We have previously shown that cGAS deficiency leads to increased intestinal epithelial inflammation via reduced autophagy. We have now show an upregulation of cGAS protein during tumorigenesis but cGAS deficiency is linked to greater inflammation-driven tumor growth. Our future research will focus on clarifying the role of cGAS and autophagy in tumorigenesis, as well as the cell specificity of this effect.

*Poster 135*

**Modifying the collagen network in ocular tissue via femto-second laser pulses**

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Collagen is the most prominent protein in the human body and plays an essential role in determining the mechanical properties of tissue. The organization of collagen fibers varies throughout our bodies, which effects the mechanical properties of the corresponding organs. Therefore, to fully understand organ physiology and pathology, we must also understand the collagen fiber organization and the corresponding mechanical properties. The study of collagen mechanical properties is often done at one of two extremes experimentally at the micro and macroscale. The microscale involves understanding the properties of individual collagen fibrils or fibers, or the macroscale involves understanding the mechanical properties of macroscopic tissue. What is much more difficult is studying the heterogeneity of the collagen fiber network and the role of that heterogeneity in tissue mechanical properties somewhere between the two extremes. In the Laboratory of Ocular Biomechanics, we have done work to characterize the collagen fiber orientation in the sclera and lamina cribrosa of ocular tissue and then used modeling to understand the role of various collagen network structural properties on the mechanical properties. In this poster, I will present a new method for controlling the mechanical properties of collagen fibers with high spatial resolution (< 2µm). I will show preliminary results using a femtosecond laser source to modulate the collagen fiber structure. This method will allow for increased understanding of the collagen network heterogeneity, to see how a microscopic change can affect the mechanical properties locally and on a larger scale within the tissue. In this poster I will demonstrate the photomodulation of single collagen fiber beams in the lamina cribrosa of fresh sheep eye sections. The section is modulated between two silicone sheets. The use of silicone sheets is important as it will allow sections to be place in uniaxial or biaxial stretching devices so that the section can be imaged and photomodulated under load, giving insight into the photoinduced mechanical changes.

*Poster 136*

**Quantification of hard exudates using color fundus photographs based on deep learning Pix2Pix GAN image translation model**

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Diabetic retinopathy is one of the leading causes of blindness. Hard exudates (HEs) are one of the important clinical indicators of the presence of DR and they can be visualized using color fundus (CF) photographs. Accurate segmentation of hard exudates assumes significance in the quantification and monitoring of DR progression. In this study, we attempted a novel image translation method based on Pix2Pix generative adversarial networks (GAN) deep learning model to segment HEs in CF.

This study is performed based on a retrospective dataset of 150 color fundus images taken from subjects diagnosed with DR. We adopted Pix2Pix GAN model which is developed for translating an image pixel-by-pixel into any targeted image. Here we plan to translate CF image to the corresponding HE-segmented CF image. For the generator, we used the residual encoder-decoder (ResUNet) model. Ground-truth segmentations required for training were obtained based on our previously validated semi-automated method. In segmented images for training, HEs were marked in blue and Pix2Pix learns the blue regions. Both generator and discriminator are optimized for mean squared error (MSE) loss functions. The train-test split was 120:30. Segmentation performance is evaluated using Dice coefficient (DC) between the ground-truth and the Pix2Pix GAN segmentations.

On the 30 eyes, the proposed method achieves an average Dice score of 91.47% against ground truth segmentation. The proposed Pix2Pix-GAN-based approach demonstrated close agreement with ground-truth segmentation. This method remains generalizable and can be adapted to other segmentation tasks.

*Poster 137*

**Choroid layer segmentation using OCT B-scans: An image translation approach based on Pix2Pix generative adversarial networks**

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Dysfunction of the choroid layer is associated with various posterior segment eye diseases such as age-related macular degeneration (AMD) and central serous chorioretinopathy (CSCR). For accurate disease screening, clinicians seek a quantitative assessment of the choroid layer based on ubiquitous optical coherence tomography (OCT) images. To this end, we attempted a novel image translation deep learning approach to accurately segment the choroid layer using OCT images.

This is a retrospective study involving 994 OCT B-scan images of healthy subjects. Motivated by the performance of the Pix2Pix generative adversarial network (GAN) architecture to translate natural images pixel-by-pixel in relation to the target images, we trained a Pix2Pix GAN model with the residual encoder-decoder network as a generator to map OCT images with the corresponding choroid annotated images. Train-test split is 747:247 where test data is blind to training. For training, ground-truth labels of the choroid (inner-boundary: red color, outer-boundary: blue color) are obtained using our previously validated exponentiation method where all are verified by an expert grader. Only images with accurate choroid segmentation are considered as ground-truth. Performance analysis is performed based on the Dice coefficient (DC) between the algorithmic and ground truth segmentations.

On the 247 test images, the proposed method achieved a mean Dice coefficient of 97.50%. Visual comparison indicated close agreement between the proposed and ground-truth choroid segmentations.

The proposed choroid layer segmentation method based on Pix2Pix GAN demonstrated close agreement with ground truth segmentation. This study showcases the potential application of Pix2Pix GAN in various image segmentation tasks.

*Poster 72*

# $ST2^+$ reparative regulatory T cell therapy for inflammation reduction and tissue repair after transplantation

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**Introduction:** Tissue damage leads to the release of sequestered molecules such as IL-33 that alert the immune system to the injury. IL-33 then stimulates cells expressing the IL-33R/serum stimulation-2 (ST2) to promote tissue repair in part via the expansion of reparative $ST2^+$ T regulatory cells (Tregs). $ST2^+$ Treg repair mechanisms involve the secretion of the stem cell growth factor amphiregulin and the type 2 cytokine IL-13. We describe the attempts to expand and harness reparative human (hu) and mouse (mu) IL-33-stimulated $ST2^+$ Tregs as novel cell therapy after transplantation.

**Methods:** B6 FoxP3-$RFP^+$ mice received IL-33 injections for 10 days. Spleen $ST2^+$ or $ST2^-$ $CD4^+$ $CD25^{hi}$ $CD127^{lo}$ $RFP^+$ Tregs were FACS sorted and expanded with beads with IL-2 +/- IL-33 for 21 days. Similarly, $CD4^+$ $CD25^{hi}$ $CD127^{lo}$ FACS sorted hu Tregs were expanded with L-cell-based artificial antigen-presenting cells (aAPCs) or beads with IL-2 +/- IL-33. Phenotype, in vitro and in vivo functions were assessed. Treg-specific gene methylation profiles were assessed on expanded hu Treg with IL-2+/- IL-33.

**Results:** IL-2 and IL-2/IL-33 stimulated mu and hu Tregs expanded comparably for 21 days. IL-33-stimulated Tregs upregulated ST2, CTLA-4, and PD-1 expression, and increased IL-13 secretion, and had comparable in-vitro suppressive capacity as IL-2 expanded ones. IL-33-stimulated mu Tregs accelerated wound repair in a fibroblast scratch assay compared to IL-2 alone. Hu Tregs expanded with IL-2 and IL-33 showed reduced methylation of the ST2 and CTLA-4 genes compared to IL-2 expanded Tregs. Delivery of IL-33-stimulated ST2+ Tregs offered increased protection against tissue injury.

**Conclusions:** IL-33 can expand suppressive and reparative mu and hu Tregs that remain stable, upregulate ST2, CTLA4, PD-1, and produce IL-13. Methylation data of hu Treg suggest that IL-33 stimulation does not result in profound Treg instability but increases the expression of ST2 and CTLA-4 and other suppressive and reparative genes. Importantly, the transfer of IL-33-stimulated Treg provided protection after tissue injury.

*Poster 7*

**A genome-wide association study suggests new susceptibility loci for primary antiphospholipid syndrome**

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Primary antiphospholipid syndrome (PAPS) is a rare disease characterized by the presence of antiphospholipid antibodies and the occurrence of thrombotic events and pregnancy complications. Although the etiology of PAPS is incompletely understood, numerous studies have suggested a role for genetic susceptibility in this disease.

The goal of our study was to identify novel susceptibility loci associated to PAPS by performing a large genome-wide association study (GWAS) including five different populations of European ancestry.

A total of 482 PAPS patients and 5,006 controls from five independent populations were included. Genotyping was performed using the Illumina Immunochip, Global Screening Array, and the Infinium Human Core array. PLINK software was used for quality control and association analyses adjusting for population stratification. Genotype imputation was performed using the TOPMed Imputation Server and the TOPMed version R2 reference panel. We meta-analyzed seven million single nucleotide polymorphisms (SNPs) across populations and were able to validate two genome-wide significant ($p<5x10^{-8}$) associations near the *STAT4* and *HLA-DQA1* genes, and identified 43 additional suggestive ($p<1x10^{-5}$) associations. *In silico* functional approaches revealed significant pathways enriched by the genetic associations discover. Further, genetic similarity analysis suggests a close relationship between the genetic susceptibility of PAPS with the genetic basis of lupus and autoantibody-associated neurologic disorders such as neuromyelitis optica (NMO).

Our results provide new insights into the genetics of PAPS and highlight the role of the immune system in the pathogenesis of the disease. Genetic similarity to NMO was unexpected and probably has implications for the incompletely understood neurological manifestations frequently encountered in PAPS.

*Poster 73*

**Western diet dampens T regulatory cell function to fuel hepatic inflammation in nonalcoholic fatty liver disease**

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**Background and aims:** The immunosuppressive T regulatory cells (Tregs) regulate immune responses and maintain immune homeostasis, yet their functions in nonalcoholic fatty liver disease (NAFLD) pathogenesis remains controversial.

**Methods:** Mice were fed a normal diet (ND) or a western diet (WD) for 16 weeks to induce NAFLD. Diphtheria toxin injection to deplete Tregs in Foxp3DTR mice or Treg induction therapy in WT mice to augment Treg numbers was initiated at twelve and eight weeks, respectively. Liver tissues from mice and NASH human subjects were analyzed by histology, confocal imaging, and qRT-PCR.

**Results:** WD triggered accumulation of adaptive immune cells, including Tregs and effector T cells, within the liver parenchyma. This pattern was also observed in NASH patients, where an increase in intrahepatic Tregs was noted. In the absence of adaptive immune cells in Rag1 KO mice, WD promoted accumulation of intrahepatic neutrophils and macrophages and exacerbated hepatic inflammation and fibrosis. Similarly, targeted Treg depletion exacerbated WD-induced hepatic inflammation and fibrosis. In Treg-depleted mice, hepatic injury was associated with increased accumulation of neutrophils, macrophages, and activated T cells within the liver. Conversely, induction of Tregs using recombinant IL2/aIL2 mAb cocktail reduced hepatic steatosis, inflammation, and fibrosis in WD-fed mice. Analysis of intrahepatic Tregs from WD-fed mice revealed a phenotypic signature of impaired Treg function in NAFLD. Ex vivo functional studies showed that glucose and palmitate, but not fructose, impaired the immunosuppressive ability of Treg cells.

**Conclusions:** Our findings indicate that the liver microenvironment in NAFLD impairs the ability of Tregs to suppress effector immune cell activation, thus perpetuating chronic inflammation and driving NAFLD progression. These data suggest that targeted approaches aimed at restoring Treg function may represent a potential therapeutic strategy for treating NAFLD.

*Poster 138*

**Exploring the feasibility of remote cardiac auscultation using earphones**

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Virtual visits (a.k.a., telehealth) have been promoted in response to the COVID pandemic since early 2020. Despite its convenience, the current virtual visit practice barely relies on video observation and talking. The specialist, however, cannot accurately assess the patient's health condition by listening to acoustic cardiopulmonary signals emanating from the patient's heart with a stethoscope.

This poster tries to address this key missing component in video visits by proposing Asclepius, a hardware-software solution that turns the patient's earphones into a stethoscope, allowing the specialist to hear the patient's fine-grained heart sound (i.e., PCG signals) in video visits. To achieve this goal, we contribute a low-cost plug-in peripheral that repurposes the earphone's speaker into a microphone and uses it to capture the patient's minute PCG signals from her ear canal. As the PCG signals suffer from strong attenuation and multi-path effects when propagating from the heart to ear canals, we then propose efficient signal processing algorithms coupled with a data-driven approach to de-reverberate and further correct the amplitude and frequency distortion in raw PCG receptions.

We implement Asclepius on a 2-layer PCB board to evaluate its performance with 30 volunteers. Our extensive experiments show that Asclepius can effectively recover Phonocardiogram (PCG) signals with different types of earphones.

*Poster 74*

**Loss of the cell adhesion molecule MPZL3 drives ovarian cancer metastasis**

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Myelin protein zero-like 3 (MPZL3) is a transmembrane protein with homology to other immunoglobulin-like (Ig) family of cell adhesion molecules (CAMs). While it has been reported that altered Ig-CAM expression plays a role in epithelial ovarian cancer, the function of MPZL3 has not been investigated in tumor cells. Interestingly, TCGA data demonstrates that chromosomal loss of the MPZL3 locus (11q23.3) is frequently detected in several cancer types, including clear cell and high grade serous ovarian cancers.

To elucidate the role of MPZL3 loss in ovarian cancer, we knocked down MPZL3 via RNAi in OVCA433 cells and examined transcriptome-wide effects by RNA-seq. Our preliminary data demonstrates that loss of MPZL3 resulted in a strong epithelial-mesenchymal transition (EMT) gene-expression signature, a key feature of metastasis. Reduction of CDH1 (E-cadherin) and increases in CDH2 (N-cadherin) and VIM (Vimentin) expression following MPZL3 loss were validated using sqRT-PCR and western blotting. We found that MPZL3 knockdown caused changes in mitochondrial and lipid droplet distribution, while having no effects on lipid uptake in ovarian cancer cells. Moreover, MPZL3 knockdown led to enhanced colony formation in glucose-deficient conditions, indicating that MPZL3 loss provides cancer cells with a survival advantage in low glucose environments.

We predict that loss of MPZL3 rewires metabolism for cancer cells to utilize fatty acids as an alternate fuels source. A change in fat metabolism is a key feature of ovarian cancer cells as they metastasize to the omentum, yet the link between altered metabolism and EMT has not been established in ovarian cancer. We thus hypothesize that loss of the Ig-CAM MPZL3 is a driver of ovarian cancer metastasis by regulating both EMT and fat metabolism. By deciphering the mechanisms by which MPZL3 loss drives EMT and metabolic changes, this will provide further insight into ovarian cancer metastasis, thus generating opportunities of developing new treatments for the patients with low MPZL3 expression as an approach for precision cancer medicine.

*Poster 8*

**Neutrophils support antifungal activities in glucose depleted tissues via PYGL-mediated breakdown of intracellular glycogen storage**

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Neutrophils are critical for controlling systemic candidiasis (candidiasis), a bloodstream nosocomial infection caused by the fungus *Candida albicans*. Neutrophils rely solely on extracellular glucose to fuel their energy demanding antifungal activities at tissue sites. However, *C. albicans* consumes glucose. Hence, encountering fungal pathogens in glucose-depleted tissues exert metabolic challenges for neutrophils. It is not known how neutrophils dynamically reprogram their metabolic pathways to function in glucose-deprived tissues in candidiasis. We show that, neutrophils activate glycogen phosphorylase, liver form (PYGL), an enzyme essential for the breakdown of intracellular glycogen storage (aka glycogenolysis) in low glucose during candidiasis. *C. albicans* regulates glycogenolysis in neutrophils in dectin-1/Syk pathway dependent manner. Consequently, selective blocking of PYGL function inhibits fungicidal activities of neutrophils only when glucose availability is low. Finally, treatment with Beta-2 adrenergic receptor agonist, an approved drug and potent activator of PYGL function, improved antifungal activities of neutrophils in low glucose. Overall, these results provide mechanistic insights on how glycogenolysis supports compensatory metabolic reprograming required for antifungal function of neutrophils in the glucose-depleted infected tissues.

*Poster 75*

**Analysis of alternative methods of gap junction plaque formation**

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Gap junction plaques internalize in a process that results in the release of double-membrane vesicles, annular gap junctions, into either of two contacting cells. Though the canonical role of internalization is to degrade the gap junction proteins connexins and downregulate cell-cell communication, the rapid assembly of plaques observed after mitosis and during wound healing is inconsistent with the time required for new connexin synthesis and delivery to the cell surface during plaque formation. We thus hypothesize an alternative method of gap junction plaque formation that involves the recycling of annular gap junctions to the cell surface. Events related to annular gap junction processing are poorly understood. Here we analyze gap junction behavior and fate, specifically recycling, using immunocytochemistry (ICC), transmission electron microscopy (TEM) and time-lapse imaging. While ICC and TEM reveal details of junction structures in SW-13 adrenal cells, we expressed a photoconvertible-tagged Cx43, mEos3.2-Cx43, to track the dynamics of the structures over time. In these cells typical gap junction structures were observed and were converted from green to red, allowing us to track converted junction structures over time. The return of annular gap junctions to the plasma membrane was observed, typically in close proximity to preexisting plaques. Annular gap junctions were observed fusing to one another or moving toward the cell surface and gradually taking on the typical linear shape of plaques. These results support the hypothesis that connexins in annular gap junctions may contribute to gap junction plaque formation by delivering “old” connexins to the cell surface rather than via new protein synthesis. In future studies, we will use pulse-chase biotinylation and image tracking results to develop computational models of gap junction structure behavior in control conditions and experimental conditions known to alter cell-cell communication or result in rapid gap junction plaque formation, e.g. at the end of the mitosis or during wound healing. Supported by NSF MCB 2011577

*Poster 9*

**Tumor-intrinsic p38 signaling as a therapeutic target to overcome non-T cell-inflamed tumors and immunotherapy resistance**

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We previously identified WNT/β-catenin (CTNNB1) and an atlas of molecular alterations that drive the non-T cell-inflamed phenotype and immune-checkpoint inhibitor (ICI) resistance across cancers. To refine our analysis, we have subsequently separated tumor types by clinically relevant stratifications, such as human papilloma virus (HPV) in head and neck squamous cell carcinoma (HNSCC), to identify immune-exclusion mechanisms associated with specific patient populations. P38 MAPK is a known regulator of dendritic cells (DCs) and myeloid cells however a tumor-intrinsic immunomodulatory role has not been previously described.

Using the T cell-inflamed gene expression signature we previously defined, we integrated tissue RNAseq from 395 HPV- HNSCCs in The Cancer Genome Atlas (TCGA) with single-cell RNAseq from two independent HNSCC studies. We identified 67 pathways as activated in non-T cell-inflamed tumors from the HPV- cohort of HNSCC, 59 of which were independently validated. This included CTNNB1 from our prior work and p38 MAPK, the therapeutic target in our ongoing clinical trial (NCT04074967). CTNNB1 and p38 pathway molecules both showed inverse correlation with CD8A protein abundance from the Clinical Proteomic Tumor Analysis Consortium. We observed a significant enrichment of pathway expression only in tumor cells ($p<0.05$) from both HNSCC scRNAseq studies and dominantly in non-T cell-inflamed tumors. Using an accumulative scoring system integrating bulk tissue and single cell sequencing data, we prioritized seven pathways as strongly connected in non-inflamed tumors, with the top regulators as CTNNB1 and p38, among others. EMT6 and CT26 murine models demonstrated improved survival with the addition of p38 inhibitor to ICI relative to ICI monotherapy. Ongoing treatment of patients with anti-PD1 refractory tumors with combination Nivolumab and anti-p38 inhibitor yielded major and durable clinical responses.

p38 is a novel tumor-intrinsic mechanism that drives immune exclusion. P38 inhibition enhances ICI and can overcome anti-PD1 resistance in patients.

*Poster 10*

**Biochemical conversion of acinar cells into β-like cells in the pancreas**

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β-cell depletion outpaces β-cell generation which overall decreases the number of insulin-producing cells, and a shortage of insulin becomes evident. The generation of insulin-producing cells to compensate for their absolute or relative shortage in type 1 (T1D) and type 2 (T2D) diabetes is a good therapeutic strategy. Thus, a cure for diabetes should entail replacement of insulin-producing β-cells. Accordingly, there have been tremendous efforts throughout the years to generate new β- cells either through proliferation of pre-existing β-cells or by neogenesis using different sources such as embryonic stem cells, duct cells, non-β-cells residing in the endocrine islets, or acinar cells. One potential treatment for this disease would be direct conversion of pancreatic acinar cells into β-cells in sufficient numbers to restore and maintain euglycemia. Focal adhesion kinase (FAK) is a non-receptor tyrosine kinase that mediates integrin signaling, a major group of proteins involved in the interaction between the cell and the extracellular matrix (ECM). Our lineage tracing studies along with transcriptomic characterization demonstrate that treatment of adult mice with a small molecule that specifically inhibits kinase activity of focal adhesion kinase results in trans-differentiation of subset of peri-islet acinar cells into insulin producing β-like cells. The acinar-derived insulin producing cells infiltrate the pre-existing endocrine islets, partially restore β-cell mass, and significantly improve glucose homeostasis in diabetic mice and non-human primates (NHPs). These findings are directly applicable to our full understanding and potential treatment of diabetes and may provide a key understanding of how to generate new functional β-cells from acinar cells.

*Poster 11*

**Preserved mitochondrial activity in hemorrhagic shock + trauma (HS/T) after exercise**

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**Introduction:** Exercise training has proven benefits in many disease states, particularly those involving the immune and metabolic systems. We have shown exercise training mitigates early sequela of hemorrhagic shock + trauma (HS/T) in mice, preserving homeostasis and organ function. The mechanism for immunometabolic alterations post-exercise in response to HS/T is unknown.

**Methods:** Male C57BL/6 mice were exercised on a treadmill daily for 4 weeks (n=4) or maintained with normal activity (n=4). Mice were subjected to pseudofracture and 2 hours of hemorrhagic shock (HS/T) and sacrificed 4 hours after resuscitation. Bone marrow mononuclear cells (BMMCs) were isolated and analyzed with scRNA sequencing, identifying over 20,000 genes (10X). Plasma was analyzed by LC-MS/MS identifying 914 proteins. Transcriptomic and proteomic layers were harmonized and compared between exercise and sedentary groups.

**Results:** Analysis of scRNA and proteomic data yielded 5390 and 326 molecules, respectively, with significant differential expression between exercise and sedentary conditions in HS/T. The exercise cohort had significantly higher expression of pathways related to mitochondrial translation (79 associated genes), mitochondrial iron-sulfur protein biogenesis (16 associated genes), and electron transport chain (67 associated genes). Exercise group BMMC transcriptomics showed increased mitochondrial fatty acid beta-oxidation (16 associated genes). In each case, the number of associated genes describes the genes that mapped to the corresponding KEGG or Reactome pathway and had significantly increased expression ($p<0.05$).

**Conclusions:** The increased markers of standard mitochondrial activity with exercise may indicate increased mitochondrial robustness in response to HS/T after exercise training. Furthermore, the enhanced transcriptomic signatures for mitochondrial fatty acid beta-oxidation in BMMCs may be unique to this compartment. These results will guide future work to elucidate mechanisms for exercise-related preservation of mitochondrial function in HS/T.

*Poster 139*

**Evaluation of air-powered shopping scooters in grocery stores**

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Objective: The purpose of this study was to further previous research and gather additional information regarding the usage of motorized shopping scooters as well as feedback for improvements to an air-powered scooter. Methods: Online surveys were used to assess individuals' shopping characteristics and experience using the motorized scooters and to gather feedback from store employees regarding their experience. K-Means clustering analysis was used to determine user demographics who chose to use the air-powered scooter versus the electric powered scooter while shopping. Results: A total of 127 individuals provided informed consent, 65 individuals from Site 1 (Giant Eagle, Homestead, PA) and 62 individuals from Site 2 (Giant Eagle Market District, Shadyside, PA). 120 participants met the inclusion criteria and completed the survey. K-Means clustering found that age, type of personal mobility device, shopping bill total, and frequency using a motorized shopping scooter to be significant factors in whether individuals chose to use an air-powered scooter or electric-powered scooter. Conclusion: Motorized shopping scooters are in high demand and used by a wide variety of individuals, yet electric-powered scooters are commonly unavailable due to having dead batteries or all the devices being in use. Air-powered scooters may serve as a practical replacement for the current electric-powered scooters found in grocery and retail stores.

*Poster 115*

**Fugl-Meyer Assessment predicts and classifies motor recovery in patients with chronic post-stroke hemiparesis using cervical spinal cord stimulation**

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We have previously demonstrated that electrical stimulation of the cervical spinal cord using implanted epidural electrodes (SCS) can improve the upper limb motor function of individuals with chronic post-stroke hemiparesis. Although immediate rehabilitative effects were clearly observed while SCS was delivered, the magnitude of motor function improvement was significantly different across participants. In this study, we used Fugl-Meyer Assessment (FMA) to characterize changes in upper extremity motor function of 4 participants with chronic post-stroke hemiparesis who received cervical SCS throughout 4 weeks. All participants were implanted with two lead electrodes spanning from C3 to T1 spinal levels on the contralesional side. Stimulation was administered 5 times per week while strength and motor control tasks were performed. Sensory and motor upper extremity FMA scores were measured a week before the implantation of the SCS electrodes (baseline), as well as in the 2$^{nd}$ (mid-study) and 4$^{th}$ (end-study) weeks after implantation. Importantly, FMA scores were measured during stimulation on and off conditions. Our results showed a strong linear association ($r^2 = 0.98$) between the change of FMA motor scores after 4 weeks (i.e., difference of FMA motor scores at baseline and end-study) and the baseline FMA sensory scores, which suggests that patients with *less* sensory impairment have *more* potential for motor recovery using SCS. Interestingly, FMA motor scores were more increased for proximal arm muscles compared with distal hand muscles. We also found that FMA motor scores increased over time for both stimulation on and off conditions, indicating that motor function recovery may be consolidated over the course of SCS and motor practice. Taken together, these results show that the FMA sensory scores can be used to predict and motor function recovery promoted with SCS, and FMA motor scores to measure motor recovery over the relevant time courses. These results may help with stratification of stroke patients and customization of effective rehabilitation protocols using SCS.

*Poster 76*

**The processing of telomeric 8-oxoguanine by OGG1 and MUTYH glycosylases promotes cellular senescence**

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Telomeres cap and protect linear chromosome ends and their integrity is crucial to preserve genome stability and ensure sustained cell proliferation. DNA damage caused by oxidative stress from environmental exposure and endogenous sources accelerates telomere shortening and dysfunction, which can contribute to tumorigenesis and aging. Base excision repair (BER) enzymes process the highly prevalent oxidative lesion 8-oxoguanine (8-oxoG), which forms preferentially at telomeric repeat sequences due to their susceptibility to oxidative damage. 8-oxoG DNA glycosylase (OGG1) excises 8-oxoG opposite cytosine, while MutY-homolog (MUTYH) removes the adenine misincorporated opposite 8-oxoG. By exploiting a unique tool that selectively targets the telomeres with formation of 8-oxoG, we demonstrated that acute telomeric oxidative base damage is sufficient to trigger premature cellular senescence in non-diseased cells in the absence of telomere shortening. Here we investigated the role of 8-oxoG processing by BER enzymes at telomeres in cellular senescence induction. Looking at different senescence phenotypes upon telomeric 8-oxoG induction in OGG1 and MUTYH deficient non-diseased cells, we found that OGG1 or MUTYH knock out partially, and double knock out completely, rescued telomeric 8-oxoG induced senescence, cytoplasmic chromatin fragments (CCFs) formation and DNA damage response (DDR) activation. BER deficiency also prevented replication stress and related telomere fragility. Moreover, treatment with Poly(ADP-ribose) polymerase-1 (PARP1) inhibitor Olaparib, to retain PARP1 at sites of targeted 8-oxoG damage, revealed that BER proficient cells experienced an exacerbated senescence induction compared to the cells lacking one of the enzymes, whereas the double knock out cells were completely unaffected. These data suggest that BER processing of telomeric 8-oxoG and resulting formation of toxic repair intermediates, interfere with telomere replication and drive telomere dysfunction, thus triggering cellular senescence.

*Poster 77*

**The spaceflight dichotomy between the flat and the long bones**

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It is known that space travelers are at high risk for bone loss. Exposure to conditions of microgravity results in elevated bone resorption and reduced bone formation. However, not much is known about the responsible cellular and molecular mechanisms. The mouse calvaria, with its suture enriched of skeletal stem cells (SSCs), represents an ideal model to study the effect of microgravity on SSCs and their role in bone homeostasis and regeneration. Therefore, we developed a mouse model in which we investigated the effect of spaceflight on flat and long bones, and on the regeneration of a sub-critical size defect.

Sixteen 8-week-old female C57BL/6 mice were randomly distributed in two groups: one group of 8 mice that did not undergo surgery (untreated mice) and one group of 8 mice that underwent a surgery to create the 0.5 mm bone defect. After 45 days in space, mice returned to Earth and calvarial tissues and long bones were collected and analyzed. The same treatments and analyses were performed on sixteen ground control mice.

µCT analysis of the calvaria showed a higher bone volume fraction for the flight group. On the contrary, and consistent with what has been previously reported, long bones exhibited significant lower bone mass in spaceflight mice. ScRNA-seq data indicate that lack of gravity is associated to a higher level of cell proliferation markers expression in the osteogenic lineage of the calvarial sutures, and a lower level of expression of β-catenin, which is known to be involved in the osteogenic differentiation, in comparison to ground control. This trend is completely reverted with the creation of the defect.

This study indicates that, during spaceflight, bone homeostasis (bone mass) and bone regeneration of the calvarial bones are not negatively impacted. The differences with the well documented loss of bone mass and impaired bone regeneration observed in the long bones highlighted a significant spaceflight dichotomy between the flat bones and the long bones. The changes in the gene expression profile due to the microgravity needs to be deeper investigated and analyzed.

*Poster 78*

**CASTOR1-mTORC1 pathway controls colon epithelial homeostasis and repair by regulating interleukin IL-6/STAT3-mediated inflammation**

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The mammalian target of rapamycin (mTOR) signaling pathway integrates signals of both environmental and growth factors to regulate cell growth and survival. However, how mTORC1 responds to acute inflammatory signals to regulate bowel homeostasis and repair is poorly studied. Cytosolic arginine sensor for mTORC1 subunit 1 (CASTOR1) is a newly discovered arginine sensor, which negatively regulates mTORC1 activity. In this study, we generated a CASTOR1 knockout mouse model and used it to investigate the role of CASTOR1-mTORC1 pathway in acute inflammatory bowel disease (IBD). We found that mice with CASTOR1 knockout were resistant to body weight loss, maintained intact intestinal barriers, and showed increased cell proliferation and decreased epithelial apoptosis during dextran sulfate sodium (DSS)-induced intestinal epithelial injury and acute colitis. Mechanistically, CASTOR1 knockout promoted intestinal crypt proliferation and regeneration by inducing interleukin-6-associated reparative inflammation, STAT3 activation. Furthermore, treatment with berberine chloride induced mTORC1 activation and relieved IBD impairment. Conversely, treatment with rapamycin enhanced the process of acute inflammation in wild type mice. Together, these findings provided evidence that CASTOR1 deficiency and activation of mTORC1 could prevent and block intestinal inflammatory disorders. Our results indicate that CASTOR1 has protective effects on DSS-induced colitis, and facilitates colon epithelial regeneration by regulating mTORC1 and IL-6-STAT3 pathway, thus providing a new target for the treatment of colitis-related diseases.

*Poster 47*

**Defining diurnal and nocturnal periods in 24-h ambulatory blood pressure monitoring: comparing three measurement methods**

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Ambulatory blood pressure monitoring (ABPM) use is increasing in research and clinical practice due to its diagnostic superiority over clinic BP. However, little work has examined discrepancies in using intervals to define diurnal (day/wake) and nocturnal (night/sleep) periods of ABP versus actigraphic measurement of these periods, and how different such measures perform in analyses. Likewise, few studies to date have examined novel ambulatory BP conceptualizations, such as area under the curve (AUC), compared to traditional derived mean values. The present study seeks to fill this gap by analyzing the agreement among three diurnal and nocturnal BP measurement methods, and comparing models of actigraphy-derived sleep values predicting each BP outcome measurement for agreement.

A community sample of 239 adults, ages 21 to 70 years, completed a follow up assessment of the North Texas Heart Study. Each participant completed two days and one night of ABPM and 2 nights of actigraphy. ABP was analyzed in three ways: (1) fixed periods of average "daytime" and "nighttime" from 07:00-22:00 and 0:00-05:00, respectively; (2) average "waking" and "sleeping" ABP values defined by actigraphic bedtime and risetime; and (3) "waking" and "sleeping" ABP values defined by actigraphic bedtime and risetime summarized using the area under the curve of BP.

Analyses revealed virtually no differences between the waking vs. daytime ABP periods, but a significant discrepancy between nighttime and sleeping systolic ABP periods ($t=-12.88$, $p<.001$). Model comparisons mirrored these results, revealing few differences in *diurnal* ABP variance explained by sleep, and less agreement by ABP measure for *nocturnal* systolic ABP variance explained by sleep.

Fixed daytime periods versus waking values of ABP are virtually identical and do not produce dissimilar results in analyses of a community sample. There is a lack of agreement between fixed-nighttime and actigraphy-based determinations of nocturnal systolic ABP. Waking/sleeping AUC of BP is not a superior summary measurement compared to mean values of ABP.

*Poster 79*

**Subcutaneous injection of IHP-102 attenuates lung vaso-occlusion in sickle cell disease mice**

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Sickle Cell Disease (SCD) is an autosomal-recessive-genetic disorder that affects ~100,000 Americans and over 3 million people world-wide. Acute systemic painful vaso-occlusive episode (VOE) is the primary reason for hospitalization of SCD patients and serves as an antecedent to acute chest syndrome (ACS), a type of acute lung injury and one of the primary reasons for mortality among SCD patients. Intravenous (IV) injection of P-selectin monoclonal antibodies have been shown to attenuate lung vaso-occlusion by neutrophil-platelet-erythrocyte aggregates in transgenic-humanized SCD mice and reduce frequency of VOE in SCD patients. However, IV route of administration requires at least an out-patient visit to the clinic, thus highlighting the need for therapies that can cut-down the health care cost by enabling self-administration (such as sub-cutaneous) by SCD patients at home during the prodromal phase of a VOE. Importantly, P-selectin dependent neutrophil-platelet aggregation and the complement pathway activation contribute to the vaso-occlusive pathophysiology in SCD. IHP-102 is a novel compound, which serves as a dual-inhibitor of both P-selectin and the complement pathway. Our initial pharmacodynamic findings in rodents revealed that P-selectin-inhibition activity of IHP-102 was detectable in the serum within 30 min following sub-cutaneous administration of 30 mg/kg IHP-102. Next, quantitative fluorescence intravital lung microscopy (qFILM) was conducted to assess the efficacy of IHP-102 in attenuating intravenous 10 μmole/kg oxy-hemoglobin (IV oxy-Hb) triggered lung vaso-occlusion in Townes SCD mice. Remarkably, and despite the described vasculopathy that would impair systemic delivery in this model, sub-cutaneous administration of either 10 or 30 mg/kg IHP-102 led to significant attenuation of lung vaso-occlusion by neutrophil-platelet aggregates in Townes SCD mice administered IV oxy-Hb. These findings are the first to highlight the therapeutic potential of a subcutaneously administrable therapy (IHP-102) in preventing VOE and ACS in SCD.

*Poster 140*

**Exploring the roles of interneuron subtypes in network dynamics**

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Neuronal responses to sensory stimuli can be strongly modulated by animal's brain state. Three distinct subtypes of inhibitory interneurons, parvalbumin (PV), somatostatin (SOM), and vasoactive intestinal peptide (VIP) expressing cells, have been identified as key players of flexibly modulating network activity. The three interneuron populations have specialized local microcircuit motifs and are targeted differentially by top-down inputs from higher-order cortical areas and neuromodulators. Optogenetic stimulation of different interneuron cell types demonstrates different impacts on neuronal population responses, such as firing rate and network synchrony. In this work, we systematically study the function of each interneuron cell type at controlling network dynamics in a spatially ordered spiking neuron network. We model top down and neuromodulatory inputs as static current applied to each neuron population.We find that the network transitions through three distinct network states, from subcircuit to weak synchrony to strong synchrony state, as we activate the excitatory or SOM population or inactivate the PV or VIP population. Further, we investigate how network responses to modulatory inputs depend on the connectivity of the SOM cells.This work provides a foundational understanding for the modulation of network activity with respect to four unique populations and testable predictions for future experiments.

*Poster 12*

**Assessing the safety of the synthetic glucocorticoid Des-Ciclesonide and its therapeutic potential in BPD**

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While synthetic glucocorticoids (sGC) like dexamethasone (Dex) possess anti-inflammatory and lung maturation activities that could be beneficial to treat preterm infants with bronchopulmonary dysplasia (BPD), their use is limited due to their adverse systemic effects. Des-ciclesonide (DesCIC), the metabolite of ciclesonide (CIC), is a potent glucocorticoid receptor (GR) agonist with tissue-specific metabolism and a higher safety profile that makes it a promising pharmacotherapy for BPD.

Within 24 hrs of birth, Sprague-Dawley pups were given five daily s.c. injections of Vehicle (Veh), 0.5 mg/kg Dex, 0.5 mg/kg DesCIC or 1.25 mg/kg DesCIC. Tissue and blood samples were collected 4 or 24 hrs after the final injection. Systemic effects were assessed by measuring weight gain, endpoint brain weight, blood glucose and serum levels of insulin-like growth factor-1 (IGF-1).

While Dex was associated with delayed weight gain and a reduction in IGF-1, both doses of Des-CIC did not trigger growth suppression or reductions in IGF-1. Hyperglycemia was evident in the Dex treated pups at 4 and 24 hrs but absent in 0.5 mg/kg DesCIC with only a modest transient elevation after 1.25 mg/kg DesCIC. Pups treated with high but not low dose DesCIC had a modest reduction in brain weight that was significantly different than that observed with Dex. RNAseq of lung and liver tissue revealed a greater number and higher level of induction of genes in the Dex compared to DesCIC group with unique pathways defined by the transcriptome in each tissue. qRTPCR confirmed the induction (e.g. FKBP5) and repression (e.g. TNF-$\alpha$) of GR target genes in liver and lung by DesCIC.

Our data suggests that DesCIC may be anti-inflammatory without producing the detrimental growth and hyperglycemic effects of Dex in neonatal rats. It also reveals that the potential enhanced safety profile of the prodrug CIC to treat BPD may be influenced by its tissue-selective conversion to a novel selective GR modulator (DesCIC), whose unique transcriptional responses may limit adverse systemic effects typically triggered by therapeutic sGCs in neonates.

*Poster 48*

**Identification of four clusters with distinct emotional and behavioral presentations in a large, representative sample of autistic youth**

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Research suggests that autistic individuals are at high risk for emotion dysregulation, aggression, self-injurious behavior, depression, and anxiety. There is a growing interest in understanding how emotional and behavioral symptoms cluster together within autistic individuals. Identifying subgroups with similar presentations would be helpful for identifying underlying mechanisms and developing treatment approaches.

The sample (total n = 1,609) included 558 psychiatrically hospitalized autistic youth from the Autism Inpatient Collection (age 12.7± 3.6), and 996 autistic youth from the Interactive Autism Network autism research registry (age 11.9 ± 3). We applied a machine learning clustering technique to item-level parent report data from the Emotion Dysregulation Inventory Reactivity and Dysphoria scales, self-injurious behavior items from the Aberrant Behavior Checklist, and items from the Anxious/Depressed, Withdrawn/Depressed, and Aggression scales of the Child Behavior Checklist.

The clustering algorithm produced four distinct groups of individuals. Results showed that the Reactivity and Aggression scales are directly proportional. Furthermore, although the fourth group shows moderate levels across all scales, the first and second groups show higher levels of Dysphoria, Anxiety and Withdrawn scales than the Reactivity and Aggression scales. The third group shows the opposite pattern: higher levels of Reactivity and Aggression scales than the other scales.

Results suggest that presence of emotion dysregulation and aggression contribute significantly to determining the cluster membership and the pattern between the emotional and behavioral presentation across these clusters.

*Poster 116*

**Decoding the fate of early acute T cell mediated rejection after renal transplantation**

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The prevalence of risk-modifiers (change in renal function (ΔseCr), BK viremia (BKV), DSA, & non-adherence (NA)) prior to or after TCMR, and their effect on long-term transplant (Tx) outcomes is unclear.

**Methods:** We assessed histology and risk-modifiers within 6mo pre- and post-T cell Mediated Rejection (TCMR) diagnosis and analyzed their effect on histological resolution (1yr) and graft loss (GL, 7y). Patients underwent serial biopsies (Bx)(for-cause+2protocol:3&12mo) plus DSA and BKV testing. To assess clinical resolution, we computed ΔseCr from nadir post-Tx→TCMR→6mo post-TCMR. NA was gauged by CNI-IPV.

**Results:** Based on 2211 Bx in 1400 patients (2013-19), 398 had TCMR in the 1st post-Tx year. While 68% of TCMR was subclinical(protocol Bx), 32% was clinical(fc-Bx). 70% of patients had follow-up Bx. 8% of patients had DSA prior to TCMR, 17% at TCMR and 11% within 6mo post-TCMR. 21% of patients had persistent DSA. 41% patients had >10% improvement in seCr from diagnosis to 6mos post-TCMR while 7% had a >30% decline. Despite therapy, only 33% of patients had seCr improve to within 10% of their nadir, suggesting non-resolution in 67%. Nonadherence was noted in 24% patients within 6mo of TCMR diagnosis. In a Cox model, persistent DSA, lack of clinical resolution, and NA were associated with worse GS. Despite full clinical resolution, complete histological resolution of TCMR was only seen in 25% patients, while 44% had persistent TCMR suggesting a clinical-histological mismatch. Moreover, histological persistence irrespective of clinical resolution was associated with worse GL. In contrast, histological resolution even with clinical persistence was associated with graft survival similar to full clinico-histological resolution. Finally, both non-adherence and persistent DSA were associated with a markedly greater histological persistence.

**Conclusion**: Histological but not clinical resolution prognosticates early TCMR. Both histological resolution and GS are affected by peri-TCMR risk modifiers such as DSA and non-adherence. These could be used to assess the need for a resolution biopsy.

*Poster 80*

**Genetic factors associated with anti-Müllerian hormone levels in Samoan women**

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Anti-Müllerian hormone (AMH) is a critical regulator of ovarian follicle recruitment and development and is commonly used to predict ovarian reserve and time-to-menopause. The use of AMH as a diagnostic marker for PCOS awaits the establishment of an international standardization reference and age- and ethnicity-adjusted cut off levels. Genetic variation that affects AMH levels might help identify new biomarkers of reproductive health. To date, only few loci (*AMH, MCM8, TEX41*, *CDCA7* and *CTB-99A3.1*) have been identified in women of European ancestry, highlighting the need to investigate genetic determinants of AMH levels in populations of non-European ancestry.

To assess genetic variation of AMH, we selected Samoan women of 25 - 51 years excluding those with a history of hysterectomy and/or ovariectomy, or who were pregnant or lactating at recruitment. Serum AMH levels were measured via ELISA assay from Ansh Labs. A genome-wide meta-analysis was performed on a total of 1103 women using genotyped and imputed variants via a Samoan-specific reference panel. Association of SNVs with MAF $\geq$ 0.01 was assessed using a Cox's proportional hazards regression adjusting for centered age and age$^2$, genetic relatedness and/or polity. The findings were followed up using FUMA and transcriptome-wide analysis (S-MetaXcan).

We present the first GWAS of circulating AMH levels in Samoan women, identifying 14 suggestive loci ($p \leq 1 \times 10^{-5}$). The strongest GWAS signal was found in *ARID3A* (rs149054433, *p* $9.87 \times 10^{-8}$) and supported by significant association in gene-based test and transcriptome-wide analysis. Notably, the kisspeptin receptor (*KISS1R*) gene and an SNV linked to age at menopause map nearby. Another noteworthy signal was detected at *TMED10* (rs7156476, *p* $4.63 \times 10^{-6}$), located near the *FOS* and *JDP2* genes which form the activating protein-1 transcription factor that regulates GnRH expression. This locus also harbors genes previously implicated in premature ovarian failure (*MLH3* and *EIF2B2*).

To validate these findings, analysis in larger cohorts of Samoans and other Pacific Islanders is needed.

*Poster 81*

**M2 macrophages and oxidative stress response are prognostic for progression-free survival of prostate cancer patients**

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Prostate cancer is the most frequently diagnosed and second leading cause of cancer death in American men. Defining the mechanisms of prostate cancer progression may lead to more effective diagnosis, prognosis, prevention and/or treatment for the disease. Immune cells and oxidative stress are thought to play an important role in prostate tumor microenvironment. However, how immune cells and oxidative stress impact prostate cancer remain poorly understood. In depth study of oxidative stress in the immune microenvironment will be needed for better understanding of the mechanisms of prostate cancer progression.

The potential role of immune cells in the microenvironment of prostate cancer and its relationship with oxidative stress were explored using public bulk and single-cell RNA-seq datasets. In addition, through machine learning, genes highly related to oxidative stress were identified and used to construct a prostate cancer prognosis model, which appears to be more accurate than the standard Gleason grading system. Our analysis also revealed down-regulation of oxidative stress response (OSR) in prostate cancer cells and associated infiltration of M0 macrophages in a large number in prostate cancer tissues, as compared to the adjacent normal prostate. More importantly, elevated numbers of M2 macrophages and associated OSR down-regulation were detected in high Gleason grade prostate cancer specimens and these molecular and cellular changes are prognostic of progression-free survival of prostate cancer patients.

The above findings suggest that M2 macrophages and OSR down-regulation are prognostic markers of prostate cancer and that M2 macrophages may promote growth and progression of prostate cancer by down-regulating OSR in the tumor cells.

*Poster 117*

**Evaluation of a model-based precision dosing platform using Bayesian forecasting method for personalized Busulfan therapy in the adult hematopoietic stem cell transplantation population with limited sampling**

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The purpose of this study was to evaluate the ability of a model-based precision dosing platform utilizing Bayesian forecasting (BF) to reliably estimate busulfan exposure and recommended doses in comparison to the current clinical practice and to determine a limited sampling strategy (LSS) to minimize the number of blood samples necessary and the inconvenience to patients.
Data from patients who received intravenous busulfan as part of conditioning regimen prior to hematopoietic stem cell transplantation (HSCT) were entered into BF software, InsightRX Nova. Estimation of busulfan exposure and dose recommendations was compared to the clinical practice values, aiming for the predefined target exposure of approximately 4X4800 µmol*min/L. Estimation performance was tested using several LSSs.
Fifty-one adult patients (23 to 65 years) provided exposure measurements estimated using 7 blood samples collected following the first dose administration. BF provided acceptable bias and precision of exposure estimations under all tested sampling strategies (<15%). The bias and precision of AUC estimates ranged from MAE of 0.7–8.5% and rRMSE of 9–14%, respectively. Two LSSs including LSS-VI (with 3, 4, 5, and 6h) and LSS-VII (with 3, 4, and 6h) predict AUC-ISS with good precision and minimal bias. These LSSs performed similarly well in an independent external validation, where the AUC and recommended doses predicted by BF using these LSSs have acceptable agreement, with those calculated by NCA using ISS (MAE ≤15%) in >80% of the cases.
Model-based precision dosing platform utilizing BF with only 3 or 4 plasma busulfan concentrations can be used to reliably estimate busulfan exposure after intravenous administration in adults undergoing HSCT. This study provides supporting evidence for the utility of Bayesian forecasting-based platforms in clinical practice as it will simplify and standardize the hospital service.

*Poster 118*

**Personalizing intravenous busulfan dosing in children undergoing hematopoietic stem cell transplantation with limited sampling strategies - experience at an academic medical center**

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Currently busulfan doses are adjusted by monitoring of the plasma concentrations using intensive sampling strategy (ISS). The area under the plasma drug concentration-time curve (AUC) is calculated by non-compartmental analysis (NCA) of 7 plasma concentrations taken after the first of 12 intravenous doses given as 2-hour intravenous (i.v.) infusion every 6 hours. The purpose of this study was to develop and validate limited sampling strategies (LSS) using 4 or less sampling points with which to accurately calculate the exposure and adjusted doses of i.v. busulfan.
Data from patients (0.08 – 20 years old) who received i.v. busulfan as part of conditioning regimen prior to hematopoietic stem cell transplantation (HSCT) were included. Estimation of busulfan exposure and dose recommendations was compared to the clinical practice values, aiming for the predefined target exposure of 600 - 900ng/mL. Estimation performance was tested using several LSSs.
LSSs using 3 or 4 plasma busulfan concentrations at 2, 3 and 6, or 2, 3, 5 and 6 hours were developed that demonstrated good precision and minimal bias with rRMSE values of 2.2 and 1.1% and MAE values of 1.7 and 1.6%, respectively, and best agreement with both AUC-ISS and adjusted doses (limits of agreement <±5%). AUC estimated by 4-sample model had the least deviation from the ISS-based reference (1.6±1.3 %), Similarly, AUC estimated by 3-sample model had very small deviation from the ISS-based reference (1.7±1.2%). When administering the doses calculated using 3-sample or 4-sample models, was simulated and the resulting AUC and Css were calculated using the observed AUC-ISS value, all patients achieved AUC and Css within the target range (<±5%).
LSSs using 3 or 4 plasma busulfan concentrations at 2, 3 and 6, or 2, 3, 5 and 6 hours can be used to reliably estimate busulfan $AUC_{0-\infty}$ after IV administration in children undergoing hematopoietic stem cell transplantation (HSCT). The application of the 3 or 4-sample LSS will benefit patients, medical staff, and minimize costs associated with therapeutic drug monitoring (TDM).

*Poster 13*

**Inhibition of vasa vasorum angiogenesis induces thoracic aortic aneurysm**

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**Objective:** Thoracic aortic aneurysm is a life-threatening condition resulting in dilatation and potential rupture of the thoracic aorta. The adventitial (outer) layer of the aorta contains numerous small blood vessels known as vasa vasorum. Previous work by our team revealed deficient vasa vasorum associated with medial hypoxia in human aortic aneurysm. We hypothesize that application of ABT-510 to the ascending thoracic aorta induces aneurysmal degeneration through its antiangiogenic effects on the vasa vasorum and consequent reduction in aortic wall perfusion.

**Methods:** The ascending aorta was exposed through a median sternotomy in New Zealand White rabbits (n=6). The proximal ascending aorta was treated with ABT-510-soaked sponge while the distal portion was treated with a vehicle control. Transthoracic echocardiography (TTE) was undertaken immediately prior to the procedure and 14 days post-operatively to measure the aortic diameter. The harvested aortic tissue was analyzed with H&E staining, Verhoeff-Van Gieson staining, and immunohistochemistry.

**Results:** TTE revealed aorta treated with ABT-510 exhibited an increase in aortic diameter over 14 days when compared with control (36.08% ± 7.35 vs 13.84% ± 3.63, p=0.031). On histologic analysis, treated aorta had decreased vasa vasorum count, decreased vasa vasorum cumulative area, and decreased vasa vasorum indexed cumulative area relative to control. Immunohistochemistry showed evidence of increased hypoxia, decreased elastin, and smooth muscle cell loss in the medial and adventitial layers of treated aorta.

**Conclusions:** Our findings suggest that inhibition of adventitial vasa vasorum via anti-angiogenic signaling and a reduction in aortic wall perfusion cause aneurysmal degeneration. Clarifying adventitial-based biomolecular pathways of aneurysm formation could enable development of targeted, pro-regenerative therapies that restore perfusion to the aortic wall and prevent the degenerative sequelae of elastic fragmentation and smooth muscle cell loss.

*Poster 119*

**Cold-sleep for long duration spaceflight**

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Optimization of physical and cognitive performance of astronauts while conserving resources is vital to successful long-duration spaceflight. Lowering metabolism for deep space missions would reduce consumables, oxygen consumption, and carbon dioxide excretion. Increasing sleep would reduce perceived mission duration and potential for psychological distress. Intravenous sedatives can reduce metabolism and wakefulness with lower core body temperature (Tc / 'cold-sleep') but are impractical for self-administration.

We recruited healthy volunteers to test if orally administered sedatives can achieve metabolic suppression.

Participants ingested a telemetry capsule to record core temperature. We measured metabolic rate (metabolic equivalents (METS)) using the canopy method of indirect calorimetry. We placed surface cooling pads on the participant's back to promote heat loss. After baseline measurements, participants ingested the sedative, and we recorded vitals for 4 hours, or until vitals returned to baseline. We assessed drug effect on metabolic rate, Tc, and other vitals by comparing each participant's data to their baseline, expressed as mean differences (95% confidence intervals, CI).

Eight participants took 1 mcg/kg of sublingual dexmedetomidine. Metabolic rate declined 16% from 0.90 (SD 0.19) METS to 0.77 (SD 0.10) METS (Δ= -0.14, 95% CI -0.25 to -0.03). Tc declined 0.6ºC (95%CI -1.0 to -0.2 ºC) from 37.1ºC (SD 0.3) to 36.4 ºC (SD 0.5). We observed mild decreases in heart rate and blood pressure. Four participants took 8 mg tizanidine orally. Metabolic rate declined by 11% from 0.92 (SD 0.10) METS to 0.82 (SD 0.09) METS (Δ = -0.10, 95%CI -0.15 to -0.05). Tc declined 0.7ºC (95% CI -1.3 to 0.0ºC) from 36.9 ºC (SD 0.06) to 36.2 ºC (SD 0.4).
Sublingual dexmedetomidine achieves many effects required for cold-sleep. However, effects are short-lasting and will require innovative dosing or other routes for long-duration use. Despite a robust effect on Tc, 8 mg tizanidine has less effect on metabolism, suggesting this dose may not be sufficient for this application.

*Poster 82*

**Evaluating positive-allosteric-modulator-induced conformational changes of $\alpha$3 glycine receptor**

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About 11-40% of US adults suffer from chronic pain, which is often treated with opioids. Due to frequent opioid misuse, alternative approaches to pain management are desperately needed. Recent studies have suggested that positive allosteric modulators (PAMs) of glycine receptors (GlyRs) are potential non-opioid analgesic alternatives. Tetrahydrocannabinol (THC), an active ingredient in marijuana, has been identified as a GlyR PAM. Early experiments discovered a series of molecules with potential affinity for the THC binding site, but their potency and efficacy as GlyR PAMs have not been fully explored. This study aims to explore the potency and efficacy of this series of molecules to better understand allosteric modulation of GlyR and to identify new, potentially better GlyR PAMs for pain treatment. Weighted ensemble (WE) simulations were used to study the impact of modulator binding (potency) to the THC binding site and the induced conformational changes to the ion channel function (efficacy). A hydrated membrane patch containing an $\alpha$3 GlyR structure with THC in the experimentally suggested binding pocket was created to test THC binding stability and analyze the induced conformational changes in the system. Subsequent molecular dynamics (MD) simulations of the $\alpha$3 GlyR with other PAM candidates were carried out to determine the optimal interactions for potency and efficacy. The study provides insight into the structural understanding of GlyR modulation, including the stability of THC binding site and the conformational changes induced by binding. The MD simulations of other PAM candidates provide insight into interactions of GlyR modulation, to try and establish a correlation between potency and efficacy. We have developed a process to computationally analyze both potency and efficacy of potential PAMs in the THC binding site. A more thorough understanding of the structural characteristics of the THC binding site will enable the discovery of more effective PAMS for the $\alpha$3 GlyR.

*Poster 49*

**Development and validation of an older adult nutrition equity index (NEI) and association with the healthy eating index (HEI) in older black and white U.S. adults**

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Objectives: We developed a novel, broader measure of nutrition equity in older U.S. adults that includes functional ability. We examined the convergent validity of the nutrition equity index (NEI) with food insufficiency (FI) via the Healthy Eating Index (HEI).

Methods: The Health, Aging, and Body Composition Study (Health ABC) is a prospective, longitudinal cohort of Medicare-eligible, community-dwelling Black and White men and women. Included participants (N = 2569, 74.7 ± 2.9 years) had baseline (1997-98) and 1 year follow-up nutrition equity-related data, and 1 year follow-up FI, key covariates, and 1995 HEI from a 108-item interviewer administered food frequency questionnaire. FI was a modified validated question on ample food amount/variety from the U.S. Department of Agriculture. NEI consisted of 8 questions (including FI): anxiety about money for food, limited money for food, hunger, home food acquisition, and difficulty: shopping, preparing meals, and carrying groceries. Participants received a point on the NEI each time a response indicated nutrition equity. Low nutrition equity was classified as 0 – 7 out of 8. Convergent validity was assessed via multivariable linear regressions between FI and NEI, respectively, with HEI, adjusted for sociodemographic, lifestyle and comorbidity factors.

Results: 13.4% of the sample had FI and 44.9% had low NEI. Black older adults were more likely to have FI (20.3%) and low NEI (59.7%) with Black women disproportionately affected ($p<0.001$ for all). FI (vs. non-FI) and low NEI (vs. high NEI) were more likely to have <high school education and lower income ($p<0.001$ for all). In age, sex, and energy-adjusted models, FI was associated with 2.5 point (95% CI: [-3.9, -1.2]) lower HEI score, and low NEI with 2.0 point (95% CI: [-3.0, -1.1]) lower HEI score. Adjusting for low education (34.8% FI; 31.1% low NEI) attenuated associations of FI and NEI with HEI.

Conclusions: Low NEI included >30% more participants vs. FI alone and showed convergent validity. NEI was similar to FI for HEI associations and identified more older adults with nutrition inequity.

*Poster 14*

**The absence of phosphorylation in amelogenin leads to acidification of forming dental enamel**

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**Introduction**: Enamel is the most highly mineralized tissue of the human body, made of densely-packed and well-arranged hydroxyapatite (HA) crystals. During its formation, it is composed predominantly of a protein matrix. These enamel matrix proteins (EMPs) are crucial to reaching enamel's thickness and unique crystalline structure, although their precise roles have not yet been fully elucidated. The most abundant of these proteins is amelogenin (AMELX). It has a single phosphorylation located on Serine 16, that is essential in controlling enamel formation. Earlier *in vitro* studies showed that phosphorylated AMELX stabilizes amorphous calcium phosphate and prevents HA crystal formation. *In vivo*, in a mouse model $AMELX^{Ser16Ala}$ knock-in (KI) which lacks AMELX phosphorylation, apatite crystals form faster and the enamel structure of KI mice is severely affected.

**Objective**: To test the hypothesis that accelerated enamel mineralization in $AMELX^{Ser16Ala}$ KI mice induces local acidification that also affects forming enamel mineral composition.

**Methods**: Mandibular incisors from 8-week-old wild-type (WT) and KI mice were isolated. They were freeze-dried and immersed in bromocresol purple (BCP) pH indicator to visualize pH differences in forming enamel of KI and WT. To assess the structural differences in EMPs and enamel mineral composition, we conducted FTIR microspectroscopy in reflectance mode.

**Results**: BCP staining showed the secretory-stage enamel was more acidic in KI vs. WT incisors. A distinct pattern of alternating low and high pH bands typical of the maturation-stage was absent in KI enamel. Consistent with a higher initial mineral density in KI enamel (unpublished), the mineral to protein ratio was greater in KI secretory stage enamel, as observed by FTIR. The ratio of acidic phosphate to phosphate was also higher in KI enamel, consistent with mineralization in an acidic environment. Together, our observations demonstrate that the lack of AMELX phosphorylation leads to acidification of secretory enamel and affects its mineral and organic composition.

*Poster 120*

**Disentangling the age effect on the relationship between white matter hyperintensities, executive function, and worry in late-life**

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Worry is a transdiagnostic phenotype encountered in multiple mental disorders and independently associated with cognitive impairment and cardiovascular disease. The role of white matter hyperintensities (WMH) in late-life severe worry is not well-characterized. We investigated the relationship between WMH, worry, executive function (EF). We collected demographic, clinical, neuropsychological, and MRI data from 110 participants (>50 yo) with varying worry severity. Worry severity was measured by Penn State Worry Questionnaire; EF was computed from trail-making and color-word interference tests; WMH volume was calculated from MRI with an automated fuzzy connected algorithm. We quantified the basic relationship between worry, EF, WMH, and age with correlation. To further characterize these relationships, we repeated the analysis with the sample stratified into old and young groups with a median age split, and we used a mixture of linear regressions to perform an age-WMH clustering analysis. Worry was negatively associated with global WMH ($\rho$=-0.27, p=0.014), though not after controlling for age ($\rho$=-0.19, p=0.078). EF was not significantly associated with age, WMH, or worry. No significant correlations were observed in the younger group between worry, WMH, EF, and age. In the older group (>60 yo), however, EF was significantly associated with WMH ($\rho$=-0.38, p=0.008) and worry ($\rho$=0.31, p=0.025). The cluster analysis revealed two latent WMH trajectories, “normal” and “accelerated” groups. The “accelerated” group showed greater worry severity than the “normal” group and a significant correlation between EF and worry. These findings demonstrate a complex relationship between worry and WMH, with age and EF playing major roles, especially with increasing age and WMH accumulation.

*Poster 83*

**Sirt5/Akt2/PGC1α signaling axis regulates lysosomal function in the retinal pigmented epithelium (RPE) cells: role in age-related macular degeneration (AMD)**

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**Abstract**: Lysosomes are critical for several homeostatic functions in the RPE cells, particularly, phagocytosis and autophagy. Decline in lysosomal function has been linked with AMD pathogenesis, but the underlying pathways regulating lysosomal function during disease progression remains elusive. We and others have recently shown that Akt2 is activated in the macular RPE of human dry AMD donors. Activated Akt1/Akt2 signaling inhibits Transcription factor EB (TFEB) nuclear activity, thereby downregulating both autophagy and lysosomal function. Herein we tried to ascertain the molecular cascade involving Akt2 activation-mediated deregulation of lysosome function and autophagy in RPE cells and its implications in AMD.

TFEB nuclear localization is diminished in RPE cells from human AMD donors with the *Cfh* Y402H (most prevalent) risk allele and Akt2 is upregulated in iPSC-derived RPE cells from the same donors. We observed that overexpression of Akt2 also inhibits the TFE3-mediated compensatory maintenance of lysosomal function in *Tfeb* KO mouse embryonic fibroblast (MEF) cells. Moreover, in a mouse model overexpressing Akt2 specifically in the RPE (*Akt2* KI) we noticed downregulation of lysosomal markers like Lamp1, Cathepsin D/L and autophagy flux in the RPE along with an atrophic AMD-like phenotype as the mice age. Human-high throughput protein-protein interaction and co-immunoprecipitation studies showed that Akt2 and Sirt5 are binding partners. Both of these proteins co-regulate one another through PGC1α and thereby modulate TFEB activity in RPE cells. Moreover, inhibiting Akt2 activation by treating mice with an mTOR-independent TFEB activator, trehalose, rescues the abnormalities in lysosomal function and autophagy in the *Akt2* KI RPE cells.

Our results show that Akt2 activity in RPE cells is regulated by Sirt5, whereas the mutual cooperation between the two proteins modulate TFEB activity/autophagy and abnormality in this regulation is associated with AMD pathogenesis.

*Poster 15*

**Hepatocyte-specific β-catenin overexpression reduces bile stasis by augmenting biliary cell-like reprogramming in murine model of intrahepatic cholestasis**

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**Background & Aim:** Cholestasis is a chronic progressive liver disease with an impaired biliary system. To develop therapeutic strategies, we aimed to target β-catenin overexpression in hepatocytes (HCs) that can mediate their reprogramming to biliary-like cells and contribute to functional de novo biliary branches that will help improve intrahepatic cholestasis.
**Methods:** Age matched WT control (Con) and TG (overexpressed β-catenin in HCs) mice, both containing ROSA26-stopflox/flox, were injected with AAV8-TBG-Cre to permanently label HCs with EYFP and then fed DDC diet for different time points. Mice were analyzed for bile flow, fibrosis, cholestatic injury and HC-biliary cell like reprogramming.
**Results:** TG mice showed significantly increased bile flow rate (BFR) as compared to Con mice after DDC diet. Liver histology and porphyrin measurement showed significantly less porphyrin accumulation in TG as compared to Con. This was concomitant with decreased hepatic bile load in TG mice than the Con. Analysis of bile homeostasis and transport genes demonstrated comparable levels of expression in TG and Con. This suggests a plausible mechanism independent of bile metabolizing genes that contributes to increased BFR observed in TG mice. HC-derived duct-like structures positive for both EYFP and Sox9 were observed more frequently in TG than Con. We also observed increased expression of hypophosphorylated active β-catenin & phosphorylated Tyr654 β-catenin in TG than Con. Furthermore, an increased number of A6-positive HCs was observed in TG as compared to the Con, indicating more number of intermediate HC phenotype undergoing reprogramming to biliary cell type in TG and hence better potential for improved injury outcome.
**Conclusion:** Mice with β-catenin overexpressing HCs showed significantly improved BFR, reduced porphyrin and decreased hepatic bile accumulation in response to the biliary toxin DDC. Using HC fate-tracing, we observed increased biliary cell markers in TG HCs that is suggestive of HC-to-biliary cell reprogramming potentially contributing to enhanced bile flow

*Poster 121*

**Direct electrical stimulation of the motor thalamus restores speech motor deficits following traumatic brain injury**

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Traumatic brain injury (TBI) is a leading cause of permanent motor disability in the United States often resulting in the complete or partial loss of speech production. While the true level of incidence is not known, studies have estimated that as many as 65% of TBI patients suffer from dysarthria. Speech therapy is the gold standard for treating language deficits despite its minimal impact on more severe cases. We posit that thalamic stimulation could improve speech motor deficits by augmenting the facial muscle activity involved in speech. Indeed, we recently demonstrated in non-human primates (NHP) and human participants that targeted stimulation of the motor thalamus drives excitatory inputs to M1 resulting in potentiate motor output via the corticospinal tract. We also observed increased facial muscle responses in NHPs with stimulation. *Here, we present show that motor thalamus stimulation facilitates speech production in two chronic TBI patients.* We performed acute stimulation studies in 2 TBI implanted subjects with mild and severe speech impairments. The participants completed speech-therapy exercises to measure their articulation and speech fluency. The mild subject repeated two-word "tongue-twister" phrases as quickly as possible over 20 seconds. The severe subject recited single words five times as clearly as possible. To quantify performance, we collected video and audio recordings. All tasks were repeated with and without paired thalamic stimulation at 50 Hz. Stimulation resulted in improved sustained sound, clearer speech, and cleaner phoneme separation, or more refined articulation. Additionally, we observed a reduction in consonant slurring and, in the mildly impaired subject, we observed a significant decrease in the number of errors in the "tongue-twister" task. These preliminary results provide promising evidence that thalamic stimulation to enhance facial motor output could pave the way for improving speech production in TBI patients and other patient populations suffering from motor speech disorders.

*Poster 16*

**Neutrophil extracellular traps induced by surgical stress regulate cancer metabolism leading to tumor growth**

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Although surgery is a crucial intervention to cure malignancies, its accompanying immune response can enhance tumor progression. Neutrophil Extracellular Traps (NETs) systemically released during surgical stress may contribute to this process. Since stressed cancer cells need a metabolic adaptation to grow, we aim to explore the direct effect of surgical NETs in regulating cancer metabolism leading to tumor growth and metastasis.

To assess the effect of surgical stress, mice were injected with cancer cells subcutaneously or intravenously followed by multiple laparotomies, with or without intraperitoneal DNAse (NET inhibitor) perioperatively. Our results showed that mice subjected to laparotomy had significantly larger subcutaneous tumors and lung metastases compared to control, which correlated with elevated levels of serum NETs markers. DNAse treatment significantly reversed these findings.

Cancer cells were also cultured *in vitro* with NETs from freshly isolated and stimulated neutrophils. NETs induced cancer cell proliferation more with addition of oleate and under hypoxic conditions. Bulk-RNAseq of cancer cells revealed activation of fatty acid (FA) metabolism pathway upon NETs treatment, paralleled with an upregulation of FA transport gene expression, fluorescent-FA uptake, and Acetyl-CoA from FA metabolism. Moreover, etomoxir (CPT1a inhibitor) reversed cancer proliferation induced by NETs.

In conclusion, surgical insult promotes tumor progression through metabolic reprogramming by systemically released NETs. Elucidating the molecular mechanism may help in finding therapeutics to prevent the protumorigenic effects of surgical stress.

*Poster 141*

**dbGaPCheckup: an R package to perform pre-submission checks of dbGaP-formatted subject phenotype files**

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Data archiving and distribution is essential to scientific rigor and reproducibility of research. The National Center for Biotechnology Information's Database of Genotypes and Phenotypes (dbGaP) is a public repository for scientific data sharing of omic-based research. To support curation of thousands of complex data sets, dbGaP has detailed submission instructions that investigators must follow when archiving their data. We developed dbGaPCheckup, an R package which implements a series of check, awareness, reporting, and utility functions to support data integrity and proper formatting of the subject phenotype data set and data dictionary prior to dbGaP submission. For example, as a tool, dbGaPCheckup ensures that the data dictionary contains all fields required by dbGaP, and additional fields required by dbGaPCheckup; the number and names of variables match between the data set and data dictionary; there are no duplicated variable names or descriptions; observed data values are not more extreme than the logical minimum and maximum values stated in the data dictionary; and more. The package also includes functions that implement a series of minor/scalable fixes when errors are detected (e.g., a function to reorder the variables in the data dictionary to match the order listed in the data set). Finally, we also include reporting functions that produce graphical and textual descriptives of the data to further reduce the likelihood of data integrity issues. Our innovative assistive and timesaving tool fills an important gap for researchers by making dbGaP submission of large and complex data sets less error prone. The dbGaPCheckup R package is freely available on CRAN and developed on GitHub.

*Poster 84*

**Microglial reprogramming by HV1 antagonism protects from inflammatory neurotoxicity**

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The precise mechanisms determining microglia activation's neurotoxic or neuroprotective role remain poorly characterized. Metabolic changes are important for microglial phenotype determination. Metabolism, in turn, can be strictly regulated by changes in intracellular pH. We tested whether pharmacological targeting of the microglial voltage-gated proton channel 1 (Hv1), an important regulator of microglial pH, is critical for reducing inflammatory neurotoxicity while maintaining the neuroprotective components of activation. We explored the activation profile during pharmacological Hv1 inhibition using mouse primary microglia.

Lipopolysaccharide/gamma interferon-mediated activation-induced widespread production of proinflammatory mediators, as well as reactive species and phagocytic activity. In co-cultures with rat cortical neurons, the ensuing neurotoxicity was mainly attributable to the release of tumor necrosis factor alpha (TNFα), reactive oxygen species, and zinc. Strikingly, pharmacological inhibition of Hv1 largely abrogated inflammatory neurotoxicity not only by reducing the production of cytotoxic mediators but also by promoting neurotrophic molecule production and restraining phagocytic activity. Importantly, this Hv1-mediated change from a pro-inflammatory to a neuroprotective phenotype was associated with metabolic reprogramming.

Finally, Hv1 antagonism not only reduced inflammatory neurotoxicity but also promoted neuroprotection against a separate, excitotoxic injury. Our results strongly suggest that Hv1 blockers will provide an important therapeutic tool against a wide range of inflammatory neurodegenerative disorders.

*Poster 50*

**Edutainment for anti-racism education: development of an intervention for graduate medical trainees**

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**Background:** Racism is a pervasive social problem that influences medicine, highlighting the need for interventions. One promising educational technique—referred to as edutainment—utilizes clips from television shows as an instructive strategy. We conducted a mixed-methods study to assess the acceptability of edutainment for anti-racist curricula for graduate medical trainees (i.e. residents).

**Methods:** We conducted a survey of underrepresented in medicine (URM) medical faculty and focus groups with medicine, psychiatry, and pediatrics residents. URM faculty were randomly assigned 4 of 8 clips accompanied by closed and open-ended items. Focus group participants viewed selected clips and provided feedback. We calculated descriptive statistics for closed-ended survey items and employed thematic analysis for open-ended items and focus group transcripts.

**Results:** Twelve URM faculty completed the survey, providing positive feedback for all clips. Thus, we included all clips (n=8) in the focus groups. For each of the participating specialties we conducted 2 focus groups (2-11 participants each, total n=25) with participants viewing 4 of the 8 clips. Analysis of focus group transcripts found that participants were receptive to the edutainment approach (e.g. *"I really like this use of media"*) and participants thought the selected clips portrayed important issues (e.g. *"We talk a lot about how the goal should be equity..."*). While feedback as to key discussion points were similar across specialties, feedback as to the realism and acceptability of certain clips differed by specialty. Triangulation of survey and focus group results found differences in acceptability of specific clips.

**Conclusion:** Findings from this study suggest edutainment may be a promising educational modality for anti-racism education among resident physicians. However, differences between residents of different specialties and residents and attendings suggest the importance of including input from a diverse group of participants and the importance of tailored approaches to curricular design.

*Poster 51*

**Wearable but not always reliable: evaluating the reliability and validity of psychophysiological signals from wearable devices in laboratory and ambulatory settings**

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Despite the increasing popularity of ambulatory assessment, the reliability and validity of psychophysiological signals from wearable devices is unproven in daily life settings. The present study evaluated the reliability and validity of two wearable devices (Movisens EcgMove4 and Empatica E4) in the lab and daily life among adults aged 18-64, with a well-validated device (Mindware) as the laboratory gold standard. Results revealed that the ECG-based chest strap (EcgMove4) provided more reliable and valid heart rate variability (HRV) measurements than the PPG-based wristband (E4) in both laboratory (split-half reliability: 0.95-0.99 vs. 0.85-0.98; concurrent validity: 0.97-1.00 vs. 0.85-0.99; valid data rate: 93.10% vs. 26.14%) and ambulatory settings (split-half reliability: 0.95-0.99 vs. 0.85-0.98; concurrent validity: 0.95-1.00 vs. 0.75-0.98; valid data rate: 82.94% vs. 8.79%); and the poor valid data rates of Empatica PPG signals in the laboratory could be partially attributed to participants' hand movements ($r = -.27$, $p = .03$). Both wearable devices' valid data rates in ambulatory settings were lower than in laboratory settings, with a 10% decrease for Movisens ECG, 17% for Empatica PPG, and 1.5% for Empatica EDA. In laboratory settings, heart rate derived from EcgMove4 and E4 both exhibited higher concurrent validity than HRV metrics (ICCs 0.98-1.00 vs. 0.75-0.97), and number of skin conductance responses derived from E4 showed higher concurrent validity than skin conductance level (ICCs 0.38 vs. 0.09). Although the reliability and validity of wearable devices are improving, our findings reveal gaps indicating caution when selecting devices that yield consistently robust and valid signals.

*Poster 142*

**Improved 3D modeling of choroidal Haller's sublayer vasculature based on swept-source OCT scans using Phansalkar thresholding**

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The choroid, a dense vascular layer in the posterior to the outer retina, serving various metabolic functions such as supplying oxygen and nutrients to the retina layers. Studies indicate structural changes in choroidal vasculature are inter-linked with several vision-threatening diseases including age-related macular degeneration (AMD) and central serous chorio-retinopathy (CSCR). Precisely modeling choroidal vasculature enables accurate quantification of vessel-level biomarkers such as cross-section diameter of vessel. In response, we proposed and validated a methodology to improve modeling choroidal vasculature in 3D using wide-field SS-OCT volumes.

This retrospective study was performed using both healthy and diseased wide-field SS-OCT volume scans taken from the Carl Zeiss Plex Elite 9000 device. We proposed an algorithm to model Haller's sublayer vasculature. We employ previously validated 3D residual U-Net to extract the choroid layer from SS-OCT volume and further, binarization based on Phansalkar thresholding to segment the choroidal vasculature. The accuracy of proposed vessel segmentation method was validated and compared with our earlier exponentiation enhancement binarization based on subjective grading performed on 5 healthy, 5 CSCR and 5 AMD eyes. The scores provided by the grader based on the accuracy of vessel segmentation in 2D B-scans and for 3D vasculature of both methods were recorded.

The mean 2D and 3D grading scores for the Phansalkar-based vasculature are observed to be 92.67% and 94% while for the exponentiation enhancement method, they are observed to be 77.67% and 73.33%, respectively. The proposed method significantly improves the performance of choroidal vessel segmentation.

*Poster 85*

**Contribution of post-natal *Prx1* expressing$^{+}$ cells to periodontal regeneration in mouse molars**

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**Background:** Post-natal skeletal stem cells expressing Paired related homeobox 1 (pnPrx1 cells) are known to play an important role in bone regeneration. It was reported that pnPrx1 cells are present within the continuously regenerating periodontal ligament (PDL) of the mouse incisor and are involved in the formation of the mouse molars' PDL. However, the potential involvement and contribution of pnPrx1 cells to periodontal regeneration in mouse molars remain unclear. In the present study, we aimed at identifying the role of pnPrx1 cells within the mouse molar periodontium in periodontal regeneration.

**Methods:** The contribution of pnPrx1 cells to periodontal regeneration in mouse molars was evaluated by creating a periodontal fenestration defect (~2mm in length, 1 mm in width, and 0.5 mm in depth) in the buccal aspects of the mandible involving mesial and distal roots of the first molar. Defects were created in the test group (Prx1-CreEr-GFP+/TdTomato+) (n=5) and control group (Prx1-CreEr-GFP-/TdTomato+) (n=5) tamoxifen treated mice. All mandibles were harvested at 7- and 30-days post-surgery for histology/fluorescence for detection of TdTomato (red) cells.

**Results:** TdTomato (red) cells were observed in the newly formed PDL and at the areas of PDL adjacent to and around the periodontal defect of the distal root of the first molar at 7-days post-surgery in test group. Compared to the 7-days post-surgery group, more red cells were observed at 30-days post-surgery in PDL surrounding the distal root and in the newly formed bone. No red cells were observed in control group at 7- or 30-days post-surgery. Incomplete formation of PDL and cementum was observed in both groups throughout the observation period.

**Conclusion:** pnPrx1 cells contribute to the regeneration of surgically created periodontal defects of the mouse molars. Additional studies are being performed to evaluate whether these cells can be harnessed to foster periodontal regeneration.

*Poster 86*

**Evaluation of the time needed for BCG to be alive to protect against tuberculosis in intravenously BCG vaccinated SIV+ macaques**

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Effective means of improving Bacille Calmette-Guérin (BCG) immunogenicity to protect against pulmonary tuberculosis (TB) is needed, especially among people living with HIV. Intravenous (IV) administration of BCG previously showed significant enhancement of immune responses and conferred ~75% protection against TB. Using our SIV/*M. tuberculosis* (Mtb) coinfection model (to mimic HIV/Mtb coinfection) in Mauritius cynomolgus macaques (MCM), we evaluated the length of time necessary for live BCG to elicit a protective immune responses by varying the timing of anti-BCG drug (isoniazid, rifampicin, and ethambutol: HRE) initiation. MCM were infected with SIVmac239 and randomly assigned into 4 different vaccination groups 16 weeks after SIV infection: intradermal BCG (BCG ID No HRE), BCG IV with HRE started 1 week post BCG (BCG IV 1wk HRE), BCG IV with HRE started 3 weeks post BCG (BCG IV 3wks HRE), and BCG IV without HRE treatment (BCG IV No HRE). The HRE treatment lasted for 8 weeks. Airways samples were collected by bronchoalveolar lavage (BAL), whereas lung tissue and lymph node were harvested at necropsy in a subset of BCG ID and BCG IV 3wks vaccinated MCM 20 weeks after BCG but before (Mtb) challenge. The remaining MCM were challenged with Mtb Erdman strain (~15 CFU). CD4 and CD8 T cell levels increased in BAL in all IV vaccinated MCM. CD4 T cells producing IFNg, IL-2, IL-17, and TNF peaked after BCG and were maintained until necropsy. There were increased tissue-resident T cells and cytokine production in lungs of IV vaccinated MCM compared to BCG ID. No signs of BCG dissemination were apparent in any IV vaccinated MCM. We are currently evaluating protection against infection and disease in the SIV+ MCM in each vaccination group.

*Poster 52*

**Changes in negative urgency and lack of premeditation are associated with changes in externalizing behaviors in the transition from childhood to adolescence**

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Adolescence is a critical period of development when more serious externalizing behaviors (e.g., aggression, delinquency, substance use, disruptive rule-breaking behaviors) tend to emerge. Understanding the processes underlying behavioral development in the transition from childhood to adolescence would help support youth as they enter adolescence and minimize the risk of externalizing behaviors. Prior work found that higher levels of two facets of impulsivity—negative urgency (acting rashly in response to negative emotions) and lack of premeditation (acting without thinking or planning)—in childhood predicted declines in externalizing behaviors from childhood into adolescence. Therefore, we investigated whether changes in negative urgency and lack of premeditation were associated with changes in externalizing behaviors over time.

Data were from the Adolescent Brain Cognitive Development (ABCD) study. Externalizing behaviors (parent-reported Child Behavior Checklist) and impulsivity facets (youth-reported UPPS-P Impulsive Behavior Scale) were measured at 9–10 and 11–12 years old. Youth ($N$ = 6,917) were included in analyses if they had complete data. Two latent change score models—for negative urgency and lack of premeditation—were used to investigate the relationship between changes in these impulsivity facets and externalizing behaviors across timepoints.

Between 9–10 and 11–12 years old, mean levels of externalizing behaviors, negative urgency, and lack of premeditation decreased over time ($p$ < .001). In both negative urgency and lack of premeditation models, decreases in each facet were associated with decreases in externalizing behaviors ($p$ < .001).

Findings suggest a developmental relationship between externalizing behaviors and negative urgency and lack of premeditation. These facets of impulsivity may be indicators of the developmental processes underlying maturation of externalizing behaviors. Teaching youth strategies to improve negative urgency and lack of premeditation in childhood could promote greater and more robust maturational declines in externalizing behaviors as they enter adolescence.

*Poster 143*

**Restricted crossing U-turn traffic control by interval type-2 fuzzy logic**

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The work presents a novel approach to Restricted crossing U-turn (RCUT) control, based on the interval Type-2 fuzzy system (IT2FS). Conventional intersections, around the world, are bottlenecks that cause a lot of delays for users of various transportation modes (e.g. drivers, pedestrians, and bicyclists). To reduce such delays, and potential exposure to risks of traffic accidents, traffic engineers have proposed a number of innovative intersection designs. These new, alternative intersection designs are usually safer for intersection users than the conventional (e.g., four-leg) intersections. This safety improvement comes from the fact that alternative intersections usually eliminate one or more left-turn traffic movements. In addition to these safety improvements, most of the alternative intersection designs improve mobility-related performance measures (e.g., decrease control delay and/or increase throughput) over conventional at-grade intersections.

RCUT design, among other various alternative intersections, proposes dislocation of left and through movements from the main intersection for an increase in safety and efficient traffic conditions. This concept assumes the rise of two new U-turn intersections downstream from the main intersection that can be controlled by traffic lights. RCUT design is justified when U-turn traffic demands, along with demands from and to minor streets, are significantly lower than arterial street ones. The semi-actuated traffic control seems the most appropriate control mode that should be applied at the RCUT. Evaluation of semi-actuated traffic control based on IT2FS is compared with other traffic control modes. The results show that semi-actuated traffic control, based on IT2FS, can generate statistically better results than other well-known controls.

In the last decade, IT2FS has become a popular approach for solving traffic control problems. In this research, in the first step, we created fuzzy logic RCUT traffic control system based on the interval Type-1 fuzzy system (T1FS). In the next step, we developed a similar system based on the T2FS. Previous research shows that T2FS can frequently outperform T1FS in the applications to traffic control problems, which encourages us to develop IT2FS for controlling traffic lights at RCUT. Considering all traffic demand scenarios IT2FS control strategy yields 10.6 %, 13.82 % and 10.78 % lower average control delay, the average number of stops, and average queue length than the second-best traffic control, respectively.

*Poster 87*

**Gasdermin-D-dependent Neutrophil Extracellular Traps (NETs) formation promotes hemophilic arthropathy in factor VIII-deficient mice and hemophilia patients**

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**Background:** Hemophilic arthropathy (HA) is the predominant pathophysiology resulting from recurrent joint bleeding in individuals with hemophilia and leads to permanent joint damage, chronic pain and reduced quality of life. Recent evidence suggests that joint-bleeding may trigger sterile inflammation in the joints and promote the innate immune pathways activation in neutrophils.

**Aim:** To determine whether neutrophil Gasdermin-D (GSDMD)-dependent NETs formation promotes HA.

**Methods:** We adapted model of knee joint HA in FVIII-total knockout ($F8^{TKO}$) mice and collected blood samples from hemophilia patients diagnosed with HA. Scoring of the bleeding severity and histology of injured joints were conducted to assess the joint injury in mice. Imaging-flow-cytometry and ELISA assays were used to evaluate the number of circulating NETs and their markers in patients diagnosed with HA and mice with induced HA. Neutrophils from HA patients, $F8^{TKO}$ mice and matched controls were isolated. The activation of GSDMD-pathway components was evaluated in neutrophils using qRT-PCR (gene expression) and western blotting (protein levels and cleavage).

**Results:** $F8^{TKO}$ but not control mice manifested knee-joint injury and severity of bleeding 5-days post HA induction. Circulating NETs (cNETs) and cNETs markers were significantly abundant in the plasma of hemophilia patients diagnosed with HA and $F8^{TKO}$ mice following knee injury but not plasma of control humans or mice. Neutrophil genes expression of proteins contributing to GSDMD-mediated NETs formation was higher in HA patients and $F8^{TKO}$ mice with HA compared to matched controls. Western blot analysis showed activation of GSDMD pathway in neutrophils isolated from HA patients and $F8^{TKO}$ mice but not in controls.
**Conclusions:** These findings are the first to suggest that formation of cNETs contribute to pathogenesis of HA in hemophilia. Preliminary analysis of gene expressions and protein levels in isolated neutrophils under HA conditions shows potential contribution of GSDMD pathway activation to joint damage in HA.

*Poster 17*

**Evaluation of novel amniotic membrane and umbilical cord-derived nerve wraps in sciatic nerve recovery**

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Peripheral nerve injuries (PNIs) can occur due to various causes and lead to reduced sensation, muscle weakness, and impaired motor abilities. Nerve wraps are being researched to promote rapid nerve recovery after PNIs by assisting neurite extension, insulating and protecting injured peripheral nerves, reducing axonal escape rates, and the inflammatory response triggered by surgical trauma. The objective of this study is to investigate whether amniotic membrane and umbilical tissue-derived nerve wraps can improve nerve regeneration and recovery in rats. The experimental design involved using placental and umbilical cord-derived nerve wraps, polycaprolactone/glial cell line-derived neurotrophic factor nerve wrap, and bovine collagen wrap, to enhance nerve regeneration in a Lewis rat sciatic nerve transection model. Sensory recovery was evaluated weekly by measuring responses to different external stimuli, including an acetone evaporation technique, tactile stimuli (von Frey filaments), and a mechanical stimulus (rat pincher). At six weeks post-injury, all animals will be sacrificed. Tissue samples from the gastrocnemius muscle will be collected for an evaluation of cross-sections for fiber surface area and the neuromuscular junctions. Electrophysiological assessments will be performed by placing a custom-built nerve cuff electrode distal to the nerve repair site and on the gastrocnemius muscle to determine compound muscle action potentials. The neural tissues within the nerve guide will be assessed for markers of neurogenic inflammation and neuronal recovery.
As part of the preliminary study, a baseline for sensory assessments was conducted before surgical procedures through confirmation with nociceptive behaviors. Mechanical pain threshold was evaluated in five healthy Lewis rats, with an average weight of 280 g, using a commercially available rodent pincher. A threshold of 4234±1231 N was achieved, confirming the validity of the approach. Tactile threshold was assessed using von Frey filaments that applied a precise amount of force to the skin of the rats. The animals were placed on a specially designed von Frey mechanical testing rack. The test results showed a tactile threshold of 4.9±1.5 g at the 50% level, supporting literature findings and providing further validation for the approach. To assess thermal sensitivity, an acetone evaporation test was conducted. Lewis rats weighing between 240-330 g were sprayed with 0.1 ml of acetone using a spraying apparatus. The technique involved five rounds of acetone dispersion and the tallying of the positive responses. The observed response rate of 23% is within an acceptable range for this method. Following the completion of the sensory baseline study on healthy rats, the muscle tissue fixation protocol for cryosectioning was optimized. The optimal method involved perfusing a 4% PFA solution through the heart of the rat, followed by dissection of the muscle and subsequent external fixation for an additional 2 hours. Cryosectioning and staining were performed, resulting in clear and well-preserved tissue morphology. It is expected that the efficacy of nerve wraps in a rat sciatic nerve transection model will be determined upon completion of the study. The use of nerve wraps is expected to lead to enhanced nerve regeneration, functional and sensory recovery, and decreased muscle atrophy in the nerve wrap-treated groups.

*Poster 88*

**Understanding mitochondrial G-quadruplex formation and their potential as therapeutic intrabody scaffolds**

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**Background** The 16,500 base-pair mitochondrial genome contains a guanine-heavy rich mtDNA strand susceptible to G-quadruplexes (G4) formation, inhibiting replication and transcription. G4 formation is implicated in various mtDNA-related diseases which are often fatal and lack treatment. The G4 Hunter algorithm and others have predicted over a hundred G4s in the mitochondrial DNA, but their biological function and relevance still need to be fully understood.

**Methods and Results** HF1 and BG4 intrabodies are single-chain variable fragments (scFv) used to study G4s by chromatin immunoprecipitation and sequencing (ChIP-seq). For localization, our study engineered these scFvs to contain a mitochondrial transport sequence, and a human influenza hemagglutinin (HA) tag for detection and isolation. Mitochondrial scFv presence was confirmed by confocal microscopy of transduced 143B osteosarcoma with HF1 and BG4. Following preliminary data analysis, we developed clonal cell lines with matching expression levels of the intrabodies. Equalized expression levels are essential to discern sequence specificity. These selected clonal lines were subjected to the ChIP enrichment process and the enriched mtDNA analysis is underway to determine G4-specific interaction through qPCR and sequencing.

**Conclusion and Future Direction** The need to address untreatable mitochondrial diseases has made mtDNA G4s prime candidates for therapeutic targeting. ScFvs that bind G4 structures in cells will be identified through sequencing and verified by qPCR. It will be paramount to identify mitochondrial G4-specific intrabodies that stabilize mutagenic sequences and stall their replication, resulting in heteroplasmy shift and reduction of their pathogenic effects.

*Poster 53*

**Trajectories of opioid prescribing by dentists and OMFS in the US**

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**Objective:** Opioid prescribing by dentists and oral and maxillofacial surgeons (OMFS) has decreased, yet some still prescribe at high rates. Our objective was to identify high prescribers through defining trajectories of opioid prescribing.

**Methods:** The IQVIA Longitudinal Prescription dataset identified actively prescribing dentists and OMFS (≥20 prescriptions of any medication) from 2015-2019. Group-based trajectory models (GBTM) identified opioid prescribing trajectories based on annual prescribing rates (number of opioids/total number of prescriptions*100). Chi-square or Mann–Whitney U tests identified significant differences in characteristics by trajectory group.

**Results** From 2015-2019, 70,978,575 opioids were prescribed among 199,145 dentists/OMFS. GBTM identified 8 groups of prescribers. In group 1 (14.3% of prescribers), opioids comprised <1% of all prescriptions. Groups 2, 3 and 4 (15.2%, 17.7% and 13.0% of prescribers, respectively) prescribed at low rates (2015=5.5%, 12.5% and 16.9%; 2019=1.5%, 4.4% and 11.9%, respectively). Group 5 (7.9% of prescribers) decreased prescribing by 82.7% (2015=29.4%; 2019=5.1%). Groups 6 and 7 (16.3% and 12.1% of prescribers) prescribed opioids at moderate rates (2015=28.7% and 39.2%; 2019=18.1% and 28.8%, respectively). Group 8 (3.5% of prescribers) consistently prescribed at high rates (2015=54.6%; 2019=44.7%). Within this high group, 4 trajectories were identified; 3 groups did not change their prescribing while 1 group (7.5% of high prescribers) rapidly declined prescribing. Compared to a combination of the 3 groups, the decliner group had more dentists (85.4% vs 80.8%) and fewer OMFS (13.0% vs 18.4%). At baseline, decliners had more Medicaid patients (2.5% vs 1%) and a higher opioid prescribing rate (95.5 vs 91.6 prescriptions/provider/100 patients, $p<0.001$ for all).

**Conclusion:** GBTM identified consistently high opioid prescribers; a few high prescribers significantly decreased prescribing. Understanding characteristics of the decliners can inform future opioid stewardship interventions.

*Poster 18*

**Evaluation of gadolinium-gold nanoparticles for MRI- and CT-based imaging of neurogenesis in brain tissue regeneration**

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Neurogenesis is upregulated after a stroke and is quintessential to the regeneration of brain tissue. To visualize the spatio-temporal participation of newly born neural progenitors in tissue regeneration *in vivo* using MRI, neural progenitors need to be tagged with a contrast agent that affords a distinction from native tissue. Gadolinium-gold nanoparticles (GdAuNP) have been shown to be taken up into neural progenitors *in vitro* and afford a robust $T_1$-weighted MRI contrast of transplanted cells *in vivo*. However, to visualize endogenous neurogenesis, *in vivo* uptake is required and the distribution of cells throughout the tissue requires a higher level of cellular contrast. 30 μL of GdAuNP solution (500 nM) injected *in vivo* into the lateral ventricle were taken-up by neural progenitors in the subventricular zone and afforded their visualization using MRI. Three formulations of GdAuNP are being compared to determine their *in vivo* uptake into neural progenitors, as well as the magnitude to $T_1$-weighted contrast. MRI detection of GdAuNP-labeled neural progenitors was validated using microCT and fluorescence-based immunohistochemistry. Longitudinal imaging will evaluate the retention of agent within cells and their potential to track neural progenitor migration. *In vivo* longitudinal imaging of endogenous neural progenitor migration will be essential to understand how these cells contribute to *de novo* brain tissue formation.

*Poster 122*

**Bone mineral density and tibial microarchitecture changes in division I male and female cross-country runners**

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Weight-bearing physical activity can improve bone mineral density (BMD), structure, and strength, but it remains unclear if bone adaptations to prolonged physical training are similar between elite male and female endurance athletes. To compare the effect of 6 months of sport training on areal BMD (aBMD) and tibial volumetric BMD (vBMD), microarchitecture, and strength between men and women. Twenty-one male (n=12; 19.4±0.5 yr; 21.4±0.4 kg/m$^2$) and female (n=9; 18.9±0.4 yr; 20.9±0.4 kg/m$^2$) Division I cross-country runners completed HR-pQCT scans at 4% (metaphysis) and 30% (diaphysis) of total tibial length and DXA scans of the total body, lumbar spine, and hip prior to and following the competitive fall season. Generalized linear mixed modeling was used to compare changes over time between men and women, adjusting for baseline BMI. Data are presented as estimated marginal mean±SEM, $\alpha$=0.05. At the tibial diaphysis, training increased (main effects of time) cortical area (286.8±5.4, 290.3±4.9 mm$^2$, p=0.016) and perimeter (77.7±0.6, 78.2±0.6 mm, p=0.007), stiffness (307.2±6.2, 310.8±5.6 kN/mm, p=0.021), and failure load (17.5±0.4, 17.7±0.3 kN, p=0.040). Training also increased (main effect of time) total body aBMD (1.238±0.014, 1.248±0.016 g/cm$^2$, p=0.001). No main effects of time were observed for the tibial metaphysis (4%). Men had lower total and cortical vBMD, but greater cortical area, perimeter, and thickness, stiffness, and failure load than women at the tibial diaphysis (main effects of sex, p≤0.032). Men had greater trabecular area and thickness, stiffness, and failure load than women at the tibial metaphysis (main effects of sex p≤0.003). Men had greater aBMD for the total body, femoral neck, and total hip (main effects of sex p≤0.037). No significant sex*time interaction effects were observed. Six months of cross-country training elicited positive bone adaptations for the whole body and at the diaphyseal tibia, which were similar between sexes. Sex-differences in parameters of bone health may also explain differences in risk for bone stress injury between men and women.

*Poster 54*

**Association between life's essential 8 cardiovascular health scores during pregnancy and vascular integrity in the placenta**

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**Background:** Adverse pregnancy outcomes (APOs) identify pregnant people at risk of later cardiovascular disease. Poor antenatal cardiovascular health (aCVH) is associated with higher APO incidence, indicating aCVH may be related to pregnancy health. Placental pathology is also common in APOs, but it is unclear if aCVH is directly related to placental health. The aim of this study was to examine the relationship between aCVH and placental vascularization.

**Hypothesis:** We hypothesized higher (healthier) aCVH scores would be associated with higher (healthier) vascularization in the placenta.

**Methods:** Participants enrolled in the Magee Obstetric Maternal & Infant Biobank and one of two prospective observational cohort studies examining activity patterns in pregnancy. aCVH was quantified during each trimester and averaged across gestation. Components included sleep, diet, smoking, physical activity, pre-pregnancy BMI, blood pressure, 50g glucose challenge test results, and gestational weight gain. Component scores were averaged for a composite score (possible range, 0-100). Immunohistochemistry of placenta tissue was performed. Sections were stained with CD34 antibody to highlight vascular endothelial cells and counterstained with hematoxylin. Software computed the number of pixels positive for CD34 (numerator) and the total pixels (denominator); the ratio was the proportion of villous tissue occupied by fetal vessels (FV%). Linear regression associated aCVH scores with FV%.

**Results:** Placenta tissue was obtained from 64 participants (mean$\pm$SD age = 32$\pm$4.9 years). aCVH score averaged across gestation was 72.6$\pm$10.7 points and decreased significantly from the first to third trimester (72.8$\pm$12.7 vs. 65.1$\pm$11.9, $p<0.01$). FV% was 26.3$\pm$5.13 percentage points. Associations between aCVH scores and FV% approached but did not reach significance ($p<0.2$) in each trimester and across gestation.

**Conclusion:** aCVH was not significantly associated with placental vasculature, though a small sample size limits conclusions. Replication in a fully-powered sample is warranted.

*Poster 19*

**Cell-type specific contributions to the acquisition and performance of an auditory categorization task**

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Senses connect our brain with the environment, allowing us to perceive the world around us. Auditory information enters the brain via a feed forward hierarchal pathway that canonically terminates in the auditory cortex (ACtx). An open question is how this information is processed then routed brain-wide to influence behavior. Excitatory projection neurons in the ACtx are broadly comprised of three groups; intratelencephalic (IT), extratelencephalic (ET), and corticothalamic (CT). These distinct populations target nodes of the ascending pathway as well as downstream regions classically associated with decision making, action, and reward. These organizational principles allow for the shaping of auditory representations across brain-wide neural networks. We hypothesize that ACtx projection neurons provide a critical link between auditory input and behavioral output, necessary for acquisition and performance of auditory-guided behaviors. To investigate this, we trained head-fixed mice to categorize amplitude modulated noise (AM) and bilaterally silenced neural populations during sound using stGtACR2. To ensure that ACtx was necessary for successful performance of this task, we silenced all excitatory neurons *en masse* and found that inhibition biased decisions towards one spout, ultimately leading to a significant reduction in categorization accuracy. Unexpectedly, silencing of any of the 3 projection neuron classes had little effect on mice's ability to categorize the rate of AM noise, indicating that no single projection is necessary for task performance and suggesting that multiple projections may work synergistically. This apparent disconnect between cell-specific and global inhibition led us to examine other consequences of targeted inhibition across learning. We found that longitudinally inhibiting ET led to a reduction in learning rate, evidenced by increased number of trials and sessions to achieve task proficiency. Furthermore, ET mice trained to expert level had lower accuracy across sessions compared to controls.

*Poster 20*

**Postprandial changes to systemic metabolism imprint durable changes on T cell immune responses**

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While costimulatory and cytokine signals can shape an antigen-stimulated T cell immune response, it is now clear that nutrient availability and intrinsic metabolic pathways play key roles in T cell function and fate. Indeed, the effects of post-prandial metabolism on T cell function and fate are unstudied. Here we show that short periods of fasting and refeeding can have long-lasting effects on T cell immunity. Directly ex vivo, T cells from fed hosts have higher mitochondrial capacity and volume compared to T cells from fasted hosts. Remarkably, these metabolic phenotypes persist after activation and 7 days of expansion in vitro. Further, when OT-I naïve T cells from congenically mismatched fasted and fed mice were co-transferred into Vaccinia$^{OVA}$-infected hosts, fasted T cells failed to fully engage an effector response and formed dramatically fewer memory T cells after viral clearance. Metabolomic profiling of serum revealed serum triglycerides as a likely culprit for postprandial metabolic reprogramming. Chylomicrons enriched from lymphatics of fed mice were sufficient to impart metabolic reprogramming on fasted T cells in a manner antagonized by the chylomicron protein apoCIII. T cells from LDL receptor (LDLR)-deficient mouse had the metabolic phenotype of fasted T cells and were insensitive to fed serum, confirming the role for triglyceride uptake in post-prandial T cell metabolic programming. Therapeutic T cells expanded in vitro from fasted mice failed to control tumor growth compared to those expanded from fed mice. Our data suggest that T cells are exquisitely sensitive to systemic metabolic changes in the postprandial period and inherit those metabolic programs for several generations. Further, our study highlights the need to consider diet content and timing as key factors in immunology, in immune cell analysis, vaccination strategies, and the generation of cellular therapies for disease including cancer.

*Poster 89*

**Selective vulnerability and circuit integration of olfactory bulb dopaminergic neurons after olfactotoxic sensory deprivation**

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One of the hallmarks of neurogenerative diseases is selective loss of specific sub-populations of neurons. Around 40% of dopaminergic (DA) neurons in the mouse olfactory bulb (OB) undergo cell death after a month of sensory input blockade but the remaining 60% are resilient to lack of sensory input. Furthermore, OB DA neurons are continuously generated throughout life, enabling them to fully repopulate after sensory input blockade. This makes OB DA neurons an ideal model system to determine selective vulnerability and how newborn DA neurons are functionally integrated to replace previously lost neurons.

Injection of an olfactotoxic drug, methimazole, enables us to see the impact of rapid elimination followed by gradual restoration of sensory input to the OB. CA chronic *in vivo* 2-photon imaging in DAT-cre;Ai9;Ai162 and THcre;Ai9 mice that express red fluorescent morphological marker to track survival and integration, and a green genetically encoded calcium indicator to track the odor response properties of individual neurons over weeks. We found that loss of DA neurons was significantly elevated during the first week after ablation of OB sensory input and in contrast, there was no effect on DA neuron loss 7-14 days after OSN ablation.

We are analyzing whether there are differences in the vulnerability of the previously described large embryonically generated and small postnatally generated DA neurons. We are also quantifying the rate of newborn DA neuron integration before and after ablation and whether there are differences in odor response characteristics between neurons that are vulnerable or resilient to sensory disruption. Distinguishing vulnerable neurons from their resilient neighbors will help with novel targeted therapeutic strategies for the treatment of neurodegenerative diseases.

*Poster 21*

**The long noncoding RNA LUCAT1 regulates inflammatory gene expression in human macrophages**

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The so called *"dark matter of the genome"* which contains thousands of genes including long noncoding RNAs (lncRNAs), is an attractive yet largely unexplored area of research. Emerging evidence indicates that lncRNAs play crucial roles in a variety of biological processes including cell identity, tissue homeostasis and metabolism by regulating gene expression. However, their functions in immune cells such as macrophages remain largely unknown. Here, we describe novel immune functions of a lncRNA, LUCAT1 (Lung Cancer Associated Transcript 1), in regulating inflammatory responses of human macrophages. *LUCAT1,* originally discovered as NRF2-regulated gene in lung cancer, is differentially expressed in a variety of tumor cells. However, its function under physiological contexts have not been described. Our results indicate that LUCAT1 is expressed in resting human neutrophils, monocytes and macrophages, and is highly induced via ERK-MAPK-AP1 axis in TLR4-activated macrophages. *LUCAT1* gene uses an alternative polyadenylation sequence to produce two major isoforms with distinct cellular localization - a 890 bp long cytosolic transcript and a predominant, 2.5 kb long nuclear RNA. Transient perturbation of LUCAT1 expression coupled with bulk RNA-seq analysis reveal that LUCAT1 regulates the expression of several immune genes including cytokines and chemokines (*CSF3, IL24, CXCL5* and *CXCL6*), matrix metalloproteinases (*MMP1* and *MMP3*) and others. Further, our results demonstrate that the nuclear LUCAT1 and not the cytosolic transcript is functionally active in regulating immune gene expression. The nuclear LUCAT1 acts *in trans* in a RNA-dependent manner to mediate these functions by associating with chromatin to promote the transcription of its target genes. Ongoing studies are focused on identifying LUCAT1-interacting nuclear proteins and how LUCAT1 is recruited to specific genomic loci in macrophages. Together, our study highlights the emerging paradigm that lncRNAs are important components of the gene regulatory circuits of the immune system.

*Poster 123*

**Eight-year effects of lifestyle intervention on kidney outcomes adjusting for time-updated lean mass**

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**Background:** The dependence of serum creatinine on muscle mass may limit the interpretation of creatinine-based estimated glomerular filtration rate (eGFR) after weight and muscle loss. Leveraging time-updated Action for Health in Diabetes (Look AHEAD) data, including lean body mass measured by dual x-ray absorptiometry (DXA), we aim to assess the effects of intensive lifestyle intervention (ILI) on eGFR slope over 8 years.

**Methods:** We performed a post-hoc analysis of Look AHEAD data collected from 2001-2015, restricted to participants who underwent at least two DXA scans. We applied linear mixed effect models to evaluate for within-individual eGFR slope (primary outcome) and mean eGFR (secondary outcome) over 8 years. We compared the analyses with and without adjustment for time-updated lean mass measured by DXA, which was performed up to four times during follow-up. We evaluated for effect modification by baseline eGFR on slope of eGFR and mean eGFR using the Wald test.

**Results:** 1369 participants (27% of the 5145 original trial participants) who underwent at least two DXA scans during the trial were randomized to ILI (N=678) or usual care (i.e., diabetes support and education) (N=691). At baseline, mean weight was 98kg, lean mass was 54kg, eGFR (ml/min/1.73m$^2$) was 90, and 82% had normal albuminuria. In analyses not adjusted for lean mass, there was no significant between-group difference in eGFR slope (+0.08; 95%CI -0.1, 0.3) or in mean eGFR (-0.1; 95%CI -1.2, 0.9) for ILI vs usual care. In the adjusted analyses, there was also no significant difference in eGFR slope (+0.08; 95%CI -0.1, 0.3) or in mean eGFR (-0.1; 95%CI -1.2, 0.9) between ILI and usual care. Baseline eGFR did not modify the effect on slope or mean eGFR.

**Conclusion:** In patients with type 2 diabetes, BMI ≥25kg/m$^2$ and good baseline kidney function, eGFR slope did not differ between ILI and usual care with and without adjustment for lean mass. More evidence is needed to determine if accounting for changes in lean mass improves interpretation of eGFR in longitudinal studies of patients with kidney disease when muscle loss is suspected.

*Poster 22*

**Decoding the regulatory landscape of tumor-associated macrophage in breast cancer**

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The tumor microenvironment (TME) plays a critical role in tumor progression and includes heterogeneous cell types that can have pro-tumorigenic (e.g., immunosuppressive macrophages) and anti-tumorigenic cells (e.g. cytotoxic T cells). Among the most abundant cell types in the TME, an inflammatory cell population such as tumor-associated macrophage (TAM), is an influential component for tumor growth and immune-therapeutic response, which has potential to provide diagnostic and prognostic biomarkers. However, TAM-based biomarkers that predict tumor aggressiveness and the complex interaction network with cancer cells and other immune cells in breast TME remain unexplored. We characterized TAM fractions and gene signatures associated with tumor characteristics using an integrative scRNA-seq analysis of ER-positive breast tumors (n=29) from two independent public datasets. We found that TAMs were enriched in the patients with high fraction of cancer epithelial cell, but not in ones with low fraction of CD4+/CD8+ T-cells and B-cells, as well as a distinct transcriptomic landscape. Next, we examined associations between TAMs and cancer cell derived factors. Our analyses show that in cancer epithelial cells the expression of S100A11, which is involved in cytoskeleton rearrangement, cell migration, and tumor progression, is positively correlated with TAMs. In addition, epithelial mesenchymal transition (EMT), angiogenesis, TGF-β*β*, and hedgehog signaling are enriched in fibroblast and endothelial cells but depleted in immune cells. This study reveals the relationship between TAMs and clinicopathological parameters in human breast tumor and elucidates the interactions with cancer cells or other immune cells. As ongoing study, we will validate our findings in ER-positive breast cancer cell lines and organoid models using 3D *in vitro* platform.

*Poster 124*

**Understanding travel considerations and barriers for people with mobility impairments to using current mode of transportation through journey mapping**

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The aim of this study was to use a journey mapping methodology to identify travel considerations and barriers faced by people with mobility impairments (PMIs) at each stage of their trip, from considering a trip through arriving at their destination, for their current modes of transportation (private vehicle, public transportation, and paratransit). The objective was to understand and avoid pain points during the transition to autonomous driving systems. Ten PMIs participated in semi-structured one-on-one interviews. Descriptive statistics were used to analyze demographic information, and a qualitative content analysis was conducted to extract themes from the transcribed interviews. These themes were then organized by the modes of transportation used. The top four themes that emerged when considering and planning a trip were: availability of 3rd party assistance (private vehicle, public transportation, and paratransit), finding an accessible or suitable parking space (private vehicle), access to service locations (public transportation and paratransit), and transportation schedules (public transportation and paratransit). The top four travel barriers identified in locating, entering, riding, and exiting transportation, and arriving at the destination were: ingress/egress to the vehicle (private vehicle and public transportation), concerns with wheelchair securement (public transportation and paratransit), requiring 3rd party assistance (private vehicle and public transportation), and accessibility to service locations (public transportation). The study suggests that to mitigate travel considerations and barriers for people with mobility impairments, both vehicle-specific barriers and infrastructure issues should be addressed simultaneously. The findings provide valuable insights into the design and development of autonomous vehicles that better accommodate the needs of PMIs.

*Poster 125*

**Uveal melanoma immunogenomics predict immunotherapy resistance and susceptibility**

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Immune checkpoint inhibition (ICI) has shown significant efficacy in select metastatic cancers with high tumor mutational burden (TMB). However, most solid cancers have low TMB and are ICI-resistant. We hypothesized that immunogenomics of metastatic uveal melanoma (UM), a prototypic ICI-resistant cancer, would provide insights toward developing therapies for immune-resistant cancers.

Freshly resected UM metastases (n=100) were procured from patients (n=84) enrolled in NCT01814046 and NCT03467516. No patients had responses to ICI. Metastases were profiled with whole genome sequencing, total RNAseq, TCR repertoire analyses, single-cell RNAseq, and *ex vivo* tumor-infiltrating lymphocyte (TIL) culture expansion and anti-tumor reactivity testing.

Unbiased correlative principal component analysis of total RNAseq segregated metastases by canonical immune pathways. Immune axis gene loadings were used to create an unbiased enrichment score: *Uveal Melanoma Immunogenomic Score* (UMIS). Single-cell RNAseq revealed T cell-inflamed microenvironments in high versus low UMIS metastases, as evidenced by increased exhausted CD8 T cells (ratio=40.73, $P<0.001$) and antigen presenting cell function. Low UMIS tumor cells upregulated *CTNNB1* (beta-catenin) and *SNHG7* (lncRNA), which have been linked to tumor immune resistance. As validation, UMIS predicted *ex vivo* expansion of tumor reactive TIL (rho=+0.47, $P<0.001$; AUC=0.85, $P<0.001$). Interestingly, UMIS correlated with TCR diversity (rho=+0.54, $P<0.001$) but not clonality (rho=+0.02, $P$=ns). *Ex vivo* culture rescued TIL cell count and clonality ($P=0.004$). Finally, when retrospectively applied to NCT01814046, UMIS predicted magnitude of tumor regression (rho=-0.68, $P=0.001$) and survival after TIL adoptive transfer (PFS: HR=0.36, $P=0.044$; OS: HR=0.24, $P=0.009$).

We demonstrate a novel transcriptomic score, UMIS, that predicts anti-tumor reactivity and clinical efficacy of TIL adoptively transferred into patients with metastatic UM. UMIS is now being adapted for other immune-resistant cancers.

*Poster 90*

**Reducing Akt2 in Retinal Pigment Epithelial Cells Attenuates Diabetic Retinopathy**

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**Purpose:** Evidence has emerged to suggest that retinal pigment epithelium (RPE) injury contributes to the development of diabetic retinopathy (DR), the leading cause of blindness among working-age adults. However, the role of the RPE in DR remains poorly understood. Since Akt2 signaling has been implicated in diabetes and is integral to both RPE homeostasis and glucose metabolism, we investigated whether Akt2 in the RPE could influence DR.

**Methods:** *Best1*-Cre generated RPE-specific *Akt2* conditional knockout (cKO) mice were used. Diabetes was induced in *Akt2*$^{fl/fl}$ and *Akt2* cKO mice by intraperitoneal injection of streptozotocin for 5 consecutive days. Akt2 and Akt1 activities were examined in retinas from both DR patients and diabetic mice. Retinal leukostasis, and electroretinograms (ERG) were measured at a 2 month duration of diabetes (4 months of age). Mice at an 8 month duration of diabetes (10 months of age) were used to evaluate retinal capillary degeneration.

**Results:** We found that Akt2 and Akt1 activities were reciprocally regulated in the RPE of DR patients and diabetic mice; *Akt2* cKO inhibits a diabetes-induced increase in retinal leukostasis. Retinal capillary degeneration, and ERG abnormalities caused by diabetes were also attenuated in *Akt2* cKO mice.

**Conclusions:** Our results suggest that targeting Akt activity in the RPE may be a novel therapeutic strategy for treating DR.

**Acknowledgements:** This work was supported by start-up funds to DS from Ophthalmology, University of Pittsburgh, the Jennifer Salvitti Davis, M.D. Chair Professorship in Ophthalmology (DS) and Research to Prevent Blindness (Ophthalmology, UPMC).

*Poster 126*

**Tumor suppressor CASTOR1 phosphorylation status predicts the survival of lung cancer patients**

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Lung cancer is the most common cause of cancer-related mortality accounting for about 25% of all cancer-related death worldwide. We have previously shown that the cytosolic arginine sensor for mTORC1 subunits 1 (CASTOR1) is a novel tumor suppressor involved in the regulation of mTORC1 pathway. Overexpression of CASTOR1 attenuates mTORC1 activation in KSHV-transformed cells, and in breast and lung cancer cells, leading to the suppression of tumorigenesis in xenograft and transgenic mouse models. A lower expression level of CASTOR1 predicts poor survival in at least 10 types of cancer. Furthermore, we have found that CASTOR1 integrates signals of both nutrients and growth factors to regulate mTORC1 and cell proliferation. Specifically, AKT-mediated phosphorylation of CASTOR1 (p-CASTOR1) at S14 leads to CASTOR1 ubiquitination and degradation, resulting in mTORC1 activation, cell proliferation and tumorigenesis. In this study, we examined p-CASTOR1 at S14 (CASTOR1-pS14) as a prognostic marker in lung cancer. Using an in-house specific antibody, we performed immunohistochemical (IHC) staining of lung cancer tissue microarrays (TMAs) composed of tumor and adjacent non-tumor tissue cores from lung cancer patients. Semi-quantitative analysis of CASTOR1-pS14 level showed that male patients with higher expression level of CASTOR1-pS14 had significantly worse overall survival (OS) and relapse-free survival (RFS), compared to patients with a lower level of CASTOR1-pS14, giving rise to about 50% and more than 90% difference in 5-year and 10-year OS or RFS, respectively. Significant difference is also found in male patients with stage I-II lung cancer who also harbor KRAS mutations. Among these patients, a higher level of CASTOR1-pS14 confers significantly worse OS and RFS compared to patients with a lower expression of CASTOR1-pS14, with 5-year OS and RFS similar to male patients with stage III-IV lung cancer.

*Poster 23*

**Dynamic causal modeling of pitch MMN in first episode psychosis reveals impaired A1-STG communication and selective dysfunction of NMDA receptors**

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Mismatch Negativity (MMN) is severely reduced to pitch (pMMN) and to duration (dMMN) deviant stimuli in schizophrenia (SZ). However, it is unclear if MMN is reduced in first episode psychosis and when the potential underlying pathophysiology starts. DCM allows inferences about biological processes in the brain from sensor-level data and is designed to explain, mechanistically, differences in the auditory network of deviance detection between groups that sensor-based analyses may miss.
We recorded pMMN and dMMN with EEG and MEG from 26 first episode psychosis (FE) individuals and 25 healthy controls (HC) and applied DCM assuming MMN source locations in bilateral A1, superior temporal gyrus (STG), and inferior frontal gyrus (IFG). Within each source neural architectures were based on the Canonical Microcircuit (CMC) model and on the conductance-based neural-mass model. Statistical comparisons were performed using Parametric Empirical Bayes.
Preliminary analyses of scalp EEG for pMMN revealed no significant differences between groups at FCz. However, CMC DCM revealed reduced connectivity between left A1 and STG sources in FE for pMMN, suggesting impaired communication of the prediction error signal to upper hierarchical levels of processing. Moreover, conductance-based DCM revealed a selective dysfunction of N-methyl-D-Aspartate (NMDA) receptors in FE in most sources, suggesting impaired NMDA-induced synaptic plasticity may account for the reduced deviance detection response.
DCM may represent a more detailed model of cortical circuit activity that can reveal the subtle functional auditory processing deficits in early psychosis, even in the face of generally within-normal-limits sensor MMN.

*Poster 91*

**The roles of PINK1 in regulating dendritic mitochondrial distribution that contribute to the maintenance and maturation of dendritic arbors and spines**

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PTEN-induced kinase 1 (PINK1) is a serine/threonine kinase that plays critical roles in mitochondrial turnover, dynamics, mitophagy, and transport. Loss of PINK1 function has been linked to autosomal recessive Parkinson's disease with cognitive and neuropsychiatric comorbidities. However, the role of PINK1 in regulating dendritic mitochondrial distribution, motility, and function that contribute to the maintenance and maturation of dendritic arbors and spines is not well understood. In this study, we investigated the effects of *pink1* knockout (KO) on dendritic architecture and mitochondrial distribution in primary mouse cortical neurons. Our findings indicated that *pink1* KO neurons exhibited a striking simplified dendritic architecture, reduced spine density, and spine maturation. We analyzed the mitochondrial density and distribution by measuring the percentage of the dendrite length that was occupied by mitochondria. We observed a decrease in mitochondrial density in the dendrites of KO neurons, with a higher degree of variation in mitochondrial occupancy among different types of branches. Preliminary analysis suggests that certain classes of spines in KO neurons are less likely to have mitochondria distributed near the base of the spine. Taken together, our results suggest that dysregulated mitochondrial distribution contributes to dendritic injury and spine immaturity in mutant PINK1 pathogenesis.

*Poster 144*

**Mild hypoxia over laminar cribrosa could result from moderate IOP elevation**

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**Purpose:** Glaucoma is characterized by progressive degeneration of retina ganglion cells and their axons. The axon damage is believed to start within the lamina cribrosa (LC) region. This can happen at any level of intraocular pressure (IOP) and is likely to worsen if elevated IOP induces LC deformations that distort the vasculature and compromise blood flow. Our goal was to evaluate how LC oxygenation is affected by the tissue distortions resulting from elevated IOP.
**Methods:** We reconstructed the LC vessel networks of 3 healthy monkey eyes using histological sections. We also obtained in-vivo IOP-induced LC deformations from a healthy monkey using OCT images and analysis techniques while IOP was controlled. A biomechanics-based technique was used to map the OCT-derived tissue strains to local LC vessel distortions. The hemodynamics of the three vessel networks were simulated under deformations from various IOP increases. The results were used to determine the effects of IOP on LC oxygenation (PO2).
**Results:** IOP-induced deformations reduced LC oxygenation significantly. For moderate IOP elevation (20~30 mmHg), the region of severe hypoxia was small, but the mild hypoxia region increased significantly. About 40% of LC tissue suffered from mild hypoxia when IOP reached 30 mmHg. Extreme IOP increases (>50mmHg) led to large severe hypoxia regions.
**Conclusions:** Our models predicted that moderately elevated IOP can lead to mild hypoxia in a substantial part of the LC, which, if sustained, may contribute to neural tissue damage. For extreme IOP elevations, severe hypoxia was predicted, which would potentially cause more immediate damage. Our findings point to the need to advance experimental tools to determine in-vivo LC oxygenation to better glaucoma.

*Poster 127*

# Plasma p-tau231 and p-tau217 provides information on tau tangle deposition in symptomatic Alzheimer's disease individuals

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**Introduction** It has been suggested that phosphorylated tau (p-tau) at threonine 231 and 217 are markers of amyloid-β (Aβ) rather than tau pathology. However, most of the studies were conducted in cohorts composed mainly of preclinical Alzheimer Disease (AD) individuals. It remains to be elucidated if p-tau is still more associated with Aβ than tau pathology in symptomatic individuals. Our objective on this study was to evaluate the contribution of each plasma biomarker to identify brain Aβ and tau pathologies across the AD spectrum.

**Methods:** We evaluated 138 cognitively unimpaired (CU) and 87 cognitively impaired (CI) individuals with available Aβ [$^{18}$F]AZD4694-PET and tau [$^{18}$F]MK-6340-PET, plasma Aβ42/40, p-tau(at threonine 181, 217, and 231), and glial fibrillary acidic protein (GFAP), from the TRIAD cohort.

**Results**: Our results demonstrated that in the CU group only plasma p-tau231 and p-tau217$^{+}$ significantly added to the model to detect Aβ pathology, while no biomarker contribute to identify tau pathology. In the CI, plasma p-tau217$^{+}$ and GFAP significantly added to the model to identify tau and Aβ pathology, while p-tau231 only added to detect tau deposition. P-tau181, Aβ42/40, and NfL did not significantly add to the model in CU or CI groups. Voxel-wise analysis demonstrated that in CU, p-tau231 and p-tau217$^{+}$ were only regionally associated with Aβ-PET. In CI, p-tau231 provided additional information on tau tangle accumulation in AD-related regions. On the other hand, for p-tau217$^{+}$ were associated with Aβ-PET and tau-PET.

**Discussion**: Our results support plasma p-tau231 and p-tau217$^{+}$ as state markers of Aβ deposition in preclinical AD. In CI, plasma p-tau231 is mainly related to tau pathology, and p-tau217$^{+}$ appears to be linked to both Aβ and tau pathology.

*Poster 55*

**Preconception stress moderates the association between preconception emotion dysregulation and pain severity at 6-months postpartum in Black individuals**

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**Introduction:** Postpartum pain is common and associated with adverse effects on well-being and quality of life. Emotion dysregulation is a risk factor for the development of pain and is related to difficulty managing emotional responses to life stress. Black individuals are disproportionately impacted by psychosocial stressors and experience higher rates of postpartum medical complications. Thus, we examined the moderating effect of preconception stress on the association between preconception emotion dysregulation and pain severity at 6-months postpartum in Black individuals.

**Methods:** Black participants (N=180) were enrolled in a peripartum substudy of the Pittsburgh Girls Study and completed the Difficult Life Circumstances scale to evaluate life stress and the Difficulties in Emotion Regulation scale (lack of emotional awareness, limited access to emotion regulation strategies subscales) prior to pregnancy at ages 19-22 years. At 6-months postpartum, participants completed the pain severity item from the PROMIS Global-10. Maternal age at 6-months postpartum was included as a covariate.

**Results:** The association between preconception limited access to emotion regulation strategies and postpartum pain was moderated by preconception life stress (B=.051, p<.01). Tests of simple slopes indicated that limited access to emotion regulation strategies was positively associated with postpartum pain severity at high levels of preconception stress (+1 SD; B=.141, p<.001), but not at low levels. Preconception life stress did not moderate the association between lack of emotional awareness and postpartum pain severity.

**Conclusion:** Preconception emotion dysregulation, specifically limited access to emotion regulation strategies, increases risk for postpartum pain severity in the presence of life stress. Life stress may contribute to difficulty identifying the skills necessary to manage emotions, which may interfere with successful adjustment to pain; and/or unsuccessful attempts to use strategies to regulate emotions, which may increase perceptions of pain.

*Poster 145*

**All-around electromagnetic wave absorber based on Ni-Zn ferrite**

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Exploring single component, low-cost, lightweight, non-toxic, broadband, and finally a scalable effective electromagnetic wave absorber (EMA) is of high interest today to quench its expanding demand. Herein, we investigated EM wave attenuation properties of $Ni_{0.5}Zn_{0.5}Fe_2O_4$ (NZF) samples substituting $Mn^{2+}$ in place of $Fe^{3+}$ (x= 0.1, 0.2, 0.3; named as NZFM0.1, NZFM0.2, NZFM0.3) as well as $Mn^{2+}$ in $Zn^{2+}$ site (x= 0.1, 0.2; named as NZM0.1F, NZM0.2F) within a widely used frequency range of 0.1-9 GHz. Among the sample sets, NZFM0.3 and NZM0.1F shows excellent microwave absorption with a maximum reflection loss (RL) of -47.1 dB and -50.2 dB, wide bandwidth (BW) (RL < −10 dB, i.e., attenuation >90%) of 6.48 GHz and 6.52 GHz at thicknesses (t) of 7.5 mm and 6 mm respectively. Moreover, attenuation constant (α) is found to exhibit a significant increment from ∼217 Np/m for NZF to 301 Np/m for NZM0.1F with Mn-doping. The key contribution arises from magnetic-dielectric properties synergy along with enhanced dielectric and magnetic losses due to the cation rearrangement in spinel NZF. In addition, porosity is induced to the system with a controlled two-step heat treatment that promotes the total loss with multiple internal scattering of the EM wave. Besides the thickness dependent study, RL is analysed varying incident EM wave angles for the optimised NZM0.1F sample displaying its angle insensitiveness up to 50º. This study demonstrates NZM0.1F as a futuristic microwave absorber suitable for practical high-frequency applications.

*Poster 92*

**Loss of NEK1 aggravates C9orf72 repeat expansions-mediated toxicity**

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An expanded GGGGCC (G4C2) repeat in chromosome 9 open reading frame 72 (C9orf72) is the most common genetic cause of frontotemporal dementia and amyotrophic sclerosis (ALS/FTD). Heterozygous loss-of-function (LOF) mutations in NEK1 (NIMA-related kinase 1) was shown to be a causative gene in ALS with evidence of reduced penetrance. Reports suggest the co-occurrence of heterozygous LOF mutations of NEK1 in C9orf72 carriers. However, the functional implications of NEK1 in C9 ALS have not been explored. Here, we develop an *in vivo* model to assess the impact of ALS risk factor gene NEK1 on C9orf72-mediated neurodegeneration. We found that reduced levels of NEK1 protein in a *Drosophila* model of C9orf72 ALS deteriorates eye phenotype, cause neuromuscular junction defects, impairs motor function, and decrease lifespan. Furthermore, *in vitro* expression of G4C2 repeats decreases NEK1 protein and mRNA, and C9 iMNs showed reduction in NEK1 mRNA. Strikingly, reduce levels of NEK1 *in vivo* and *in vitro* elevated toxic G4C2 RNA suggesting that NEK1 may modify C9orf72 toxicity via G4C2 RNA. Interestingly, NEK1 reduction in C9orf72 alters the percent of motile mitochondria and transport. Our data provide evidence that the reduce levels of NEK1 is a potential causative factor of C9-ALS toxicity.

*Poster 93*

**The contributions of endoplasmic reticulum stress and reactive oxygen species to hearing loss in the cochlea**

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Hearing loss is a common condition that affects a significant portion of the population around the world. However, there are no biological therapies for hearing loss and many of the underlying pathophysiological mechanisms that cause hearing loss are not well understood. Hearing loss doubles in frequency with each decade of life. Aging and numerous environmental factors contribute to hearing loss. Although there are many distinct factors that can interfere with auditory function, there are common molecular mechanisms that are thought to underlie the pathophysiological impacts of hearing loss-associated insults. Endoplasmic reticulum (ER) stress and excessive reactive oxygen species (ROS) are associated with multiple forms of hearing loss. However, the precise contributions of ER stress and ROS to hearing loss, and the molecular mechanisms associated with ER stress in the cochlea are unclear.

To investigate ER stress in the cochlea, we are developing a pharmacological model of cochlear ER stress using either systemic or local delivery of Tunicamycin, an antibiotic and anticancer drug. We demonstrate that Tunicamycin induces hearing loss in adult mice, and upregulation of numerous ER stress-linked genes in both pre-hearing and adult mice. Tunicamycin-induced ER stress causes significant increases in ROS in multiple cochlear cell types. Finally, we observe that Tunicamycin affects expression of TMC1, the pore-forming subunit of the hair cell mechanotransduction channel, which is essential for hearing. Our data suggest that ER stress and ROS induced by Tunicamycin affects hearing through specific regulation of TMC1, and provide an intriguing mechanism that may underlie cochlear pathophysiology in hearing loss.

*Poster 94*

**Functional dissection of Atoh7 cis-regulatory elements in genesis of retinal ganglion cells**

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Atoh7 is critically required for retinal ganglion cell (RGC) specification, differentiation, and optic nerve growth. Atoh7 is transiently expressed in retinal precursor cells. However, the detailed roles of Atoh7 cis-regulatory elements governing RGC specification and differentiation remain elusive. In humans, loss of a remote regulatory element nearby Atoh7 is associated with optic nerve atrophy and RGC deficiency. Here, we tested the requirement of Atoh7 enhancer elements in the developing mouse retina. We generated two mouse enhancer knockouts (KOs) that targeted different enhancer regions of the Atoh7 chromatin landscape. In one mutation that lacked a distal enhancer of Atoh7 (Atoh7-RE), RGCs are formed normally. Paradoxically, transcriptomic analysis at E14.5 retina revealed that whereas Atoh7 expression is significantly downregulated, its downstream gene regulatory network remains robustly expressed. To evaluate these data at a deeper level, we performed Sc-RNA-seq analysis. Data revealed that Atoh7 expression is disproportionally affected in cell clusters, as a considerable amount of Atoh7 transcript remains expressed in retinal precursors cells at E14.5 retina. Importantly, Atoh7-RE knockout mice exhibited a dysregulation in axonogenesis genes and a disruption in RGC axon innervations to the brain. Another mutation that lacked the entire Atoh7 enhancer landscape (Atoh7-EN-KO) resulted in a complete loss of RGCs and the absence of optic nerves due to a strong downregulation of Atoh7 and gene regulatory network that is important for RGC genesis. Taken together, our results illuminate that the Atoh7 enhancer landscape is essential for RGC development and axonogenesis and revealed differential requirements of Atoh7 enhancer elements in mice and humans.

*Poster 95*

**Viral and cellular N$^6$-methyladenosine (m$^6$A) epitranscriptomes during KSHV primary infection and essential roles in viral infection and replication**

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N$^6$-methyladenosine (m$^6$A), the most prevalent modification on messenger RNA (mRNA), plays an important role in all stages mRNA biogenesis and functions including pre-mRNA splicing, pri-miRNA processing, mRNA export, mRNA stability, translation modulation and mRNA degradation. We have previously mapped the viral and cellular m$^6$A epitranscriptomes during Kaposi's sarcoma-associated herpesvirus (KSHV) latency and reactivation, and observed abundant m$^6$A modifications on KSHV transcripts as well as global reprogramming of cellular m$^6$A epitranscriptome. In this study, we examined the kinetics of viral and cellular m$^6$A epitranscriptomes during KSHV primary infection of primary human umbilical vein endothelial cells (HUVEC). We found dynamic m$^6$A modifications on viral transcripts that were correlated with the expression of KSHV transcripts and replication. In addition, KSHV induced dynamic reprogramming of cellular epitranscriptome during primary infection, regulating pathways that control cell survival and viral replication. Knockdown of m$^6$A reader YTHDF2 reduced KSHV replication whereas overexpression of YTHDF2 enhanced KSHV replication during KSHV primary infection. RNA immunoprecipitation (RIP)-qPCR revealed direct bindings of YTHDF2 to KSHV lytic transcripts ORF45, ORF57 and ORF59. Knockdown YTHDF2 resulted in elevated expression level of interferon-stimulated genes including IFIT1, IFIT2, and IFIT3 upon KSHV infection. IFIT1, IFIT2, and IFIT3 transcripts stability were increased upon YTHDF2 knockdown, indicating YTHDF2 mediates their degradation during KSHV primary infection, which facilitating KSHV infection and replication. These results reveal a pivotal role of m$^6$A modifications in KSHV primary infection and provide rich resources for the community.

*Poster 24*

**Visualization of SV2A in complex with anti-epileptic drugs by cryo-EM**

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Synaptic vesicles are small membrane-enclosed vesicles, that store various neurotransmitters at presynaptic terminals and release them by calcium-triggered exocytosis. The synaptic vesicle 2A (SV2A) is a transmembrane glycoprotein present in synaptic terminal throughout the brain and is implicated in the regulation of synaptic vesicle exocytosis and neurotransmitter release. SV2A is predicted from its sequence to be a transporter related to the major facilitator superfamily (MFS) of transporters and has been shown to function as galactose transporter in yeast cells, but its biological function remains obscure. Additionally, SV2A serves as a specific binding site for certain antiepileptic drugs (AEDs) implicated in the treatment of epilepsy, which is one of the most debilitating neurological disorders in the USA. Unfortunately, these drugs also cause many side-effects in epileptic patients and fail or even worsen the epileptic symptoms in patients with mutated SV2A. The aim of my research is to elucidate the architecture of SV2A in a complex with an AED molecule by single particle cryo-EM, and further characterize high-affinity drug binding sites and conformational states through structure guided mutations. Towards this end, I have mapped a strategy for expression and purification of SV2A, quantitative binding between SV2A with AEDs and nanobodies using scintillation proximity assays (SPA), and surface plasmon resonance (SPR), respectively. I have determined a cryo-EM structure of SV2A in complex with nanobody and a drug related to PSV. The structure indicates that the interactions of drug with SV2A stabilize an outward-open conformation and laid the foundation to understand the quantitative drug binding, regulation, and modulation of SV2A conformational states through structural and functional methods.

*Poster 56*

**Foreign military withdrawal, male migration, and female education**

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Foreign military withdrawal is an economic and political shock to an occupied nation. This paper studies the effects of a major foreign troop evacuation on female participation in higher education. In the aftermath of the 9/11 attacks, the U.S. launched the War on Terror campaign that led to collapse of the Taliban regime and installation of a democratic system in Afghanistan. After a decade of military occupation, between 2011-2016, the United States implemented a conditions-based transfer of security responsibilities to local security forces and evacuation of international troops. I exploit geographic and topographic barriers for the shipment of military equipment from Afghanistan's districts to regional military airbases as the source of variation for physical repatriations of troops. I use the least-cost travel distance between districts and the nearest logistic hubs to instrument the departure of forces. Withdrawal of foreign forces resulted in an 85% increase in female university participation, while male participation did not change. I show that a decline in economic opportunities and massive male emigration explain this difference.

*Poster 142*

**Sonoreperfusion with fibrin-targeted phase shift microbubbles for the treatment of microvascular obstruction**

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**Introduction:** Mortality from AMI has decreased in recent years; however, microvascular obstruction (MVO) occurs frequently, ultimately limits myocardial salvage, is associated with rising post-AMI CHF, and has no definitive therapy. To address this urgent unmet need, we have developed an image-guided acute therapy, termed 'sonoreperfusion' (SRP), that resolves MVO via ultrasound-targeted microbubble cavitation (UTMC). We previously used standard MBs with fibrin-targeting and demonstrated enhanced reperfusion compared to standard MBs with non-targeting. However, phase shift microbubbles are much smaller than standard MBs, (~200 nm vs. 3 µm, and may allow greater clot penetration and perhaps more effective SRP. Thus, we compared the SRP efficacies of fibrin-targeted phase shift microbubbles (FTPSMB) (Microvascular Therapeutics, Inc), to standard size fibrin targeted microbubbles (FTMBs)
**Methods:** MVO of the rat hindlimb (n=6) was created by injecting porcine microthrombi into the left femoral artery under contrast-enhanced ultrasound (CEUS) guidance. DEFINITY® MBs (Lantheus Medical Imaging) were infused (2 mL/hr) through the right external jugular vein for CEUS. Following 10 min of stable MVO, a transducer was positioned vertically above the hindlimb to deliver therapeutic US pulses during concomitant administration of FTMBs/FTPSMBs (3 mL/hr). CEUS cine loops with burst replenishment were obtained at baseline (BL), 10 min post-MVO, and after each of the two SRP treatment sessions (TX1, TX2) and video intensities were analyzed (MATLAB_R2021a)
**Results:** FTPSMB treatment resulted in a greater increase in the blood volume (dB) and flow rate (dB/sec) than FTMB after each 10-minute treatment session
**Conclusions:** US-guided FTPSMB cavitation causes more rapid and complete reperfusion of rat hindlimb MVO than FTMB, likely owing to their small size and more effective thrombus penetration. Studies to explore the underlying molecular mechanisms associated with SRP treatments are underway.

*Poster 26*

**Human probiotic bacteria cell surface-associated protein mineralized hydroxyapatite incorporated in porous scaffold for bone cell growth and differentiation**

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Nowadays, synthesis of biocompatible hydroxyapatite nanoparticle (HANP), which is a key component in bone tissue engineering scaffolds is a high demand. This study explains a facile route of biogenic HANP synthesis through mineralization of the cell surface-associated protein (CSP) from the human probiotic lactic acid bacteria (LAB) *Lactobacillus rhamnosus* GG. CSP extract from the LAB (consisting of ~66 kDa, ~47 kDa, ~40 kDa and ~25 kDa protein) was biomineralized to yield spindle-shaped HANPs having an average particle length of 371 nm as evidenced in FETEM analysis. CSP-mineralized HANPs (CSP-HANPs) were also characterized by FTIR and BET analysis, while XRD and SAED analysis indicated their crystalline nature. Mechanistic studies suggested that the ~25 kDa CSP (F4SP) was the key player to act as a mineralization template. In contrast to CSP-HANPs, F4SP-mineralized crystalline HA was plate-shaped having an average length of 1.68 μm and breadth of 0.95 μm. Given that CSP-HANPs were non-toxic to cultured HEK 293 cells and osteoblast-like MG-63 cells. Chitosan-gelatin (CG) scaffold incorporated with 15% w/v CSP-HANP (H-CG) was generated and tested for bone cell growth. H-CG exhibited a favorable physicochemical property i.e., pore size distribution (160–230 μm), overall porosity (~84%) and biodegradation profile as compared to control chitosan-gelatin scaffold. H-CG scaffold was conducive to osteogenesis and rendered enhanced proliferation, alkaline phosphatase (ALP) activity, calcium mineralization and heightened marker gene expression (ALP, Col I, Runx2 and OCN) in seeded MG-63 cells. CSP sourced from a safe probiotic LAB is thus a viable and effective mineralization template for synthesis of biocompatible HANPs that can be leveraged for bone tissue engineering applications.

*Poster 96*

**Sex differences in the microbiome-gut-brain axis are time-of-day dependent**

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Circadian rhythms orchestrate many essential homeostatic processes. Emerging evidence points to the gut microbiome and its metabolites as a novel class of circadian entrainment signals, but the molecular mechanisms by which diurnal rhythms in microbial metabolites contribute to host physiological processes remain unknown. Moreover, circadian rhythms are influenced by sex, and circadian disruption is often associated with sex-specific disease risk. Despite clear links between sex-specific processes and lifelong health trajectories, studies on circadian rhythms primarily use male mice or collapse both sexes into one experimental condition. We applied an integrated multi-Omics approach combining longitudinal analyses with dietary manipulation, metabolomics, transcriptomics, single-cell immunophenotyping, and machine learning to specifically examine the hypothesis that sex differences in the microbiome-gut-brain axis are time-of-day dependent.

We identified sex-specific differences in diurnal rhythms in the intestinal microbiota, the metabolites they produce, and the expression of host genes, with more pronounced effects in females. The magnitude of these sex differences varies by time of day. These time-of-day and sex-specific patterns were abolished entirely in germ-free mice. This suggests that an intact microbiome is necessary for synchronizing sex differences across the microbiome-gut-brain axis. Further, transitioning mice to a high-fat, low-fiber diet abolished circadian rhythms in microbiota, metabolites, and host gene expression entrained by a chow diet. The high-fat, low-fiber diet generated new diurnal rhythms in the microbiota and host transcriptome. We show that circadian rhythms in the crosstalk between microbiota and their hosts are sex-specific and that diet plays an essential role in maintaining these differences.

*Poster 97*

**Impact of local oxidative stress on centromere homeostasis and involvement of PARP1/2 in centromere integrity preservation**

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Centromeres are vital for proper cell division, as they mediate kinetochore assembly and chromosome segregation. Numerous studies have shown that defective centromeres are associated with cancer, and aging. Yet, cellular stress that arises from exposure to endogenous and environmental sources including oxidative stress (OS) are likely to cause centromere instability (CIN). One major consequence of OS is the induction of oxidative DNA damage that is repaired through the base excision repair pathway (BER) in which poly(ADP-ribose) polymerases PARP1/2 play a major role. During BER, PARP1/2 recognize and bind to the DNA single-strand breaks (SSBs) intermediate generated by the removal of the damaged base through the combined activities of a specialized glycosylase and endonuclease APE1. However, PARP1/2 targets at centromeres and their specific roles in preserving centromere integrity are not clear. Using the fluorogen activated peptide (FAP) that generates exclusively 8oxoG lesions, specifically at centromeres, we will be able to delineate the molecular roles that PARP1/2 fulfills to prevent CIN under OS conditions. Our preliminary data reveal a significant PARylation activity localized at the centromere as well as the efficient recruitment of BER proteins. Our RNA-seq data revealed expression changes in cell cycle and cell death pathways in cells at 24h and 48h post-treatment. More specifically, we identified Mis18BP1, required for CENP-A recruitment and normal chromosome segregation during mitosis, which previously found in a PARylated protein screen. We therefore aim to identify the Mis18BP1/ PARP enzymes interaction in response to OS.

*Poster 27*

**Increased synaptic input on D1R MSNs and MSN hyperexcitability in the dorsomedial striatum in the Fmr1 KO mouse**

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Fragile X Syndrome is a monogenic disorder caused by a decrease in Fmr1 gene and FMR1 protein expression. Loss of function of Fmr1 causes hyperactivity, motor coordination problems, anxiety and sensory over-reactivity throughout life. These behaviors are strongly regulated by the striatal network however, there is very little research investigating changes in striatal cell connectivity and function due to loss of Fmr1. Studies in humans with Fragile X Syndrome have found decreased corticostriatal connectivity suggesting that striatal neurons may have decreased synaptic input. Structural and functional characteristics of medium spiny neurons (MSNs) in the dorsal striatum were measured using patch clamp electrophysiology in Fmr1 -/y (knockout) and Fmr1+/y (wildtype) adult male mice. The frequency of mEPSCs, a measure of synaptic input, and active cell properties such as firing frequency, rheobase, and firing rate were measured in MSNs. Additionally, we assessed whether there are differences in direct pathway, dopamine receptor 1 (D1R) positive, and indirect pathway, D1R negative, MSNs since these distinct pathways work together to during normal striatal function. There was increased variability in mEPSC frequency in the Fmr1-/y MSNs suggesting that loss of Fmr1 causes increased variability in the number of excitatory synaptic connections onto MSNs. A separate experiment found that when MSNs were sorted into D1R positive and negative cells, we found that D1R positive, but not negative cells, had an increased number of mEPSCs, suggesting an MSN subtype specific increase in excitatory synaptic innervation. Lastly, we measured functional properties of D1R and D2R MSNs using current clamp electrophysiology. FMR1 MSNs had decreased spike threshold and rheobase, and had voltage dependent changes in membrane resistance compared to WT mice in both D1R and D2R MSNs. These results suggest that FMR1-/y MSNs are hyperexcitable thus more likely to fire action potential in response to the same input compared to FMR1+/y MSNs. We are currently assessing whether an antipsychotic that is commonly prescribed to treat behavioral issues in FXS decreases MSN excitability in Fmr1-/y mice. Hyperexcitability of the striatum could be one reason for changes in behavior seen in FXS.

*Poster 98*

**Mice lacking gamma-ENaC palmitoylation sites maintain benzamil-sensitive $Na^+$ transport *in vivo*, despite markedly reduced open probability of typical ENaC-like channels**

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Epithelial $Na^+$ channels (ENaCs) control extracellular fluid volume by facilitating $Na^+$ absorption across transporting epithelia. *In vitro* studies showed that Cys-palmitoylation of the γ ENaC subunit is a major regulator of channel activity. We tested whether γ subunit palmitoylation sites are necessary for channel function *in vivo* by generating mice lacking the palmitoylated cysteines ($\gamma^{C33A,C41A}$) using CRISPR-Cas9 technology. ENaCs in dissected kidney tubules from $\gamma^{C33A,C41A}$ mice had reduced open probability compared to wild type (WT) littermates maintained on either standard or $Na^+$-deficient diets. Male mutant mice also had higher aldosterone levels than WT littermates following $Na^+$ restriction. However, $\gamma^{C33A,C41A}$ mice did not have reduced amiloride-sensitive $Na^+$ currents in the distal colon or benzamil-induced natriuresis compared to WT mice. We observed no differences in γ subunit expression or processing between WT and $\gamma^{C33A,C41A}$ mice. We also identified a second, larger conductance cation channel in the distal nephron with biophysical properties distinct from ENaC. The activity of this channel was higher in $Na^+$-restricted $\gamma^{C33A,C41A}$ versus WT mice and was blocked by benzamil, providing a possible compensatory mechanism for reduced prototypic ENaC function. We conclude that γ subunit palmitoylation sites are required for prototypic ENaC activity *in vivo*, but are not necessary for amiloride/benzamil-sensitive $Na^+$ transport in the distal nephron or colon.

*Poster 28*

**Structure-based design and synthesis of 2-substituted spiro[4.5]decanes as transient receptor potential melastatin (TRPM8) antagonists**

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Transient receptor potential Melastatin 8 (TRPM8), also known as the cold menthol receptor, is a non-selective Ca2+ permeable ion channel involved in somatosensation. Aberrant sensory response contributes to chronic pain; cold allodynia and hyperalgesia are major sensory phenotypes in response to innocuous and noxious cold, respectively, in patients with neuropathic and chemotherapy-induced pain syndromes. Oxaliplatin-induced cold hypersensitivity as manifest with the acetone test is absent in Trpm8-/- mice. Our previous structure-activity relationship (SAR) efforts uncovered spiro[4.5]decan-8-yl analog VBJ103, [hTRPM8 IC50 (Ca2+ flux): 2.4 ± 1.0 nM, hTRPM8 IC50 (electrophysiology): 64 ± 4 nM], which causes dose-dependent inhibition of cold pain responses in wild-type C57BL/6 mice with peripheral neuropathy induced by the chemotherapy oxaliplatin. Our molecular dynamic studies suggest VBJ103 binds tightly to the ligand-binding pocket of the voltage sensor-like domain (VSLD) of TRPM8 defined by icilin with a binding free energy -11.0 kcal/mol. The cyclopentyl group of VBJ103 is positioned towards a key Arg842 residue (distance: 3.4 Å) in the VSLD, one of two residues crucial for voltage-dependence. We describe our efforts towards the synthesis of 2-substituted spiro[4.5]decanes to enhance interactions with Arg842 using structure-based design enabled by the cryo-electron microscopy structures of Trpm8. Our retrosynthetic approach for annulation of the cyclopentyl ring involves allylation of the ethyl cyclohexanecarboxylate intermediate followed by Wacker oxidation, intramolecular aldol condensation and hydrogenation. This synthetic approach will generate the spiro[4.5]decan-2-one synthon for further derivatization including 2-substituted analogs of VBJ103.

*Poster 29*

**Chronic negative allosteric modulation of α5 $GABA_A$ receptors causes subtle homeostatic synaptic changes that suggest an overall dampening of neuronal excitability *in vitro***

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α5 subunit-containing GABA type-A receptors (α5 $GABA_ARs$) are enriched in the hippocampus and play critical roles in neurodevelopment, synaptic plasticity, cognition, and memory. α5 $GABA_AR$ preferring negative allosteric modulators (α5 NAMs) show promise mitigating cognitive impairment in preclinical studies of conditions characterized by excess GABAergic inhibition, including Down syndrome and memory deficits post-anesthesia. However, previous studies have primarily focused on acute application or single-dose α5 NAM treatment. Here, we measured the effects of chronic (7-day) *in vitro* treatment with the imidazobenzodiazepine L-655,708 (L6), a low efficacy and highly selective α5 NAM, on glutamatergic and GABAergic synapses in rat hippocampal pyramidal neurons. We previously showed that 2-day *in vitro* treatment with L6 enhanced synaptic levels of the glutamate NMDA receptor (NMDAR) GluN2A subunit without modifying surface α5 $GABA_AR$ expression, inhibitory synapse function, or responsiveness to L6. We hypothesized that chronic L6 treatment would continue to increase synaptic GluN2A subunit levels while maintaining GABAergic inhibition and L6 efficacy, thus increasing neuronal excitation and glutamate-evoked intracellular calcium responses. Immunofluorescence experiments revealed that 7-day L6 treatment slightly increased the synaptic levels of gephyrin and surface α5 $GABA_ARs$. Functional studies showed that chronic α5 NAM treatment did not alter inhibition and that α5 NAM sensitivity was maintained. Surprisingly, chronic L6 exposure decreased surface levels of GluN2A and GluN2B subunits, concurrent with reduced NMDAR-mediated neuronal excitation as seen by faster synaptic decay rates and reduced glutamate-evoked calcium responses. Together, these results show that chronic *in vitro* treatment with an α5 $GABA_AR$-preferring NAM leads to subtle homeostatic changes in inhibitory and excitatory synapses that suggest an overall dampening of neuronal excitability.

*Poster 57*

**High rate of errors in brief pain inventory completion among adults with sickle cell disease**

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Pain is a primary concern for adults living with sickle cell disease (SCD). Accurately assessing pain severity in daily functioning is an important step in developing interventions. The Brief Pain Inventory (BPI) was developed to address limitations of previous pain inventory metrics. However, the BPI is a self-report scale dependent on patient comprehension and interpretation of items and responses to them. This study's objective was to examine patterns in how patients completed the BPI and determine whether inconsistencies were related to cognitive functioning. A secondary analysis of 71 completed BPI forms was conducted. The BPI questionnaires were evaluated for inconsistencies, which included the following: 1) indicating no pain but reporting above-zero pain intensity; 2) reporting conflicting scores on the pain intensity numeric scales; or 3) missing one or more items. Descriptive statistics of the sample and rate of errors on the BPI were evaluated. In addition, independent T-tests were used to evaluate whether the subgroup that erred on the BPI had lower cognition, evaluated by the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) and the Hopkins Verbal Learning Test-Revised (HVLTR). In this study, 21.1% of pooled BPI assessments contained at least one inconsistency. Of those, 2.8% of patients indicated no pain but reported above-zero pain, 15.5% had conflicting scores on the pain numeric scales, 2.8% repeated the same value, and 1.4% had missing items. Participants who provided inconsistent responses on the BPI had significantly lower attention and memory scores. The examination of BPIs found variability in how patients completed the scale, which contributed to inconsistencies in scoring. Results from our study suggest that careful consideration is warranted for use of the BPI in patients with SCD. Recommended revisions to the BPI include simplifying the language, shortening sentence length, and clearly specifying the time frames.

*Poster 30*

# GluA3 subunit of AMPA receptors are required to prevent synaptopathy at inner hair cell ribbon synapses in female mice

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**Background:** AMPA-type ionotropic glutamate receptors (AMPARs) mediate fast synaptic transmission at inner hair cell (IHC) ribbon synapses. Antagonism of $Ca^{2+}$-permeable AMPARs (CP-AMPARs) at IHC synapses may be sufficient to prevent synaptopathy and loss of cochlear sensitivity (Sebe et al., 2017; Hu et al., 2020). Post-synaptic AMPARs at IHC-ribbon synapses are comprised of the dimers of dimers of GluA2, 3, and 4 subunits. However, the role of each AMPAR subunit in IHC synaptic transmission is still unresolved. Our recent studies in male mice (ARO 2022) showed that lack of GluA3 reduced hearing sensitivity and wave-1 amplitude, was not observed until 8-weeks of age (García-Hernández et al., 2017). Since there are sex differences in hearing and hearing loss (Shuster et al., 2019; Villavisanis et al., 2020; Milon et al., 2018), and knowing that the GluA3 gene is in the X chromosome (Mahadevaiah et al., 2009; Trivisano et al., 2020), we hypothesized that in the absence of GluA3, and when compared to male mice, females may have an early onset of hearing loss.
**Methods:** We used female C57BL/6 wild type (WT) and *Gria3*$^{KO}$ mice from postnatal weeks 3-13, reared in animal rooms at normal 'ambient' sound levels (55-70 dB SPL) or in 'quiet' sound levels (40-55 dB SPL). Using ABRs, we examined the role of GluA3 in auditory processing. With immunofluorescence and confocal microscopy, we studied the molecular anatomy of IHC-ribbon synapses with antibodies to GluA2, GluA3, GluA4, and the presynaptic ribbon protein CtBP2/Ribeye. With transmission electron microscopy and serial section reconstruction we studied pre- and post-synaptic ultrastructure of IHC-ribbon synapses.
**Results:** ABR thresholds and wave-1 amplitude in 3-week-old female WT and *Gria3*$^{KO}$ mice were similar. However, in mice raised in ambient sound, the ABR thresholds increased, and wave-1 amplitude decreased significantly at 5-weeks and 13-weeks in the *Gria3*$^{KO}$, compared to WT mice. In contrast if the mice were raised in quiet, ABR thresholds and wave-1 amplitude were similar between *Gria3*$^{KO}$ and WT mice at 5-weeks. Confocal imaging analysis showed a similar number of paired synapses, although the numbers of lone ribbons and ribbonless synapses were increased in *Gria3*$^{KO}$ mice in ambient sound compared to WTs. As well, the ratio of GluA4 to GluA2 subunits per synapse was increased in the KO relative to WT, suggesting an increase in CP-AMPARs in the KO. Ultrastructurally, dendritic swellings were observed in afferent endings of cochlear nerve fibers on IHCs by 5-weeks of age in *Gria3*$^{KO}$, but not in WT, and only in those KO mice reared in ambient sound levels.
**Conclusions:** Our data show that lack of the GluA3 AMPAR subunit results in synapses with increased vulnerability to AMPAR-mediated excitotoxicity at ambient sound levels. This AMPAR dysregulation apparently leads to presbycusis, particularly in female mice.

*Poster 99*

**Identifying regulators of quiescence as therapeutic targets: using chemoresistance drivers in quiescence genes to develop a pathway to improve clinical outcomes**

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Quiescent cancer cells, which have reversibly exited the cell cycle, are resistant to most cancer therapies which target rapidly dividing cells. As such quiescent cells contribute to therapeutic resistance and disease recurrence. We sought to understand the drivers of quiescence in ovarian cancer as a means to identify novel therapeutic targets which either (i) prevent quiescent cells from re-entering the cell cycle and could thus delay recurrence, or (ii) could eradicate cells to prevent recurrence. We used a novel single cell microfluidics culture approach to identify and retrieve quiescent cells. Single cell RNA sequencing was done using CellSeq2. SiRNA knockdowns were used to confirm a role in quiescence. DNA content and Fucci cell cycle reporter assays (p27 and CDT1) we used to the impact on cell cycle. Real time imaging was used to assess proliferation. Western blotting and qPCR were used to assess gene expression. Pharmacologic inhibitors of quiescence associated genes were used to assess therapeutic potential in vivo. RNASeq analysis of quiescent cells identified numerous genes previously linked with quiescence and several novel genes. SiRNA knockdown of targets such as nucleolin (NCL) and MYH9 confirmed loss of expression was associated with the induction of a quiescent state. Bioinformatic analysis suggested many of the quiescence associated genes are transcriptionally regulated by the Myocardin Related Transcription Factor/Serum Response Factor (MRTF/SRF) pathway. Indeed, we found CCG081, a known inhibitor of the MRTF/SRF activation, induced a dense cellular quiescent state associated with increases in p27/CDKN1B expression. Importantly, CCG081 treatment in vivo, as a maintenance therapy, significantly delayed tumor growth and improved survival. The MRTF/SRF pathway is a critical regulator of quiescence in OvCa. As such inhibition of this pathway with compounds such as CCG081 therapy could maintain a quiescent state which reduces recurrence in patients with residual disease. Together, this data suggests CCG081 is a novel therapeutic target to prevent recurrence of chemo-resistant ovarian cancer.

*Poster 58*

**Cortical tracking of continuous speech-in-noise: children's use of linguistic and acoustic information**

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Speech perception in challenging listening conditions is a critical skill in everyday life and these skills are still developing in childhood. Children must often attend to speech amongst competing speech streams, like listening to a teacher's voice in a loud classroom. Cortical tracking of temporal speech envelopes is an emerging technique for examining the neural encoding of continuous speech in challenging listening settings. Prior work suggests that children have immature cortical tracking of speech acoustics, relative to adults, especially in noise. Thus, selectively poorer cortical tracking of speech acoustics, especially in multi-talker listening situations, may underlie listening challenges in children. Attentional control is required to successfully attend to a target speech stream, yet it is unclear the extent to which children employ different listening strategies to aid their speech perception. Our goal is to examine the extent to which children can strategically engage different hierarchical levels of sound-to-meaning processes during speech perception, as a function of multiple listening challenges.

In the current study, cortical tracking of continuous speech (temporal response function) was measured using electroencephalography (EEG) in children (n=8, two males). Children listened to an audiobook across three conditions: (1) in quiet, (2) in the presence of another talker (forward speech, masked by a different audiobook narrated by a distinct make speaker), and (3) in the presence of reverse speech (a different audiobook played in reverse). These conditions vary in listening complexity: reverse speech represents masking with sub-lexical competition, whereas the forward speech brings in lexical competition. Both noise conditions were presented binaurally at 0dB SNR to the target track. Two four-choice comprehension questions were presented after each one-minute block of story to ensure participants listened to the target story.

Children had significantly higher behavioral performance in the quiet condition relative to both noise conditions, showing a group average of 90% accuracy on comprehension questions Behavioral performance was more accurate in the reverse speech condition than in the forward speech condition, with a 78% vs. 63% average accuracy, respectively. However, EEG data showed a large range of individual differences in cortical tracking of linguistics. Specifically, some children demonstrated enhanced linguistic tracking in the forward speech condition, where some showed enhanced tracking in the forward condition, even compared to quiet.

These results suggest that while children may show immature tracking to continuous speech, individual variability in strategies employed for continuous tracking (e.g., focusing on acoustic or linguistic features) may relate to individual behavioral listening skills. Leveraging a large assessment battery of auditory, speech, and language tests, our current work is examining the sources of such individual differences.

*Poster 146*

**Diverse responses of a basal ganglia output nucleus integrating multiple input streams in control and dopamine-depleted conditions**

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An interesting and common problem in neuroscience is understanding emergent dynamics in populations of neurons integrating inputs from multiple sources. This poster considers this problem in the substantia nigra pars reticulata (SNr), an output region of the basal ganglia (BG), which are a collection of brain regions central to behavioral functions such as decision making and action selection. The SNr receives input from two inhibitory pathways that exhibit very different firing properties. Understanding how the SNr integrates these inputs provides a means for understanding movement disorders related to the basal ganglia, like Parkinson's Disease. Previous work has revealed surprisingly diverse SNr responses to inhibitory inputs, possibly related to chloride regulation and associated changes in the GABA reversal potential.

This poster will connect these works through computational approaches where the results inform on SNr dynamics. This is accomplished through leveraging a novel classification algorithm, the STReaC algorithm, which helps tune a previously published mathematical model of the SNr. Here, the STReaC algorithm is used to analyze the diverse responses within SNr neurons to optogenetic stimulation of the globus pallidus externa (GPe) and striatum (STR), which are both input regions to the SNr found in the BG. This analysis, along with corresponding experimental data, tune the parameters within the SNr model where desirable parameters are determined by minimizing differences between experimental and simulated distributions of key neural firing properties. This poster will show results of the model tuned to two experimental groups, a control group, and a dopamine-depleted group to yield predictions about how the SNr respond to responses and possible alterations in dopamine-depleted conditions, including Parkinson's Disease, respectively. The poster concludes by discussing possible relevance to Parkinson's Disease and future directions of analysis between the experimental groups.

*Poster 31*

**Uremic toxin impairs B cell response against infection via inhibiting germinal center formation and antibody titers in kidney disease**

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Immunity to infections are crucial to healthy life. However, recent SARS-CoV-2 pandemic has shown that comorbidity such as kidney disease and associated uremia impairs protective immune response against infections. Uremic patients are not only susceptible to life-threatening infections, but they also exhibit poor antibody titers following vaccination. The mechanisms by which uremia negatively impacts antibody response is unknown. Using multiple mouse models of uremia, we assessed B cells response to model antigen NP-KLH in alum. We show that uremia inhibits both canonical germinal center (GC) and non-canonical extra-follicular B cells response following NP-KLH immunization. Immunized uremic mice demonstrated compromised affinity maturation, isotype switching, antigen-specific antibody secreting cells, antibody titer and T follicular helper cells response. Consequently, uremic mice exhibited diminished GC and antibody response following prime-boost immunization. B cells showed increased cell cycle arrest, apoptosis and impaired migration between light zone and dark zone in the uremic GCs. We identified uremic toxin hippuric acid as a major driver of loss of mitochondrial membrane potential leading to increased apoptosis in mouse and human B cells. Finally, we show that GC B cells, T follicular helper cells and antibody response were similarly diminished in uremic mice during influenza virus infection, a major cause of mortality in patients with kidney disease. These results shed light on how uremic toxin(s) suppress antimicrobial immunity and vaccine response in individuals with kidney disease. Knowledge gained from this study may pave the path for developing effective therapeutic and preventive strategies in uremic patients against infections including SARS-CoV-2.

*Poster 100*

**Taking the "succ" out of succinylation-mediated damage during Acute Kidney Injury: new posttranslational modifications associated with AKI protection**

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Acute Kidney injury (AKI) is an unfortunately frequent disease acquired during hospitalization with nearly 1 in 5 patients exhibiting some form of AKI. With the recent COVID-19 epidemic increasing instances of hospitalization, the burden that AKI and ensuing Chronic Kidney Disease (CKD) underlines the critical need for early detection, protection, and treatment for AKI. To attenuate AKI occurrence therapeutically we need a better understanding of the physiological and cellular mechanisms underlying damage. The most pronounced effect of AKI is on the Proximal Tubule Epithelial Cells (PTECs) which have the highest metabolic activity and are therefore most susceptible to damage after ischemia, sepsis, or transplant stress. This damage causes an increase in radical oxygen species, oxidative protein stress, and decreased functionality of mitochondrial Fatty Acid Oxidation (FAO) enzymes. Our approach to protect from long-lasting tissue damage is by modulating the metabolic regimen by increasing and activating FAO in peroxisomes, a normally underused metabolic organelle. Large classes of proteins can be modulated rapidly and reversibly through the activity of enzymes that ligate Posttranslational Modifications (PTMs). We have previously shown that succinylation of lysine residues on metabolic proteins can be protective in AKI, specifically when the activity of the desuccinylase Sirtuin 5 is inhibited. Maintenance of the succinylome in Sirtuin 5 knockout tissue requires the activity of a second PTM ligating enzyme, the deglycase Park7. Park 7 is activated by oxidative stress and has been linked to apoptotic protection and minimizing CKD through reduction of Advanced Glycation Endproducts. Mass spectrometry analysis of kidney lysates point towards a protective combination of activated Park7 and deactivated Sirtuin 5 increasing peroxisomal FAO. We conclude that a rapid and effective target for AKI treatment can be found by analyzing the PTM landscape of these cells, we hope to harness this mechanism to develop novel therapies for AKI.

*Poster 147*

# Regional brain inflammation associates with longitudinal brain atrophy in Alzheimer's disease continuum

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**Introduction:** Neuroinflammation typically involves the activation of microglial cells in the brain and has been linked to Alzheimer's disease (AD) pathology. However, how microglial activation influences brain neurodegeneration in individuals across the AD continuum is poorly understood. Here, we aimed to investigate the influence of microglial activation in longitudinal brain atrophy in individuals across the AD continuum. We hypothesize that high levels of regional brain inflammation predict widespread brain atrophy.

**Methods:** We assessed 95 individuals from the TRIAD cohort (60 cognitively unimpaired and 35 cognitively impaired) with available [$^{11}$C]PBR28-PET, a measure of microglial activation, and a 2-year longitudinal MRI (mean = 2.07 years). We generated grey matter voxel-based morphometry (VBM) images using SPM12 and DARTEL, smoothed with a Gaussian kernel of full-width half maximum of 8mm. We built the uncorrected ($p < 0.05$) association matrix between the [$^{11}$C]PBR28-PET SUVR and longitudinal VBM ROIs (z-score) with the β-estimates from linear regressions accounting for age, sex, and diagnosis. We divided the [$^{11}$C]PBR28-PET levels into terciles to generate the averages of longitudinal VBM changes.

**Results:** Baseline ROI-based [$^{11}$C]PBR28-PET levels associate with longitudinal brain atrophy in distinct brain regions. The inferior temporal cortex was the region where [$^{11}$C]PBR28-PET levels were better associated with widespread longitudinal brain atrophy in AD-related regions, including the amygdala, insula, and the superior temporal cortex, independently of global amyloid load and tau. Accordingly, individuals with higher [$^{11}$C]PBR28-PET levels in the inferior temporal cortex presented increased longitudinal brain atrophy compared to individuals with lower [$^{11}$C]PBR28-PET.

**Conclusion:** We identified increased baseline [$^{11}$C]PBR28-PET levels in the inferior temporal cortex that were highly associated with longitudinal brain atrophy in individuals across the AD continuum. Our results demonstrated that higher levels of inflammation in key brain regions could predict widespread longitudinal brain atrophy, suggesting that microglial activation has a detrimental impact on AD-related neurodegeneration progression.

*Poster 128*

**Preliminary results from an ongoing pilot clinical trial testing the effects of spinal cord stimulation on motor function in humans with Type 3 SMA**

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Spinal Muscular Atrophy (SMA) is a rare genetic disease occurring in 1/6-10K newborns. SMA manifests with different degrees of severity in babies (SMA Type 1), children (Type 2), and young adults (Type 3) and is caused by deletion of the Survival Motor Neuron 1. The hallmarks of SMA are dysfunction and death of select spinal motor neurons, muscle atrophy and severe motor deficits. Although available treatments are effective to stop the progression of the disease, they are unable to significantly alleviate motor deficits. A series of remarkable studies in SMA mice have shown that motor neuron dysfunction emerges as a maladaptive response to loss of excitatory synaptic inputs from sensory afferents. The same studies also showed that pharmacologically increasing the activity of sensory afferents on motor neurons, improves motor neuron function. Unfortunately, this approach results in nonspecific undesirable effects that are toxic in the long term. To overcome this impasse, we propose to develop an electrical stimulation paradigm. Specifically, we hypothesize that targeted SCS of the primary sensory fibers - restricted to vulnerable muscles in Type 3 SMA patients, will increase sensory synaptic input on motor neurons thus enhancing motor function. Here we describe the preliminary result from an ongoing pilot trial using temporary off-label epidural implants (29 days NCT05430113). We show that, over the course of 4 weeks, SCS therapy can increase leg joint torques (up to +180%) and single motor neuron firing rates, indicating that SCS is improving motor neuron function while reporting no side effects.

*Poster 101*

**Effect of target tissue innervation on the phenotype of iPSC-derived nociceptive afferents**

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Pain is one of the most prevalent symptoms in medical settings and is associated with decreased quality of life and opioid addiction, making it one of the costliest health problems in the U.S. Despite advances in our understanding and management of pain, progress in therapy development has been hampered by a number of barriers, among them difficulties in translating from preclinical models to humans. Induced pluripotent stem cells (iPSC) offer a promising solution to this problem. Furthermore, subject-specific iPSCs for tissue derivation could serve as a platform for personalized medicine. However, despite significant advancements in protocols for iPSC-derived sensory neuron generation, the phenotype of iPSC-derived nociceptive afferents (iPSC-NAs) lacks critical features of these neurons in the intact organism. For example, our data shows that iPSC-NAs generated using a standard differentiation protocol (Lampert,2022) express nociceptive markers (NaV1.8, TRPV1) but little functional protein. Given the importance of the target tissue on afferent properties, its absence during iPSC-NA generation could explain the failure to achieve an appropriate phenotype. Hence, our objective is to determine whether providing a target tissue for iPSC-NA differentiation will facilitate the generation of sensory neurons with functional nociceptor properties. We will use a two-chambered microfluidic polydimethylsiloxane system that allows differentiating iPSCs to innervate target tissues (urothelium, keratinocytes, and synovium) via microfluidic channels. Functional iPSC-NA properties will be assessed using calcium imaging and microelectrode arrays. This platform may enable a viable strategy to address a major hurdle in the wider adoption of this potentially powerful technology.

*Poster 32*

**Myoglobin sustains cardiomyocyte fatty acid oxidation and limits initial infarct size**

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During the early phase of cardiac ischemia reperfusion, oxidation of fatty acids in heart muscle cell (cardiomyocyte) mitochondria correlates with larger infarct size. However, a clinical strategy to inhibit cardiomyocyte specific fatty acid oxidation has not been considered. Myoglobin (Mb), a heme protein endogenously expressed in cardiomyocytes, is known for its classic role in oxygen storage and delivery. Recently Mb has been predicted to directly bind fatty acids, though the physiological significance of this binding is unknown. We hypothesized that loss of Mb is an effective strategy to inhibit cardiomyocyte fatty acid oxidation and prevent early infarct expansion. We mutated myoglobin by site directed mutagenesis (K45M) to prevent its binding to fatty acids without affecting its oxygen binding. By Seahorse XF analysis, gene silencing of Mb or expression of K45M mutant Mb in H9C2 cells, a cardiac cell line decreased cellular fatty acid oxidation. We next exposed cells to hypoxia for 5 hours followed by reoxygenation for 1 hour to mimic clinical ischemia and analyzed by flow cytometry for apoptosis (Annexin V). Following hypoxia-reoxygenation, H9C2 cells expressing endogenous Mb had increased levels of Annexin V indicating elevated apoptosis compared with cells lacking Mb or expressing K45M mutant. We generated a novel *in vivo* mouse model of conditional Mb depletion (Mb-cKO), and subject them to 30 minutes of ligation and 24-hour reperfusion of the LAD artery, and measured infarct size. At 24 hours following ischemia reperfusion injury, Mb-cKO mice developed smaller infarct size by TTC staining, and had fewer apoptotic cells compared to control mice expressing Mb. Isolated cardiomyocytes from Mb-cKO mice showed reduced maximal mitochondrial respiration. Our data uncover a novel role for Mb as an endogenous driver of cardiomyocyte fatty acid oxidation and determinant of early infarct size. Current studies are examining Mb's function as a fatty acid binding protein in regulating early ischemic events, with the aim of targeting this mechanism to prevent infarct expansion.

*Poster 102*

**Biased G protein-coupled receptor 3 (GPR3) signaling potentiates amyloid plaque clearance in an Alzheimer's Disease mouse model**

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Glial cell activation and neuroinflammation precede and drive the pathological changes commonly observed in Alzheimer's Disease (AD) brains. Glial cells express several G protein-coupled receptors (GPCRs) that mediate neuroinflammation. We recently determined that GPR3 is abundantly expressed in astrocytes and microglia in the *App*$^{NL\text{-}G\text{-}F}$ AD mouse model. Moreover, we determined that G protein-biased GPR3 signaling, which maintains G protein-signaling while suppressing β-arrestin 2 signaling, leads to an increase in amyloid plaque compaction and the area occupied by both microglia and astrocytes, suggesting a previously undescribed role for biased GPR3 signaling in glia and regulation of amyloid-β (Aβ) clearance.

To investigate the effect of biased GPR3 signaling on the activation and function of glia *in vivo*, we performed FACS analysis of astrocytes and microglia from G protein-biased GPR3 AD mice and GPR3 control mice. We determined that astrocytes ($CD45^{-}ACSA^{+}$) exhibit a 2.3-fold increase in the reactive glial marker GFAP in biased GPR3 AD mice. Interestingly, astrocytes from both genotypes have a similar phagocytic capacity of MX04-labeled Aβ despite elevated levels of GFAP. However, microglia ($CD11b^{+}CD45^{int}$) display a 2.1-fold increase in Aβ phagocytic capacity and elevated levels of TREM2. TREM2 induces the progressive transition of microglia from a homeostatic to a reactive state in both murine and human brains and is associated with increased microglial phagocytic capacity, reduced spread of Aβ pathology, and restricted Aβ-induced neurodegeneration. Collectively, these studies provide compelling evidence for the putative involvement of biased GPR3 signaling in astrocytes and microglia in the attenuation of the Aβ pathology in AD.

Our results will likely unravel novel mechanisms involved in the regulation of brain innate immune response and neuroinflammation in AD through GPR3 signaling. Importantly, this study may also offer great promise on the use of biased agonism as a prospect for targeted therapeutics to slow or perhaps prevent AD.

*Poster 33*

**Touch and go: analysis of mouse gait for the investigation of pain**

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**Introduction:** Acute pain and chronic pain are a major public health concern. More than 116 million people in the U.S. are affected and this number is expected to increase as the population is aging. Preclinical research aimed at improving the reliability of diagnosis, evaluation, and treatment of pain is needed to address this growing problem. A knowledge gap exists for pain and treatment evaluation between humans and animals; the latter relies on experimenter's subjective judgements of elicited animal responses, rather than animals' own intrinsic behaviors.

**Hypothesis:** We hypothesize that by training the Long Short-Term Memory (LSTM) networks, a machine deep learning algorithm, we will be able to detect and diagnose animals experiencing differing degrees of inflammatory or neuropathic pain.

**Methods:** We developed a high-throughput gait recording and analysis device using the high spatial and temporal resolution touchpad technology. Spontaneous walking of mice in an open field is registered by individual touches as the animals explore the arena. Gait features are extracted from the touch data to train a LSTM model to differentiate between painful and non-painful conditions so that animals with and without pain or with and without analgesia treatment can be identified and quantified.

**Results:** Using naïve and complete Freund's adjuvant (CFA)-induced inflammatory pain as a test system, we extracted several features in the gait analyses showing behavioral dichotomy between naïve and CFA mice. When using animals not used in the training, the model currently has a high prediction accuracy for pain but only marginal accuracy for non-painful states. We are refining the model to achieve > 95% accuracy in all predictions.

**Conclusions:** Further testing and verification will be required but we are close to fully developing an affordable high-throughput assay to assess pain behavior using the animal's intrinsic behavior rather than relying on subjective analyses of elicited behaviors.

*Poster 34*

**Soluble LAG-3 is a promising surrogate marker of type-1 diabetes development and immunotherapeutic efficacy**

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Type-1 diabetes (T1D) is an autoimmune disease in which CD4+ T cells initiate autoimmune attack of pancreatic islet beta cells. Cell intrinsic inhibitory receptor such as CTLA-4, PD-1, and LAG-3 (lymphocyte activation gene-3) regulate immunological checkpoint. Our previous findings indicate that LAG-3 negatively regulates T cell proliferation, cytokine production, metabolic homeostasis which is tightly coupled with mitochondrial biogenesis and metabolic quiescence. LAG-3 is structurally homologous to CD4 and possesses a greater affinity for their shared ligand, MHC class-II and must be cleaved off the T cell surface for full T cell activation, by the metalloprotease A disintegrin and metalloprotease 17 (ADAM17). In the context of T cell activation, the shedding of soluble LAG-3 (sLAG-3) provides a surrogate marker of self-reactive T-cell activation and a predictive biomarker of T1D progression. Our recent research findings revealed that sLAG-3 levels drop with diabetic progression in NOD mice. sLAG-3 levels indicate autoimmune activation of naive T cells during diabetes progression as well as providing B lymphocytes with the necessary help for differentiation and autoantibody production, which is associated with early development of diabetes. Our current findings on sLAG-3 along with quantification of islet-associated tetramer positive CD4+ T cells from NOD mice support its use as an earlier marker of the break in self-tolerance before autoantibodies, allowing strategies for earlier therapeutic intervention. Therefore sLAG-3, tetramer expression in CD4+ T cells and autoantibody generation can be used as a surrogate marker of type 1 diabetes development and to assess the efficacy of T-cell targeted immunotherapy.

*Poster 129*

**Verbal working memory recall relationships with gray and white matter structure and function in inpatient adolescents with bipolar disorder and other psychopathology**

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**Introduction:** Verbal working memory (WM) dysfunction is common and debilitating and may be associated with neural abnormalities in structure and function in those suffering with mood and affective disorders and other psychopathology. Identification of specific abnormalities in neural structure and function among inpatient participants may inform our understanding of verbal recall dysfunction and may suggest treatment targets.

**Methods:** Structural and functional MRI, along with cognitive testing data were acquired in a subsample (n=51, mean age=15.47, SD=1.12, 36 females) of inpatient participants with Bipolar Disorders (BD) and other psychopathology from an ongoing study (R01MH121451). Penalized regression with cross validation followed by linear regression identified whole brain gray and white matter measures and uncertain-anticipation-related functional measures (SPM12: BD>OP, $p<0.001$ uncorrected, threshold≥10 voxels) related to verbal WM recall. We applied linear regression to the test sample of 24 inpatient adolescents with unspecified B D to determine whether the model derived from the discovery sample is still able to accurately predict memory patterns in at risk group .

**Results:** Verbal WM recall was negatively related to left posterior cingulate thickness (B=-16.855, FDRq≤0.001), left angular cortex uncertainty related activity (B=-0.92, FDRq=0.04), fractional anisotropy in right striato-fronto-orbital tract (B=-25.712, FDRq=0.033), and positively related to left pericalcarine cortical volume (B=1086.47, FDRq=0.007). The linear regression model was able to predict verbal working memory in the test sample (B=27.779, p=0.002).

**Conclusion:** Positive relationships between left PCC thickness and verbal working memory recall in at-risk of bipolar disorder group, compared to negative relationships between them in BD and OP groups may reflect a differential role of default mode network across diagnoses and risk and may suggest differences in referential processing and introspection across diagnoses. Longitudinal studies are needed to identify progression of these differences over time.

*Poster 35*

**Rift Valley fever virus displays high permissivity for trophoblast cell lines and requires Lrp1 for efficient infection**

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Rift Valley fever virus (RVFV) is a pathogenic arbovirus endemic to Africa and the Saudi Arabian Peninsula. It causes disease in livestock and humans and is associated with abortion storms and late term miscarriages. Low density lipoprotein receptor-related protein 1 (Lrp1) was recently identified as a host-entry factor for RVFV. However, the role of Lrp1 in vertical transmission is not understood. To address this knowledge gap, we characterized the expression of Lrp1 in placental tissue and addressed the role of Lrp1 in RVFV infection of placenta cell lines. Using human placenta cell lines and tissue from various mammalian species, we measured Lrp1 levels by both western blot and immunofluorescent staining. The placental cell lines expressed Lrp1, although the expression levels were not consistent across each cell type. Furthermore, Lrp1 is highly expressed in mouse and human placenta tissue. Surprisingly, Lrp1 was not as highly expressed in rat placenta tissue. JEG-3, JAR, and HTR-8 cell lines were highly permissive to infection with pathogenic RVFV (ZH501 strain). Following infection with an MOI 1.0, RVFV replicated well in JEG-3, JAR and HTR-8 cell lines reaching titers of approximately $10^6$ PFU/mL by 24 hours post infection (hpi) and up to $10^8$ PFU/mL by 72 hpi. We then assessed dependence on Lrp1 by using a high-affinity ligand for Lrp1, murine receptor associated protein (mRAP). The trophoblast cell lines were pre-treated with mRAP followed by infection with RVFV. Treatment with mRAP reduced viral titers in JEG-3 and JAR cells by approximately 2 logs following RVFV infection, although the effect on infection of HTR-8 cells was less clear. These results suggest that Lrp1 may play a role in RVFV infection of trophoblast cell lines, and other entry factors may also contribute to the high tropism of RVFV for placental tissue.

*Poster 130*

**Fertility preservation for young girls – when your zip code is more important than your diagnosis**

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**OBJECTIVE:** Although ovarian tissue cryopreservation (OTC) for fertility preservation (FP) is no longer considered experimental by ASRM from 2019, cost and socioeconomic status may still impact patient access to this service. This study aimed to assess the socioeconomic backgrounds of patients with different options of OTC access, and who either completed or declined FP.

**MATERIALS AND METHODS:** Retrospective analysis of pre-peripubertal girls (≤14 y/o) referred to the UPMC fertility preservation program for OTC since 2011. Patients in the Free group (n=54) cryopreserved ovarian tissue at UPMC for free under an IRB-approved research protocol, while patients in the Pay group (n=129) had oophorectomy at a collaborating institution and paid a fee to have tissue shipped, processed and cryopreserved at UPMC. Zip codes were used to determine annual income, and a Distressed Communities Index (DCI) was calculated. The DCI combines 7 economic indicators to generate a single index score ranging from 0 to 100 (with 100 being the most distressed). A sub-analysis compared patients who completed FP treatment to those who declined, among those undergoing OTC at no cost under the research protocol.

**RESULTS:** The age did not differ significantly between the Free and Pay groups. The Pay group had higher income (80,444±27,618 vs 64,635±21,930, p<0.001), education (35±17% vs. 28±14%, p=0.01) and lower distress (36.49±29.07 vs 51.93±27.42 p=<0.001). In the Free group, 62% of individuals belonged to the top two per-quartile DCI groups compared with only 34% in the Pay group. The per capita of prepubertal girls receiving OTC for FP, was two times higher in the Free group than that in the Pay group (6.7 vs 3.7 per 100,000 individuals). A sub-analysis was conducted among patients who were designated to receive treatment at no cost, comparing those who completed FP treatment to those who declined. The results showed that the group who declined had lower annual income, higher DCI, and a lower percentage of individuals with a bachelor's degree or higher education.

**CONCLUSIONS:** The Free group population had more socioeconomic distress, but a higher per capita utilization of OTC. We suspect the socioeconomic cost barrier to FP was reduced for patients in the Free group population because OTC service cost was free, allowing for greater access despite socioeconomic status. Even when OTC for FP is free, the population that declined FP treatment appeared to be in more socioeconomic distress, perhaps due to consideration of long-term storage fees, which are not covered by the study. These data suggest that there is a potential financial disparity in access to FP care and this is exacerbated by the fact that FP services are rarely covered by insurance in the US.
The study's findings highlight the impact of socioeconomic status on access to OTC for FP, which may help guide future efforts to improve equity and accessibility for this important procedure.

*Poster 36*

**Role of lifespan interventions on the regulation and progression of Alzheimer's disease**

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Converging evidence delineates a bi-directional network connecting autophagy and inflammation that becomes progressively dysregulated with age and accelerated by pathological conditions. Systemic inflammation is a biomarker of this dysregulation, as exemplified by its prevalence in many aging-related disorders including cardiovascular disease, diabetes, cancers, and neuroinflammation in neurodegenerative disorders including Alzheimer's Disease (AD). Furthermore, the risk for AD is increased by co-morbidity with other aging-related disorders, with systemic inflammation as a common shared condition. We hypothesize that interventions that target the shared feature of systemic inflammation in the biology of aging and AD, via regulation of the autophagy-inflammation network, may have potential as therapeutic agents for the prevention of conversion to disease pathogenesis in AD as well as improve healthspan and longevity in aging populations. The present studies were undertaken to investigate the processes underlying dysregulation of the autophagy-inflammation network in aging biology; in order to identify mechanisms that precede the pathogenesis of AD and are distinct from normal healthy aging. For these studies, we crossed a mouse model of familial early onset amyloid plaque deposition (5XFAD) with a mouse genetically engineered with reduced mTOR expression to ~25% of WT levels ($mTOR^{\Delta/\Delta}$). We then generated chimeric mice using neuronal-, microglial-, and astrocyte-specific Cre lines, resulting in mTOR expression reduced to 25% of WT except in the specific $Cre^{+}$ cell type. Ongoing studies in these lines include longitudinal functional assessments (e.g frailty, behavior, cognition) and biomarkers of aging, inflammation, and AD. In parallel, we are interrogating the contribution of AMP-activated protein kinase (AMPK) as a critical regulator of the mTOR pathway. AMPK has been demonstrated as anti-inflammatory which may suggest that the mechanisms by which mTOR inhibition regulates inflammation may be dependent upon AMPK signaling. For these studies, a novel brain penetrable AMPK activator compound (BC1618) is being administered chronically to mice with genetic risk for late-onset AD, and their WT controls. These lifespan studies will allow us to compare the beneficial effects of BC1618 in normal healthy aging and in attenuating AD disease progression; and in comparison to the beneficial effects of rapamycin treatment. Results from these studies will provide both mechanistic insights into the autophagy-inflammation network involved in regulating normal healthy aging and in AD disease progression, and evaluate novel interventions administered prophylactically prior to AD disease pathology that target components of the autophagy-inflammation network as potential therapeutic agents for the treatment of AD.

*Poster 103*

**Therapeutic effects of extracellular vesicles with TGFβ3 cargo on scar-reducing corneal wound healing**

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Corneal blindness due to scarring/opacities is a leading cause of visual impairment. The mainstay of care is corneal transplantation, which is greatly affected by the global shortage of donor tissues. Our pre-clinical studies have shown that cell-based treatment with corneal stromal stem cells (CSSC) suppressed scar formation in mouse models of anterior stromal injury. A plausible mechanism is the expression of anti-fibrotic transforming growth factor β3 (TGFβ3) by transplanted CSSC. Here, we studied the therapeutic potential of CSSC-derived extracellular vesicles (EV) carrying TGFβ3 mRNA in the scar management of mouse corneas. In primary human CSSC cultures under various non-toxic stress conditions, CSSC-EV with TGFβ3 upregulation was specifically detected in response to (1) pro-inflammatory condition by culturing cells in M1 macrophage conditioned media, and (2) serum deprivation. Other stresses, such as $H_2O_2$-mediated oxidative stress, transient heat shock, and tunicamycin-induced metabolic stress, did not affect TGFβ3 expression. Importantly, CSSC-EV with TGFβ3 upregulation was corresponded to the full-length TGFβ3 mRNA transcripts. *In vivo*, EV collected after CSSC cultures under serum-deprivation or M1 pro-inflammatory stimulation were applied in a drop of fibrin gel to mouse anterior stromal wound after mechanical ablation. At day 10 post-treatment, corneas were evaluated with anterior segment optical coherent tomography. Compared to wound control with fibrin gel only, EV-treated corneas exhibited stability and significantly less opacities with reduced fibrosis, as demonstrated morphologically and by fibrosis marker expression (qPCR and immunostaining). In conclusion, a single treatment of CSSC-EV enriched with TGFβ3 mRNA in fresh corneal stromal wounds could exhibit a scar-reducing tissue healing and preserve the vision.

*Poster 131*

**Microglial activation strongly associates with neuropsychiatric symptoms in aging and Alzheimer's disease continuum**

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**Introduction:** Previous studies showed that microglial activation (MA) plays a key role in the emergence of psychiatric symptoms as well as in the clinical progression of Alzheimer's disease (AD). However, little is known whether MA is also associated with the development of neuropsychiatric symptoms (NPS) typically encountered in patients with AD. Thus, we aim to investigate the association of MA and NPS in individuals across the AD continuum.
**Methods:** We assessed 109 individuals (70 cognitively unimpaired, 39 cognitively impaired) from the TRIAD cohort who underwent clinical assessments with the Neuropsychiatry Inventory Questionnaire (NPI-Q) and had positron emission tomography (PET) for amyloid-β (Aβ) ([$^{18}$F]AZD4694), tau tangles ([$^{18}$F]MK6240) and MA ([$^{11}$C]PBR28) at the same visit. Linear regression tested the association between biomarkers accounting for age, sex, and cognitive status. A leave-one-out approach was used to assess the contribution of each NPI-Q domain to the results.
**Results:** NPI-Q severity score was significantly associated with [$^{11}$C]PBR28 in the frontal, cingulate, inferior temporal, and precuneus cortices. These associations survived correction for Aβ and tau levels (b = 7.69, $P$ = 0.01). Notably, MA predicted NPS with higher magnitude than Aβ or tau using PET values from regional (b = 0.72, $P$ = 0.01) as well as global Ab and temporal tau (b = 0.68, $P$ = 0.01). Leave-one-out analysis showed that irritability had the greatest contribution (22.8%) to NPS severity in patients, followed by nighttime disturbances (19.3%), and agitation (14.1%). MA also showed a significant association with the caregiver NPI-Q distress score (b = 5.72, $P$ = 0.03). Interestingly, microglial-related irritability had the greatest contribution (33.95%) to caregiver distress compared to other NPI-Q domains.
**Conclusion:** Our results support that MA plays a key role in the development of NPS in AD. These results suggest that MA biomarkers can be useful to identify distinct NPS and that the development of drugs targeting MA could potentially alleviate NPS in AD patients.

*Poster 37*

**Association between perceived physical fatigability and cognitive performance: study of muscle, mobility and aging (SOMMA)**

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Higher perceived fatigability—the quantification of vulnerability to fatigue in relation to specific intensity and duration of activities—is associated with poorer central nervous system health, including lower volumes of the thalamus and hippocampus. Understanding whether perceived fatigability is related to cognitive function may inform interventions to prolong independence and improve cognitive outcomes. We sought to examine the association between perceived physical fatigability and four cognitive function assessments in older adults. At baseline, SOMMA participants completed the Pittsburgh Fatigability Scale (PFS) Physical subscale (range 0–50; higher scores=greater fatigability) and four cognitive function assessments [Digit Symbol Substitution Test (DSST), Montreal Cognitive Assessment (MoCA), Trails Making Test Part B (TMT-B), and California Verbal Learning Test (CVLT)]. Analysis of variance was used to compare characteristics across PFS severity strata (0–4, 5–9, 10–14, 15–19, 20–24, and ≥25). Linear regression quantified associations between PFS and cognitive assessments adjusting for site, age, sex, race, education, and marital status. In the 873 participants (59% women; age 76.3±5.0 years; 85% White), mean PFS score was 15.8±8.7. Prevalence of cognitive impairment was 53% mild (MoCA 18–25), 1.9% moderate (10–17), and 0% severe (<10). Across PFS severity strata, number of correct DSST items was fewer (59 in 0–4 and 51 in ≥25) and number of seconds to complete TMT-B was slower (107 in 0–4 and 135 in ≥25), both $p<0.01$; MoCA and CVLT did not vary. After adjustments, for each 4-point higher PFS (greater fatigability), the number of correct items on the DSST decreased by nearly 1 [β coefficient=-0.83, 95% confidence interval (CI): -1.22, -0.43] and the time to complete the TMT-B increased by 2 seconds (β=2.11, 95% CI: 0.28, 3.94). PFS was not associated with MoCA and CVLT. We demonstrate for the first time that greater perceived physical fatigability may be indicative of subtle cognitive changes, particularly in executive function.

*Poster 59*

**Understanding suicide in sexual minority youth: neural reactivity to social feedback as a moderating influence**

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Suicide is the 2nd leading cause of death in youth. Sexual minority youth (SMY) are at elevated risk but mechanisms of this disparity are poorly understood. Suicide theories highlight social factors (e.g., belonging, burdensomeness) as key predictors. Minority stress due to sexual orientation (e.g., victimization, rejection) may be a unique factor that alters sensitivity to social feedback and heightens suicide risk. Social feedback processing is mediated by neural social reward/threat circuitry. Thus, it is critical to examine the interaction of sexual minority identity and minority stress with functions in neural social regions on suicide risk.

75 youth (52% SMY; aged 14-22) from a larger study were matched based on age, sex, and race/ethnicity. They completed surveys assessing current depression, sexual-orientation victimization history, and suicidal ideation (SI). A block-design fMRI task assessed neural reactivity to rewarding and ambiguous social feedback (vs. baseline) from unfamiliar peers. Using SPM12, we extracted principal eigenvariate in ROIs relevant to social reward/threat processing: left/right ventral striatum, medial prefrontal cortex, left/right temporoparietal junction (TPJ), precuneus, and left/right amygdala. Moderation analyses were run in SPSS PROCESS.

SMY reported higher depression (p=.034), victimization (p=.001), and SI (p=.017). Neural reactivity to social feedback moderated the link between sexual minority identity and SI. After correcting for multiple comparisons, SMY (vs. non-SMY) with lower activity in left TPJ to both rewarding (p=.001) and ambiguous (p=.004) feedback reported greater SI. Victimization history did not moderate these associations.

Findings suggest enhanced suicide risk in SMY with lower reactivity to social feedback in a key region of neural social circuitry, regardless of victimization history. Findings extend beyond sources of disparity to explain mechanisms of individual differences in suicide risk among SMY and have clinical implications for identifying those at risk who may benefit from intervention.

*Poster 104*

**Influence of the synthetic cannabinoid agonist on normal and inflamed treated cartilage**

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**Introduction:** Recently, many states in the US legalized the use of medical marijuana in clinical use . Importantly, studies in animal models of arthritis showed that cannabinoids might attenuate joint damage[1]. However, the underlying mechanism has not been completely understood. Interleukin-1β (IL-1β), is known to be associated with the pathogenesis of osteoarthritis. We hypothesize that cannabinoids can mitigate the detrimental effect of IL-1β on cartilage, thus reducing the progression of osteoarthritis.

**Methods:** Human chondrocytes were isolated from healthy articular cartilage. To generate cartilage in vitro, chondrocytes were pelleted and subjected to 14 days chondrogenic culture (Fig. 1A). After that, pellets were treated with different concentrations of Win( Win-55,212-2,a synthetic cannabinoid agonist) for 2 days . [Study 2] To simulate cartilage degradation observed in OA, we first treated pellets with IL-1β (10ng/ml) for 2 days and then applied Win for another 2 days.

**Results:** [Study 1] After treatment, the gene expression level of SRY-Box Transcription Factor 9 (SOX9) in cartilage was significantly increased in the 1μM group. There were otherwise no significant changes in all other tested genes and GAG results after the treatments of Win. However, results from Interleukin 6(IL-6) ELISA showed that condition medium from 0.01 and 0.1 μM groups contained significantly more IL-6 than other two groups. [Study 2] As shown in Fig. 2A, IL-1β treatment (Win 0-Win 1 group) suppressed the expression of chondrogenic genes, including Aggrecan(AGG), Type-II collagen (COL2) and SOX9, and significantly promoted the expression of IL6 and Nuclear factor kappa B(NF-κB), when compared to untreated control groups. Moreover, we noticed a significantly increased production of IL-6 expression in medium and loss of GAG in all IL-1β-treated groups. The results collectively indicated the establishment of in vitro cartilage degradation model. Results from real-time PCR showed that Win, at all tested doses, was not able to suppress the expression of IL6 and NF-κB, or restore the expression of chondrogenic genes. However, Win at 1 μM significantly reduced the accumulation of IL6 in the medium. The GAG assayed showed that Win treatment might attenuate the loss of GAG due to IL-1β treatment. However, no statistical difference was observed . To examine the potential anti-inflammatory properties of Win in our system, we measured the protein level of P-P65. The preliminary data showed that Win at 0.01 and 0.1μM might reduce P-P65 level, which needs to be further validated in the future.

**Discussion:** In normal engineer cartilage, whether cannabinoids can be beneficial or detrimental to osteoarthritic cartilage is uncertain with current evidence. For IL-1β insulted cartilage, the IL-6 ELISA results showed evidence of downregulated inflammation with a relative higher dose of Win. However, low dose of Win showed a beneficial influence on the phenotype of IL1-β-insulted cartilage, indicated by suppressing cartilage degradation (GAG loss) and NF-κB pathway. Therefore, the mixed results were found in this study. Whether cannabinoids can be a druggable target to treat OA requires further investigation.

**Significance/Clinical Relevance:** This work investigates the influence of medical marijuana on normal versus interleukin-1β treated cartilage, and highlights the need for careful consideration when using the anti-inflammatory properties potential of medical marijuana to treat osteoarthritis.

*Poster 60*

**Exploring nursing home medication administration processes**

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**Background**: Medication administration processes (MAP) are a central element of nursing home (NH) care. MAP is a complex and difficult process because of the burden of correctly administering and monitoring hundreds of medications among dozens of residents multiple times daily. Satisfaction with MAP has been exacerbated after the COVID pandemic due to staffing shortages, increased workload, low morale, and increased patient acuity. This study interviewed staff nurses (LPN/RN) to understand their perspectives on MAP and the usability of the MAP charting system.

**Methods**: This is a qualitative, semi-structured interview study. Nurses who have worked in NHs for at least six months were eligible. Nurses were recruited through local nursing homes and interviews were conducted by telephone. Qualitative analysis was performed using constant comparison analysis. The codes were guided by the system engineering initiative for patient safety framework. Atlas.Ti was used to track and organize the generated codes. Data was collected between December 2022 to February 2023. $20 research incentive was offered to individuals who completed the interview.

**Results**: 12 staff nurses, aged 25 to more than 75 years old participated. Most of the participants were female (n=11, 92%), worked ten years and above (n=7, 58%), and obtained an RN degree (n=11, 92%). First, participants reported significant non-user-friendly charting systems for MAP for NH residents. Second, participants stressed the importance of organization, teamwork and communication, and outstanding leadership to efficiently complete MAP activities. Third, MAP depended on people who have varied training and background. Finally, participants experienced severe short-staffed conditions in the nursing home environment.

**Conclusions and implications**: Our study found multiple and varied problems with MAP in NHs. The redesign of MAP should focus on user-friendliness, efficiency, and the wide range of educational and technological backgrounds of users.

*Poster 105*

**CD8+ lymphocytes contribute to immunologic bottlenecks in Mycobacterium tuberculosis infection**

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Meta-analysis of human data suggests that M. tuberculosis (Mtb) infection confers protection against subsequent exposure. Our lab demonstrated this in a controlled experimental system using non-human primates. As the best model for natural immunity against TB, it is crucial to explore the mechanisms behind concomitant immunity. We hypothesized that CD8+ T cells would play a key role in preventing disease following secondary challenge.

Chinese cynomolgus macaques were challenged intrabronchially with a genetically barcoded library (LibP) of Mtb, allowing an adaptive response to develop. After clearing the primary infection with anti-TB drugs, CD8+ cells were depleted in half of the cohort. This was followed by a secondary Mtb challenge with a unique barcoded library (LibS). PET-CT scans enabled tracking of lesions that developed after secondary exposure. All granulomas and lymph nodes were isolated at necropsy and sequenced to determine the bacterial source (LibP vs. LibS). Spectral flow cytometry was used to analyze lymphocytes in individual tissues isolated at necropsy, examining the cytokines and other effector molecules produced by that may drive protection in the presence and absence of CD8+ cells.

Our data support that CD8 depleted animals lose the ability to contain Mtb after reinfection. CD8+ cells do not appear to prevent establishment of infection independently. However, the bottleneck restricting dissemination, especially to lymph nodes, is loosened in the absence of this population. Thus, CD8+ lymphocytes are critical to immunological protection against Mtb and should be considered as a key component for vaccine and host directed therapy development.

*Poster 106*

**Does intravenous vaccination with self-killing BCG lead to development of an immune response that protects against Mycobacterium tuberculosis?**

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The use of intradermal BCG offers limited protection against tuberculosis. Intravenous (IV) administration of BCG has shown to produce robust protection against infection in a non-human primate model of tuberculosis. However, the duration of exposure and time required to elicit an efficacious, protective response is unknown and safety of IV administration of live BCG remains a concern. Here we investigated whether a self-limiting strain of BCG (killswitchBCG; ksBCG) could induce protective responses in macaques. ksBCG uses two transcriptionally repressible lysins; the inducer doxycycline is required for repression, with the absence of inducer leading to BCG death. Our first study demonstrated the robust in vivo killing of ksBCG once doxycycline is stopped IV ksBCG elicited a robust, multicellular immune response in airways and lung up to 8 weeks, similar to standard BCG. In the current study we are assessing protection conferred against *Mycobacterium tuberculosis* with IV ksBCG. Mauritian cynomolgus macaques (MCM) were vaccinated intravenously with ksBCG. 5 months post vaccination, animals were challenged with *M. tuberculosis* and later euthanized 12 weeks post infection. Bronchoalveolar lavages were routinely taken throughout the study, and alongside tissue samples, analyzed using spectral flow cytometry. The presence of multiple cell types (CD4, CD8, and γδ T cells, B cells, NK cells), cytokines (IFNγ, IL-2, IL-17, TNF) and cytotoxic molecules (granzymes B and K, perforin, granulysin), as well as *M. tuberculosis* bacterial burden will be assessed to determine the level of protection provided by IV ksBCG.

*Poster 38*

**Deficiency of *Trps1* impairs odontoblast function causing multiple dental defects**

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**Objectives:** Tricho-rhino-phalangeal syndrome (TRPS) is caused by mutations in the *TRPS1* gene encoding TRPS1 transcription factor. TRPS patients present numerous dental defects, including microdontia, abnormal tooth morphogenesis, and thin dentin. To understand the mechanism underlying dentin defects in TRPS, we generated *Trps1* conditional knockout mice with *Trps1* deletion in odontoblasts (*Trps1$^{Col1a1}$cKO*). Recently, we showed that dentin of *Trps1$^{Col1a1}$cKO* molars is thinner, shows a globular mineralization pattern, and has lower resistance to acid-induced demineralization. Therefore, we hypothesized that *Trps1* deficiency impairs odontoblast differentiation and function.

**Methods:** We analyzed 1$^{st}$ mandibular molars and incisors of 4wk-old WT and *Trps1$^{Col1a1}$*cKO males by micro-computed tomography (μCT) and histology (n=5/genotype). Expression of extracellular matrix (ECM) proteins, proteins involved in breakdown of ECM, membrane molecules, as well as proteins that promote and regulate ECM mineralization was analyzed by IHC.

**Results:** μCT analyses detected that *Trps1$^{Col1a1}$cKO* molars and incisors are smaller than WT and have altered morphology. Furthermore, *Trps1* deficiency results in a significant mineralization delay, decreased tissue thickness and volume of dentin and enamel. Histological analyses revealed the presence of thicker predentin and thinner dentin. There was no difference in the odontoblasts number, however impaired odontoblast organization and cell-cell interactions were detected. We uncovered elevated levels of biglycan in *Trps1$^{Col1a1}$cKO* predentin and its persistent expression in mature odontoblasts. Osx, Dmp1 and Bmp1 were downregulated in *Trps1$^{Col1a1}$*cKO odontoblasts in comparison to WT.

**Conclusion:** Our findings highlight the importance of Trps1 for the acquisition of the proper tooth size, morphology, and mineralization of dentin. The activity of Trps1 in odontoblasts is required for the downregulation of biglycan in mature odontoblasts, while supports expression of multiple proteins involved in dentinogenesis.

*Poster 39*

**Immunomodulatory functions of the gut protist Pentatrichomonas hominis in health and disease**

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Protists have been identified as part of the mammalian gut microbiome and are known to impact gut immunity and complex intestinal diseases such as infammatory bowel disease (IBD). In humans, studies mainly describe host interactions with disease-causing species, while little is known about how commensal protists impact the immune system. Protists from the Parabasalid family, including Pentatrichomonas hominis (P. hominis) have been identified in about 30% of healthy individuals; however, the role of these non-pathogenic commensal Parabasalids remains unknown. Hence, we were interested in investigating the impact of P. hominis on the gut immune landscape. To that aim, we successfully colonized mice with P. hominis in gnotobiotic housing conditions and by using an antibiotic regimen under SPF conditions. Moreover, in vitro studies demonstrated that P. hominis and more interestingly, P. hominis supernatant, are able to modulate Th1 immune responses (Tbet+), suggesting that secreted soluble molecules have immunomodulatory properties.

Given the increased incidence in IBD, we decided to study the role of P. hominis in IBD mouse models. Our results show that P. hominis supernatant prevents the development of DSS-induced colitis. By doing de-novo genome and transcriptome assembly of this protist and high-resolution mass spectrometry of the secreted supernatant we have identified immunomodulatory candidate pathways and metabolites that might be mediating this effect. Further studies are needed to understand the underlying mechanism, however our research sheds light onto the impact of protists on IBD, which is a current major gap in knowledge.

*Poster 132*

**Clinical efficacy and treatment limitations of bacteriophage therapy for recurrent *Enterococcus faecium* blood stream infections**

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Enterococci are gram-positive commensals that are intrinsically resistant to many antibiotics, and a majority of clinical *E. faecium* isolates have also acquired vancomycin resistance (VREfm). New therapeutic options are needed, and bacteriophages (phages) may expand our limited antimicrobial repertoire. A patient with recurrent *E. faecium* blood stream infections (BSIs) was enrolled in an eIND protocol at UPMC to receive a phage cocktail, in addition to standard of care. Stool samples collected throughout treatment underwent metagenomic analysis to assess treatment-related effects on the gastrointestinal (GI) microbiome. Phage-specific host immune responses were measured via ELISA. Phage-antibiotic combination therapy prolonged bacteremia-free intervals and reduced hospitalizations in this patient. Stool metagenomics highlighted a concomitant reduction in the proportion of *E. faecium* and VRE in the GI tract. BSI recurrence was preceded by increased *E. faecium* GI burden. While no phage resistance was detected, serum neutralization of both phages also preceded the final recurrence and was associated with increased IgG titers against both phages. To determine the broader applicability of the phage cocktail across diverse *E. faecium* strains within our hospital system, 89 contemporary VREfm bloodstream isolates were also assessed for phage susceptibility via the soft overlay method and underwent comparative genomic analysis. Approximately two-thirds were phage susceptible, and susceptibility loosely clustered amongst isolates belonging to the same genetic lineage. VREfm is a challenging nosocomial pathogen, and new therapeutic strategies are critical. Antibiotics combined with a phage cocktail improved clinical outcomes for a patient with recurrent *E. faecium* BSIs and altered the GI enterococcal population. These phages have relatively broad *in vitro* activity against invasive VREfm isolates in our hospital system. However, neutralizing antibody responses may be treatment-limiting, and further investigation regarding optimal phage regimens are needed.

*Poster 107*

**Diurnal rhythms in circuitry underlying motivated behaviors**

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Endogenous rhythms are present in most behaviors and physiology, and they are crucial for an organism's survival since they allow the organism to perform activities such as seeking rewards at optimal times. Interestingly, aberrations in motivated behaviors and reward-seeking are common features of psychiatric disorders such as substance use disorder (SUD). Therefore, rhythms may play a significant role in behaviors like drug-taking, and there may be certain times of the day when these behaviors are more prevalent. Additionally, rhythms may also regulate fluctuations in the neural circuitry that underlie motivated behaviors. To better understand these rhythms in the neural circuitry involved in motivated behaviors, we used electrophysiology in mice in the nucleus accumbens, a brain region that is essential for reward processing. The nucleus accumbens contains medium spiny neurons (MSNs) that have dopamine 1 receptors (DR1) and dopamine 2 receptors (DR2), as well as cholinergic interneurons that release acetylcholine. Preliminary data suggests that MSNs containing DR1 exhibit higher activity during the dark cycle in mice, which corresponds with an increase in motivated behaviors.
Conversely, cholinergic interneurons may exhibit greater activity during the light cycle. There have been limited studies that have characterized the rhythms in cholinergic interneuron activity. Therefore, the rhythms in cholinergic interneurons may be crucial for modulating the mechanisms that drive motivated behaviors. Research has suggested that cholinergic interneurons play a vital role in motivation and learning. Additionally, recent studies have suggested that fluctuations in dopamine release may be linked to cholinergic interneuron activity. Hence, the rhythms in cholinergic interneurons may play a critical role in regulating motivated behaviors and other underlying mechanisms. We are currently expanding our electrophysiology studies by utilizing drug self-administration to determine if rhythms persist in these neural mechanisms or if they are altered or eliminated. Overall, these findings suggest that rhythms exist in the circuitry that underlies motivated behaviors, such as drug-taking, and support the notion that there may be specific times of the day when these behaviors are more prevalent.

*Poster 108*

**Two classes of novel proteins facilitate efficient NLRP1b-mediated pyroptosis**

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Inflammasome is a large protein complex that detect and amplify signals from a variety of stimuli to activate inflammatory response, which results in the production of inflammatory cytokines and pyroptosis. Among various inflammasome sensors, NLRP1 is a potent and well conserved activator of inflammation. Mouse NLRP1B can be activated via site-specific cleavage by the bacterial lethal toxin, resulting in the activation of the caspase-1 and pore-forming protein GSDMD, which then triggers inflammatory cell death called pyroptosis. While major players (NLRP1B, Caspase-1, GSDMD) during NLRP1B-mediated pyroptosis have been identified and characterized, it remains elusive whether other factors are involved given the physiological significance and complexity of NLRP1B-mediated pyroptosis. To identify novel factors involved in NLRP1B-mediated pyroptosis, we performed a genome-wide CRISPR knockout screen that covers 67,405 non-essential gens in mouse macrophage RAW 264.7 cells. Two classes of novel proteins involved in phosphatidylethanolamine (PE) synthesis and m6A methylation are identified as potent factors that facilitates efficient NLRP1b pyroptosis. We discovered that WTAP and Zc3h13, two factors in modulating mRNA's m6A methylation, are required for activation of GSDMD. By coupling transcriptional analysis (RNA-seq) with CRISPR knockout results, we found potential WTAP downstream targets that may inhibit pyroptosis upon downregulation by WTAP. The other novel factor, Pcyt2 (phosphate cytidylyltransferase 2), is a rate-limiting enzyme during membrane phospholipid PE (phosphatidylethanolamine) synthesis and loss of Pcyt2 delayed pyrotptosis process. After dissecting pyroptosis signaling pathway, we found that loss of Pcyt2 compromise the GSDMD membrane insertion and the pore formation. In addition, we reconstitute pyroptosis in mouse B16-F10 cells that bypasses the GSDMD activation signaling pathway and found that loss of Pcyt2 compromises pyroptosis suggesting that Pcyt2 functions in the post-GSDMD activation steps in facilitating GSDMD membrane insertion and pore formation.

*Poster 61*

**Hair cortisol concentrations as a predictor of risk for suicidal behavior**

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**Introduction:** Suicide is the 2nd leading cause of young adult death in the United States. Yet, predicting which individuals will attempt suicide is a difficult task, with prior research finding no association between clinicians' predictions of risk and a patient's actual suicidal behavior. Hair cortisol concentrations (HCC)—which reflect HPA axis reactivity over the preceding months—provide a possible *objective, prospective* marker for risk of suicidal behaviors.

**Methods:** Our sample was comprised of living inpatients ($N$ = 110) aged 18-30 years across the spectrum of suicidal ideation and behavior, and a postmortem sample ($N$ = 80) of individuals who had died by suicide or accidental overdose. HCC over the prior 3 months was measured using a 3cm segment of hair closest to the scalp. Suicidal ideation and behaviors were assessed using clinical interview and standardized self-report measures (living sample) and psychological autopsy with next of kin (postmortem sample).

**Results:** Across the full spectrum of suicidal behavior, HCC was significantly lower among those who died by suicide compared to those with suicidal ideation ($d$ = -.87). There was also a significant trend whereas those who died by suicide had the lowest levels of HCC, followed by those with a suicide attempt, then ideation, and finally psychiatric controls, who had the highest levels of HCC. Among the postmortem sample, HCC was lower among those who died by suicide versus those who died by overdose ($d$ = -.39).

**Conclusion:** Lower HCC—which may represent a diminished ability to mount an adaptive stress response—emerged as a strong and consistent objective predictor of *future* risk for suicidal behavior among high-risk young adults.

*Poster 109*

**Immunophenotype of a mouse model of LAMA2-deficient congenitxal muscular dystrophy**

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LAMA2-CMD is a type of congenital muscular dystrophy (CMD) caused by mutations in the LAMA2 gene, which is responsible for encoding the LAMA2 protein that is crucial for maintaining healthy muscle and myogenesis. Individuals with LAMA2-CMD display low muscle tones, which then progress to significant skeletal muscle loss and fibrosis. The immune cells are important in regulating all facets of muscle homeostasis and disease, however, a comprehensive understanding of the immune cell composition in LAMA2-CMD muscle remains understudied. Such knowledge would be essential in the design and development of future therapeutic interventions for LAMA2-CMD. **The purpose of this study was to investigate the spatiotemporal dynamics of lymphocytes and myeloid cells in a LAMA2-CMD mouse model (dyW/dyW), with the aim of filling in the knowledge gap in this area.** We conducted a time-course flow cytometry analysis of peripheral blood, muscles, and lungs isolated from dyW/dyW and wildtype littermates aged 2-4 weeks. Our findings revealed an increased frequency of neutrophils and monocytes in the blood of dyW/dyW mice, as well as an increase in the infiltration of CD45+ cells into dystrophic muscles. CD4+ T cells, NK cells, macrophages, and neutrophils were found to be the major infiltrating cell types in LAMA2-deficient muscles at 2 weeks of age, suggesting their potential involvement in early muscle pathology in LAMA2-CMD. While no differences were observed in the lungs in terms of total cell counts and frequency of CD45+cells, CD4+, and CD8+ T cells, there was a reduction in the number of NK cells over time and an increase in neutrophils and macrophages at 2 and 4 weeks of age, indicating their possible role in the observed lung injury in dyW/dyW mice. In summary, our study highlights the altered frequencies of cells involved in the inflammatory process in dyW/dyW mice, which vary depending on the tissue and disease onset and provides a foundation for exploring various therapeutic interventions for LAMA2-CMD, such as gene therapy.

*Poster 62*

**Same sleep disorder but different sleep patterns: heterogeneity of multidimensional sleep health in veterans with obstructive sleep apnea**

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**Background.** Nearly one in five Veterans who receive care through the Veterans Health Administration have obstructive sleep apnea (OSA), a sleep disorder characterized by partial or complete obstruction of the upper airway during sleep. OSA is associated with a range of mental and physical health outcomes, including depression, diabetes, and cardiovascular mortality. While continuous positive airway pressure (CPAP) therapy has been shown to effectively treat obstructed airflow during sleep, CPAP may not correct maladaptive sleep patterns like irregular timing that predisposes one to experience residual daytime sleepiness and depression. Despite CPAP treatment, OSA patients may experience varied health-promoting sleep patterns that may increase risk or buffer against some of the physical and mental health consequences associated with this sleep disorder. Multidimensional sleep health (MDSH) is an emerging model described as an individual's sleep/wake profile derived from measures of sleep duration, timing, regularity, efficiency, quality, and alertness that promotes physical and mental health. While sleep health is thought to be orthogonal to sleep disorders, less is known about sleep health in sleep-disordered populations. The current investigation sought to understand individual differences in sleep health within an OSA Veteran population. Secondly, we examined the relationship between sleep health with depressive symptomatology. **Methods**. In a pilot sample of 13 OSA Veterans who were well-adherent to CPAP therapy but continued to experience excessive daytime sleepiness (mean age = 54.8, 76.9 % male, 100% White), participants collected actigraphy-derived sleep patterns for seven days via the Actiwatch 2 device. We derived four actigraphy-derived sleep variables (duration, efficiency, timing, and regularity) and applied empirically-validated cutoffs shown previously to predict cardiovascular morbidity. Depressive symptom severity was calculated from the 16-item self-reported Quick Inventory of Depressive Symptomatology (QIDS-SR). **Results.** Among the individual sleep health variables, 61.5% of participants had optimal scores for duration, 46.2% for efficiency, 38.5% for regularity, and 30.8% for timing. For total sleep health scores, 7.7% of participants had optimal scores on all 4 dimensions, 15.4% on 3 dimensions, 46.2% on 2 dimensions, 7.7% on 1 dimension, and 23.1% on no dimension. Participants with zero optimal sleep scores had significantly higher depressive scores ($M = 19.0$, $SD = 3.0$) than participants with 1 or 2 ($M = 10.0$. $SD = 3.9$) and 3 or more optimal sleep scores ($M = 11.3$, $SD = 6.0$, $p = .019$). **Conclusions.** These preliminary findings suggest heterogeneity in sleep health profiles even among a homogenous sample of symptomatic OSA patients with adequate CPAP therapy adherence. Better sleep health was associated with lower depressive symptomatology. Future work should replicate these findings in a larger sample of OSA patients with both objective and self-reported sleep dimensions and to examine whether interventions designed to improve sleep health protect against long-term health physical and mental health outcomes associated with OSA.

*Poster 63*

**Behavioral–social rhythms and metabolic syndrome prevalence in retired night shift and day workers**

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Modifiable daily routines—such as working, sleeping, and social interaction—may serve as time cues that keep endogenous circadian rhythms stably synchronized to the 24-hour day. Stability in the timing of these behaviors across days (i.e., behavioral-social rhythm regularity) contributes to health. Atypical schedules such as night shift work and major life events such as retirement are potentially associated with changes in both behavioral–social rhythms and CVD risk factors such as metabolic syndrome. This study examined whether behavioral–social rhythms are associated with CVD risk factors in retired night shift workers and day workers and explored whether past night shift work exposure moderates the associations of behavioral–social rhythm and CVD risk factors. 154 retired older adults participated in this study. Multiple logistic regression models were used to examine associations between behavioral–social rhythms and CVD risk factors. Independent variables included Social Rhythm Metric-5 score and actigraphy rest-activity rhythm inter-daily stability and intra-daily variability. The dependent variable was metabolic syndrome prevalence. More regular behavioral-social rhythms were associated with lower odds of prevalent metabolic syndrome and two of its individual components, body mass index and high-density lipoprotein. Past shift work history did not moderate the association between behavioral–social rhythms and metabolic syndrome. Behavioral–social rhythms are related to CVD risk factors in retired adults regardless of prior shift work exposure. Older retired workers may benefit from education and interventions aiming to increase behavioral–social rhythm regularity.

*Poster 40*

**Identification of Sirt-1 as a potential biomarker of iron-induced chronic liver injury in sickle cell disease**

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Sickle cell disease (SCD) is a genetic disorder affecting millions worldwide. SCD leads to several pathophysiologic complications, reducing life expectancy to 42-47 years. Red blood cell transfusions are crucial in SCD management but cause severe iron overload in multiple organs. Inflammatory markers increase in the serum and liver of iron-overloaded SCD patients. Hepatic iron homeostasis at baseline and during transfusion remains poorly understood in SCD with variable levels of iron regulators both in children as well as in adults, for.

We studied iron homeostasis in Townes sickle cell mice at baseline and post iron overload by administering iron dextran. Townes mice exhibited significant liver iron accumulation at baseline. Hepcidin was reduced, while ferroportin and ferritin levels increased in sickle mice both at baseline and post hepatic iron overload. Iron-loaded macrophages were enriched in SCD mouse livers. Depletion of hepatic Kupffer cells using clodronate liposomes caused liver injury, hepatocyte death, systemic and hepatic iron accumulation, and mortality, suggesting their protective role in iron homeostasis. Mechanistic analysis revealed a protective pathway involving heme oxygenase 1 (HO-1)-sirtuin-1 (SIRT1)-p53 signaling in myeloid cells, potentially alleviating iron-induced chronic liver injury in SCD. Kupffer cell-generated HO-1 stimulated SIRT1 activation in SCD mouse hepatocytes, preventing iron-induced senescence and cell death via P53 signaling.

Loss of Kupffer cells drastically reduced HO-1 expression, leading to reduced SIRT1, accelerated senescence, and cell death in the SCD liver. Elevated SIRT-1 levels were detected in SCD patients' blood serum at baseline which was significantly reduced upon repeated blood transfusion. Our ongoing work aims to utilize modulation of the HO-1-SIRT1-p53 signaling pathway as a therapeutic option against iron-induced chronic liver injury in SCD.

*Poster 110*

**Longitudinal characterization of gangliogenesis in the chick retina: peeking at the HAA**

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The visual high acuity area (HAA) is a specialized retinal region present in species highly reliant on their sense of vision. It is usually located in the center of the retina and presents a series of unique features governing the gift of sharp vision, such as an absence of rod photoreceptors and substantial increased retinal ganglion cells (RGCs) density. The presence of an anatomical pit minimizing the scattering of light makes the human fovea a highly sophisticated HAA.

The chick retina is a powerful model to study fovea development because of two unique attributes absent in most animal models used in visual research: presence of an HAA and exceptionally embryonic accessibility. We have previously described how, in the chick, retinoic acid (RA), a classic signaling pathway regulating many aspects of embryogenesis, is modulated in the developing HAA. Specifically, the absence of RA due to localized expression of its catabolizing enzymes defines the limits of the presumptive HAA.

To better understand the molecular and cellular mechanisms underlying HAA development, we manipulated RA signaling by *in ovo* pharmacological injection of all-trans RA (atRA) during the period of foveogenesis and performed bulk RNAseq of central retina 18hours after. Analysis comparing atRA- and DMSO-injected retinas revealed differentially expressed genes mostly related to RGCs differentiation. We then carried out a comprehensive longitudinal study based on flat-mount retinal preparations to evaluate RGCs genesis, maturation and distribution. The use of unreported antibodies in chick retina labeling RGCs populations, combined with advanced imaging analysis of flat-mounts and regional areas, allows the possibility of identifying HAA-specific attributes linked to RGCs and provides insights into mechanisms regulating acquisition of increased RGCs density in the HAA.

*Poster 64*

**A state space grid analysis of mother-preschooler interactions: associations with maternal borderline personality disorder**

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Mothers with borderline personality (BPD) experience difficulties in relationships with offspring, with negative sequelae for offspring development. Mothers with BPD are less supportive and more unsupportive; however, only one study evaluated the temporal qualities of interactions and measured child behavior. This study explored mother-preschooler interactions to understand the contexts that are particularly challenging for mothers with BPD and how the mother-child system behaves in these contexts.

Mothers with features of BPD (BPD; n=39) and healthy control mothers (HC; n=36) completed an interaction with their child, including three tasks that elicited 1) child frustration, 2) mother frustration, or 3) play. Mothers' behaviors (support, nonsupport) and children's affect (positive, negative) were coded. Trajectories of interactions were plotted on a state space grid and estimates representing the structure, temporal dynamics, and sequences of dyadic behavior were calculated.

Across contexts, BPD dyads got stuck in more negative dynamics whereas HC dyads spent more time displaying positive dynamics. BPD mothers were less likely to respond to child negative affect with support and their offspring were more likely to respond with negative affect to mothers' nonsupport. During naturalistic play, HC mothers were more variable in their responses.

Results of his study demonstrate that quantifying dynamic dyadic behavior is more informative than focusing on global metrics of mothers' behavior alone. While previous findings related to maternal BPD and unsupportive behaviors were replicated, results revealed contingencies between mother-child and specific sequences of dyadic behavior relevant to maternal BPD.

*Poster 41*

**Generalizing the analysis pipeline to facilitate neuroscience data reuse**

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The field of neuroscience has seen rapid growth in recent years, leading to the generation of vast amounts of data that require sophisticated tools for processing and analysis. The Brain Image Library (BIL)[1] hosts thousands of whole brain datasets that are regularly uploaded by Brain Initiative investigators. While most of the data serves a specific purpose for a particular experiment, it has the potential to be reused to make new unbiased discoveries. To facilitate such reuse, we have developed a pipeline that generates high-level data representations that are easily searchable, comparable, and visualizable both in raw data space and in the common coordinate framework. This effort is aligned with the Brain Initiative goal of mapping every cell in the brain. The pipeline includes tools for soma or nuclei detection, cell classification, neuron binarization, and image registration. The pipeline is designed to be highly flexible, with multiple tools for each of these analysis steps, allowing researchers to easily plug-and-play different analysis methods. For detection tasks, we have both traditional and deep learning-based tools. For classification, we use neural networks based on resnet-50[2] architecture, implemented in fastai[3]/pyTorch, and trained on a large number of manually annotated cells. For binarization, we employ a random forest pixel classification approach with a generalized pretrained model. For image registration, we use a 3d automated optimization based method, but also consider deep learning-based approaches. The pipeline is scalable and designed to run on multiple machines, enabling efficient parallel processing of large datasets. The resulting high-level annotations will allow researchers with varying levels of technical expertise to easily access and use the data, advancing our understanding of the brain.

[1] brainimagelibrary.org
[2] Deep Residual Learning for Image Recognition / https://arxiv.org/abs/1512.03385
[3] fastai: A Layered API for Deep Learning / https://arxiv.org/abs/2002.04688

*Poster 111*

**Evolutionarily transient coding sequences affect phenotype and fitness**

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Eukaryotic model organisms translate thousands of open reading frames (ORFs) outside of annotated coding sequences, suggesting the possibility that the proteome is much larger than annotated. However, the biological consequences of this translation are poorly understood. Using hundreds of published ribosome profiling studies, we constructed a high-resolution map of translation in *S. cerevisiae* and then investigated the evolutionary dynamics and phenotypes associated with unannotated ORFs. We find that over 18,000 unannotated yeast ORFs are translated, substantially more than the around 6,000 canonical yeast genes. Conducting an extensive comparative genomic analysis at both the population level and between species, we find that fourteen unannotated translated ORFs are evolutionarily conserved genes previously missed by annotation due to short length. However, the vast majority of unannotated ORFs show no evidence of conservation, comprising a population of short-lived ORFs that appear to be evolving close to neutrally.

Despite lacking signatures of selection, we detected stable protein products of hundreds of evolutionarily transient unannotated ORFs using microscopy. Employing a genetic screen in which ORF start codons were disabled, we find that 14% of tested transient ORFs provide fitness benefits in at least some environmental conditions. Around 100 annotated yeast genes also appear to be evolutionarily transient, with nearly identical evolutionary and structural properties to the unannotated transient ORFs, and several express proteins with well-characterized biological roles. Overall, our analysis reveals the existence of a vast pool of evolutionarily transient coding sequences that can have major consequences for organism biology during their short lifespans.

*Poster 148*

**Correlating patterns of IOP-induced axon deformation within lamina cribrosa with the glaucoma tissue loss at the minimum rim**

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**Purpose:** Elevated intraocular pressure (IOP) is a primary risk factor for the development and progression of glaucoma, but the mechanisms of how elevated IOP contributes to neural tissue damage and glaucoma are not yet clear. A good spatial agreement between an acute mechanical insult and chronic neural tissue damage would suggest a promising candidate mechanism. Our goal was to identify the acute lamina cribrosa (LC) insult in best agreement with the clock-hour pattern of neural tissue loss at glaucoma onset.

**Methods:** A non-human primate was subjected to elevated IOP, first acutely in a compliance test (IOPs of 10 and 40mmHg), then chronic until glaucoma onset. OCT images were acquired in each condition. Chronic neural tissue loss was defined by the decrease in minimal rim width (MRW) between baseline and glaucoma onset. We compared regional (24 half-hours) decreases in MRW with the acute IOP-induced deformations at baseline. Several mechanisms of injury were considered, including conventional stretch, compression, shear, and effective strain. We also considered recently a proposed axon-centric analysis technique to distinguish IOP-induced insults into components longitudinal and transverse to the axons. Using statistical optimization techniques, we then determined a threshold value for each potential injury mechanism that results in the best agreement between MRW decrease and axons subjected to insult above the threshold.

**Results:** Agreements between MRW changes and LC insults were highly variable. Maximal longitudinal axonal compression had the closest fit a 17.8% normalized difference for a threshold of 10.7%. Conventional insult types had significantly worse agreements with MRW changes. For example, the best effective strain agreement (threshold=15.5%) was 62% worse.

**Conclusions:** Our results point to longitudinal axon compression as a potential mechanism relating IOP-induced LC distortion and glaucomatous vision loss. An axon-centric approach produced closer agreements than the conventional one. We are currently extending the analysis to more animals.

*Poster 133*

**Returned to care and never returned to care among loss to follow up glaucoma patients: an intelligent research in sight (IRIS) registry retrospective cohort analysis**

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Purpose: Loss to follow up (LTFU) in glaucoma can lead to disease progression and vision loss. We previously identified risk factors for LTFU in glaucoma using the IRIS® Registry (Intelligent Research in Sight). The purpose of this study is to examine this LTFU population to determine factors associated with eventual return to care after a period of LTFU.
Methods: This retrospective cohort study from the IRIS® Registry (Intelligent Research in Sight) examined a cohort of 1,053,134 patients with primary open-angle glaucoma or glaucoma suspect who had an encounter in 2014. Follow up patterns were assessed through 2019, and LTFU was defined as exceeding 1 year without an encounter. Within the LTFU group, patients were considered to have "returned to follow up" (RTFU) if they presented again for an encounter after a lapse in care or "never returned" (NR) if they had no further encounters after LTFU within the study period. Baseline demographic and clinical characteristics were compared between RTFU and NR subgroups.
Results: Among 1,053,134 glaucoma patients, 582,351 (55%) had at least one episode of LTFU over the 6-year study period. Within the LTFU group, 42% (242,238) RTFU and 58% (340,113) NR. Factors associated with NR vs RTFU including older age (mean 70.6 vs 64.2 years), unknown or no insurance (73% versus 27%), history of glaucoma laser or surgery (71% versus 29%), visually impaired (76% versus 24% with visual acuity 20/200 or worse), and severe stage glaucoma (77% vs 23%) ($p$<0.001 for all). Among those who RTFU, most returned within 2 years of last appointment (68.2%).
Discussion: Over half of all glaucoma patients in this cohort became LTFU at some point over the 6-year study period. Compared to the LTFU patients who returned after a lapse in care, those who never returned were older, uninsured, more visually impaired, and had more severe glaucoma.
Conclusion: Among glaucoma patients with LTFU, those who did not eventually return to care have greater visual morbidity and disease severity than those who returned after a lapse in care.

*Poster 42*

**Optical control of protein function via genetic code expansion in chick embryos and in human retinal organoids: towards manipulation of retinoic acid signaling for control of foveogenesis**

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The fovea is a highly specialized area of the retina responsible for our central sharp vision used for tasks including reading abstracts and recognizing faces. The molecular mechanisms underlying fovea development, however, remain poorly understood, mostly due to the lack of a tractable laboratory model system. Approaches for regenerating the fovea are highly desirable for treating vision loss, but are limited due to this gap in knowledge and technical limitations.

Recently, our group discovered multiple proteins that pattern retinoic acid signaling to regulate the development of the fovea. To recapitulate these developmental cues at the proper place and time, we have developed a technique for light-controlled protein activation. Specifically, we have developed a method for genetic code expansion in chick embryos and in retinal organoids derived from human induced pluripotent stem cells. Using this technique, we have installed masked amino acid residues into specific sites in multiple proteins in both model systems. These masked residues, when irradiated with nontoxic UV or violet light, are converted to natural lysine or tyrosine residues. When used to block critical residues, this technique thus enables optical control of protein function.

By mutating critical residues in Cre recombinase, we demonstrate spatial and temporal control of DNA recombination in a chick retina model. Further, we show that the extent of protein activation may be tuned by varying the length of irradiation. Optical control of Cre recombinase function is a powerful tool for controlling gene expression in these and other systems. Additionally, we are further assessing the use of this technique for control of retinoic acid signaling in chick embryos and human retinal organoids during the foveogenesis period. We expect this method to pave the way for cuing fovea development in human retinal organoids and therefore lead to the generation of a highly desirable high-throughput model system amenable to study foveal diseases and to investigate vision restoration approaches.

*Poster 43*

**Heritable effects of metabolic stress on CD8 T cell function**

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CD8 T cells are critical for immune responses to cancer. In this context, CD8 T cells are exposed to a metabolically active tumor tissue where certain nutrients are scarce. The unique nutrient environment in tumors shapes T cell responses, but little is known about the long-term, heritable effects of tumor metabolic stress on CD8 T cells. To examine the heritable effects of metabolic stress on T cells, CD8 T cells were cultured *in vitro* in media deficient in different individual metabolites or Tumor Interstitial Fluid-like Media (TIFM), a novel cell culture medium that mimics the nutrient environment of tumor interstitial fluid in murine pancreatic ductal adenocarcinoma (PDAC). Naïve CD8 T cells were activated for 24 hours in control media, then transferred to metabolically stressful media for 48 hours. After metabolic stress, cells were returned to nutrient replete control media for four days. On Day 7, cells were restimulated with αCD3/αCD28. Upon restimulation, cells treated with TIFM or arginine-deficient media had fewer TNFα+IFNγ+ cells, and cytokine positive cells produced less TNFα or IFNγ, respectively. IL-2 expression was also lower in CD8 T cells exposed to arginine-deficient media or TIFM. In these experiments, cells were removed from metabolic stress four days prior to restimulation. In that time, stressed cells expanded more than 25-fold since they were removed from stress. Thus, this assay examines the distant descendants of the cells exposed to metabolic stress. To further test the long-term effects of metabolic stress in vivo, stressed cells were adoptively transferred into Vaccinia-OVA infected mice and examined 11 days post infection. Stressed cells had fewer IFNγ+TNFα+ cells upon restimulation despite proliferating at the same rate as control cells, consistent with the in vitro data. Collectively, these data provide evidence that CD8 T cell responses are influenced by past metabolic stress, and these changes are heritable. Future work will identify mechanisms through which CD8 T cells “remember” past metabolic stress and the functional effects on metastatic disease.

*Poster 65*

**Social characteristics of racial minority stroke survivors and perceived discrimination: a secondary analysis**

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African American and Black persons experience significant disability after stroke. They also experience high external stressors, including discrimination, which may attenuate stroke recovery. Further understanding of the associations among external stressors, disability severity, and attenuated recovery are important to reduce healthcare disparities and improve patient outcomes. However, persons of the same race may not have the same lived experiences due to variations in social characteristics such as age or education. Associations between these social characteristics and perceived discrimination may offer insights into the recovery of older adult African American and Black stroke survivors. We conducted a secondary analysis of 11 poststroke patients who were recruited to participate in the Black Lived Experiences of Stroke Study (female=10, age=67±11, ≥2 years post-secondary education=63%) to assess the frequency of perceived discrimination. Perceived discrimination was measured with the Everyday Discrimination Scale survey. We examined correlations between age and education and perceived discrimination using Cramer's V tests. Semi-structured participant interviews were also examined for examples of perceived discrimination during poststroke healthcare. Nine participants reported perceived discrimination post-stroke. Analyses revealed a clinically meaningful but not statistically significant relationship between chronological age and perceived discrimination (Cramer's $V$=0.62, p=0.12). There was no meaningful relationship between educational level and perceived discrimination. All participants reported challenges with communication, missed diagnoses and poor delivery of care poststroke. Findings support the need for additional research to examine the relationships between participants' social characteristics and perceived discrimination among Black and African American stroke survivors, and the impact on stroke recovery.

*Poster 66*

**Black youths' positive feedback regarding geographic ecological momentary assessment research designs**

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**Introduction:** Geographic ecological momentary assessment (GEMA) includes repeated measurements over time and collection of location information, allowing researchers to assess life as it is lived and observe within-person variations. While this approach offers many methodological advantages, it is often assumed that invasiveness and respondent burden would limit participants' engagement, particularly among Black youth, given the historical exploitation of Black people in research. The inclusion of Black youth in GEMA studies may be crucial to understanding the role of contextual supports and challenges in their development. While studies have assessed youths' GEMA completion rates and technical challenges, none have directly assessed Black youths' perceptions of participating in a GEMA study. To address this, gap, we engaged Black youth in a study where they were asked to complete brief daily surveys and assessed their perceptions of participating in the study in both an exit survey and qualitative interviews.
**Methods:** Twenty-five Black youth (14-19 years old, 58% female) were recruited from community-based-youth-serving programs who had witnessed community violence within the past three months for a study exploring stress and coping following violence exposure. Participants completed a baseline survey, GEMAs three times daily for two weeks, and an exit survey assessing overall satisfaction. Youth also completed a one-on-one interview via zoom assessing satisfaction and perceptions of the data collection process. Analyses included descriptive statistics (exit surveys) and thematic analysis of interview transcripts.
**Results:** In the exit surveys, 86% (19/22) of the youths agreed that participating in the study was a good experience and the majority (95%; 21/22) agreed that they would participate in this study again if given the chance. Furthermore, all of the youths reported they would recommend this study to a friend. When asked in the interview what they enjoyed most about participating in the study, one theme that emerged was that the youths felt "safe" and "secure" (Participant 3) knowing that we were tracking them. Another youth stated, "it was just check up on me…I guess that kinda boost my emotions. I felt happy after" (Participant 8). Another reoccurring theme centered on helping with the study: "The fact that I knew I was gonna be helping for something, even if it's little help, I still knew I was gonna be helping a lot cause as you can tell I love being a help" (Participant 09). Another youth reflected, "it made me feel like I was doing something important." (Participant 11). Lastly, many of the youths reported enjoying completing GEMA surveys because it allowed them to express their emotions and feelings: "I like how I can get my feelings [out] during the survey" (Participant 06).
**Conclusions:** Contrary to assumptions, the youths in our study expressed high satisfaction in completing GEMAs because it made them feel safe, helpful, and emotionally expressive. Youth violence intervention programs could adapt GEMA-based approaches to guide individualized support and engagement following violence exposure.

*Poster 134*

**Gastrointestinal bleeding reinforcement learning agent for blood product transfusion**

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**Introduction:** Blood product resuscitation is crucial to the treatment of gastrointestinal hemorrhage (GIH). Current guidelines emphasize single blood products and lab thresholds, neglecting balanced resuscitation and patient-specific factors. We developed a Gastrointestinal Bleeding Reinforcement Learning Agent (GaBRieL) using Q-learning, a technique in Markovian decision-making, to infer an optimal transfusion strategy from retrospective data.

**Methods:** We analyzed 4,630 ICU admissions for GIH from the MIMIC IV dataset. GaBRieL was trained to make hourly binary transfusion decisions, optimizing PRBC, FFP, and PLTs for inpatient mortality. Decisions were based on clinical states as defined by K-means clustering on structured HER data. Evaluation was performed on a 20% validation set.

**Results:** GaBRieL adopted a more liberal transfusion strategy than clinicians, with transfusion of PRBC at higher hemoglobin levels [8.91 vs 8.06, p<.0001], FFP at lower INR levels [1.61 vs 2.23, p<.0001], and PLTs at higher platelet levels [130 vs 66.5, p<.0001]. Deviations from GaBRieL correlated with higher mortality [28.2% vs 17.2%, p<.0001], longer ICU [11.6 vs 11.3 days, p<.0001] and hospital stays [31.4 vs 25.0 days, p<.0001], and increased time on vasoactive agents [93.2 vs 77.2 hours, p<.0001], but lower total ventilator time [66.7 vs 71.6 hours, p<.0001].

**Conclusions:** GaBRieL's more liberal and balanced resuscitation strategy may reduce ICU and hospital stays and limit vasopressor time, but at the cost of ventilator time. This contrasts with studies supporting restrictive transfusion. Further work is needed to explain the observed benefits and drawbacks of GaBRieL's strategy and assess the potential for guiding resuscitation at the bedside.

*Poster 44*

**Hepatocyte tropism is a key determinant in the pathogenesis of Rift Valley fever virus**

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Rift Valley fever virus (RVFV) causes mild to severe disease in humans and livestock. Outbreaks of RVFV have been reported throughout Africa and the virus has spread to continents outside Africa. Previous studies have demonstrated that RVFV directly infects the liver, and elevated transaminases are a hallmark of fatal RVFV infection. However, the specific contribution of hepatocyte infection to viral pathogenesis remains poorly understood. To address this, we generated a recombinant miRNA-targeted virus with limited replication in hepatocytes. MicroRNAs are short non-coding RNAs that regulate mRNA expression by targeting them for degradation; miRNAs are evolutionarily conserved and tissue specific. To study the liver tropism of RVFV, we generated RVFVmiR-122 with an insertion of four repeats of sequences targeted by miR-122, a miRNA highly expressed in the hepatocyte, into the genome of the RVFV S segment. We also generated RVFVmiR-184 with an insertion of sequences targeted by miR-184, a mosquito-specific miRNA, as a control. We observed restricted replication of RVFVmiR-122 *in vitro* in primary mouse hepatocytes compared to RVFVmiR-184. C57BL/6 mice infected with control RVFVmiR-184 died of acute hepatitis as expected, whereas mice infected with RVFVmiR-122 survived acute hepatitis and instead developed late onset-encephalitis that paralleled viral RNA load in these tissues. Mice infected with RVFVmiR-122 had 3-4 logs less viral RNA in the liver compared to those infected with RVFVmiR-184 at 3-4 dpi. Additionally, RVFV-specific immunohistochemistry revealed restricted viral spread within the liver in RVFVmiR-122 infected mice, demonstrating that the change in disease manifestation is due to restricted hepatocellular replication of RVFVmiR-122. Importantly, miR-122 KO mice developed acute hepatitis following infection with RVFVmiR-122, demonstrating the specificity of the phenotype. Together, our data suggests that liver tropism is a key determinant of RVFV pathogenesis.

*Poster 112*

**Long non-coding RNA LHX1-DT regulates cardiomyocyte differentiation through H2A.Z-mediated LHX1 transcriptional activation**

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Long non-coding RNAs (lncRNAs) play widespread roles in many cellular processes. However, the precise mechanisms for their regulation the early-stage cardiomyocyte differentiation are less understood. Here, we identified *LHX1-DT* as a lncRNA divergently transcribed from a bidirectional promoter of LIM Homeobox 1 (LHX1). We found that *LHX1-DT* was nuclear-localized and transiently expressed along with LHX1 during early differentiation of cardiomyocytes. *LHX1-DT* downregulation blocked the mesodermal lineage commitment but did not affect self-renewal of human embryonic stem cells (hESCs). The phenotype was rescued by overexpression of LHX1 into the *LHX1-DT*$^{-/-}$ hESCs. Mechanistically, *LHX1-DT* physically interacted with RNA/histone-binding protein PHF6 during mesoderm commitment and efficiently replaced conventional histone H2A with a histone variant H2A.Z at the promoter region of LHX1. In summary, our work reveals a novel lncRNA, *LHX1-DT*, mediates exchange of H2A.Z and H2A at the promoter of LHX1 to regulate cardiomyocyte differentiation.

*Poster 67*

# Enhanced ATN-GFAP clustering: differentiating clinical phenotypes in cognitively impaired individuals

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**Background:** The ATN classification system assumes a sequential model of disease progression. However, there are groups of individuals in the same ATN category that exhibit a predominance of abnormality (higher burden) of one of the biomarkers, creating heterogeneous ATN groups regarding pathological predominance. Thus, we tested the hypothesis that individuals clustered by ATN biomarker abnormality predominance may offer an alternative to groups defined using biomarkers cut-offs.
**Method:** We assessed 103 cognitively impaired individuals (CDR>=0.5) from the TRIAD cohort with available measures of plasma phosphorylated tau-181, brain MRI, amyloid PET, and tau PET. We used the K-means algorithm to stratify participants into three clusters. We compared the clusters on composite measures of memory, executive functioning, language, and visuospatial processing. To examine the utility of the discovered clusters, we compared them to traditional ATN categories in the prediction of neuropsychological measures. We did so by creating three categories: patients positive on all three ATN biomarkers, patients positive on two of the three biomarkers, and patients positive on either one or none. Additionally, we created an inflammation, amyloid and tau deposition probabilistic map anchored on young controls (n=51, mean age=23.74).
**Results:** We uncovered 3 clusters: an amyloid predominant (AP) cluster, a tau/phosphor-tau predominant cluster (TP), and a cluster showing no predominance with low levels on all biomarkers (CN). Notably, levels of neurodegeneration and inflammation were similar between the AP and TP clusters. The AP cluster significantly differed from the CN cluster in memory only. Participants in the TP cluster had significantly lower scores in memory, executive functioning, language, and visuospatial processing than the other two clusters. In comparison, using threshold-based ATN groups showed milder differences in memory and executive functioning, and no differences in language and visuospatial processing. Furthermore, cluster membership moderated the relationship between various biomarkers, to the point of reversing the direction of correlation.
**Conclusion:** Our results highlight the biological heterogeneity present within the Alzheimer's disease continuum and that the pathological predominance of amyloid and tau is associated with different disease phenotypes. Approaching dementia patients with an eye on the predominance of pathology rather than cutoffs for abnormality may provide a better understanding of AD pathological subtypes.

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