RESEARCH RETREAT — 2021

TRANSFORMATIVE TECHNOLOGY IN ENGINEERING AND MEDICINE

Thursday, March 11 at 9:30 a.m.
Friday, March 12 at 8:15 a.m.
Welcome to the Johns Hopkins 2021 Department Of Medicine/Whiting School of Engineering Research Retreat

The Department of Medicine/Whiting School of Engineering Research Retreat is about connections. Connections among researchers in both schools, connections between mentors and mentees, and connections between leadership and the adventurous researchers that elevate the discovery community.

Our annual retreat is the largest forum for the presentation of research by Department of Medicine and Whiting School of Engineering faculty and trainees and one of the largest research events throughout Johns Hopkins. The retreat has a long-standing history in the Department of Medicine, but has seen its greatest success since joining forces with the Whiting School of Engineering in 2018.

This year, we will explore “Transformative Technology in Engineering and Medicine.” The keynote address will be given by Dr. Jennifer Doudna, who won the 2020 Nobel Prize for Chemistry. Other featured speakers include Taekjip Ha, PhD, Bloomberg Distinguished Professor from the Department of Biophysics and Biophysical Chemistry and Alison Moliterno, MD, Associate Professor of Medicine. We will also continue our Spotlight on Research sessions featuring short presentations from select faculty from medicine and engineering.

We know this year’s retreat looks a little different in its virtual format, but we are confident the exposure to the bold and creative research directions at Johns Hopkins will fuel new collaborations and capitalize on the investigative strengths of each enterprise.

Enjoy the connections!

Mark E. Anderson, MD, PhD
William Osler Professor of Medicine
Director, Department of Medicine

T.E. (Ed) Schlesinger, PhD
Benjamin T. Rome Dean
Whiting School of Engineering

Claire Hur, PhD
Assistant Professor of Mechanical Engineering
Retreat Committee Co-chair

Nicholas Zachos, PhD
Associate Professor of Medicine
Retreat Committee Co-chair
KEYNOTE SPEAKER

Jennifer Doudna, PhD

Li Ka Shing Chancellor's Chair
Professor of Chemistry & Molecular and Cell Biology
University of California at Berkeley

Dr. Jennifer A. Doudna is the Li Ka Shing Chancellor's Chair and a Professor in the Departments of Chemistry and of Molecular and Cell Biology at the University of California, Berkeley. Her groundbreaking development of CRISPR-Cas9 as a genome-engineering technology, with collaborator Emmanuelle Charpentier, earned the two the 2020 Nobel Prize in Chemistry and forever changed the course of human and agricultural genomics research.

This powerful technology enables scientists to change DNA — the code of life — with a precision only dreamed of just a few years ago. Labs worldwide have re-directed the course of their research programs to incorporate this new tool, creating a CRISPR revolution with huge implications across biology and medicine.

In addition to her scientific achievements, Doudna is a leader in public discussion of the ethical implications of genome editing for human biology and societies, and advocates for thoughtful approaches to the development of policies around the safe use of CRISPR technology.

Doudna is an investigator with the Howard Hughes Medical Institute, senior investigator at Gladstone Institutes, and the President of the Innovative Genomics Institute. She co-founded and serves on the advisory panel of several companies that use CRISPR technology in unique ways.

She is a member of the National Academy of Sciences, the National Academy of Medicine, the National Academy of Inventors, and the American Academy of Arts and Sciences. Doudna is also a Foreign Member of the Royal Society and has received numerous other honors including the Breakthrough Prize in Life Sciences (2015), the Japan Prize (2016), Kavli Prize (2018), the LUI Che Woo Welfare Betterment Prize (2019), and the Wolf Prize in Medicine (2020), and the Nobel Prize in Chemistry (2020). Doudna's work led TIME to recognize her as one of the “100 Most Influential People” in 2015 and a runner-up for “Person of the Year” in 2016. She is the co-author of A Crack in Creation, a personal account of her research and the societal and ethical implications of gene editing.
FEATURED SPEAKERS

Taekjip Ha, MA, PhD
Bloomberg Distinguished Professor of Biophysics and Biomedical Engineering, Johns Hopkins University

Dr. Taekjip Ha is a Bloomberg Distinguished Professor of Biophysics and Biomedical Engineering at Johns Hopkins University and an investigator with the Howard Hughes Medical Institute. He develops and uses single molecule and single cell measurement tools to study life at high resolution.

Dr. Ha received a bachelor in Physics from Seoul National University in 1990 and Physics Ph.D from University of California at Berkeley in 1996. After postdoctoral training at Stanford, he was a Physics professor at University of Illinois at Urbana-Champaign until 2015.

Dr. Ha serves on Editorial Boards for Science. He is a member of the National Academy of Science and a fellow of the American Academy of Arts and Sciences. He received the 2011 HoAm Prize in Science.

Alison R. Moliterno, MD
Associate Professor of Medicine, Division of Hematology

Dr. Alison R. Moliterno is internationally recognized for her seminal work on the molecular mechanisms and clinical characteristics that define myeloproliferative neoplasms. Her group discovered aberrant tyrosine kinase signal transduction pathways in MPN, including acquired and constitutional mutations in MPL and JAK2 genes. Since establishing her research program in MPN, she has built a unique observational cohort of over 600 MPN patients with which to define the modern epidemiology, natural history, and molecular pathogenesis of these disorders. Her work spans both translational and basic arenas, including the relationship of genotype to phenotype in MPN in humans and murine models.

Dr. Moliterno received her undergraduate degree from Cornell University and her medical degree from the State University of New York at Buffalo. After completing postgraduate training in internal medicine and hematology at Johns Hopkins Medical Institutions, she joined the faculty in the Department of Medicine, Hematology Division and was in the inaugural class of Doris Duke Clinical Scientists. Dr. Moliterno maintains an active hospital-based clinical practice focused on myeloproliferative neoplasm (MPN) and other hematologic conditions, and serves the hospital as attending physician and consultant for inpatients with a broad range of hematologic conditions. Dr. Moliterno mentors undergraduates, medical students, residents, and fellows in both research and clinical settings. Her basic and translational research efforts are dedicated to elucidating molecular underpinnings of myeloproliferative neoplasms (MPN) and developing more effective treatment strategies.
Ada Hamosh, MD, MPH
Professor of Genetic Medicine; Professor of Pediatrics
Dr. Frank V. Sutland Professor of Pediatric Genetics

Ada Hamosh MD, MPH, the Dr. Frank V. Sutland Professor of Pediatric Genetics, is the Clinical Director of the McKusick-Nathans Department of Genetic Medicine at Johns Hopkins University School of Medicine, the Scientific Director of Online Mendelian Genetics in Man (OMIM®), and the Co-Chair of the Phenotype Review Committee of Baylor-Hopkins Centers for Mendelian Genomics (CMG), a U.S. National Human Genome Research Institute-funded project to identify the genes responsible for known and novel Mendelian disorders. Dr. Hamosh received her BA in Biology from Wesleyan University, MD from Georgetown University, and MPH from Johns Hopkins School of Hygiene and Public Health. She completed a pediatrics residency and clinical and clinical biochemical genetics fellowship at Johns Hopkins Hospital. Dr. Hamosh has authored over 110 papers and serves on several international committees representing genome-phenome relationships as well as phenotype ontologies, including the International Rare Disease Research Consortium, the Human Variome Project, the ClinGen Project, the Global Alliance for Genomic Health (GA4GH), and the Human Genome Organization (HUGO).

She and colleagues developed PhenoDB (http://phenodb.org), a web-based tool for the collection, storage, and analysis of standardized phenotype and genotype data for use in the CMG project that is freely available to all for clinical and research use, and GeneMatcher (http://genematcher.org), a website to enable matches of clinicians and researchers with an interest in the same gene. GeneMatcher includes over 10,000 submitters from 93 countries and more than 50,000 cases. Matches through GeneMatcher have resulted in over 402 publications describing over 300 novel disease genes. GeneMatcher is a founding member of the Matchmaker Exchange (MME). Dr. Hamosh serves on the steering committee of the MME and represents it on the steering committee of the GA4GH.

Sophie Lanzkron, MD, MHS
Professor of Medicine, Division of Hematology; Professor of Oncology

Dr. Sophie Lanzkron is a Professor of Medicine and Oncology at Johns Hopkins University School of Medicine. Dr. Lanzkron is a hematologist and Director of the Sickle Cell Center for Adults. She received her BA from Brandeis University and she graduated from the Albert Einstein College of Medicine where she was inducted into the AOA honor society. She did her residency at the University of Maryland and her hematology fellowship at Hopkins. She is internationally recognized for her pioneering research on the optimal care and management of patients with sickle cell disease. She has served on the National Institutes of Health, Expert Panel in the Management of Sickle Cell Disease and on the American Society of Hematology's Sickle Cell Guideline Panel. She is co-Chair of the Clinical Trial Sub-committee for the NIH’s Cure Sickle Cell Initiative and Chair of the American Society of Hematology’s Clinical Trial Network’s Patient Engagement Committee. Her research focus is on improving the quality of care provided to this historically underserved population and she is considered an expert in health services research in sickle cell disease. Dr. Lanzkron's research is currently supported through grants from PCORI and HRSA.
FEATURED PANELISTS

**Enid Neptune, MD**

*Associate Professor of Medicine, Pulmonary & Critical Care*

Dr. Neptune is a pulmonary, critical care physician with expertise in genetic disorders of the matrix with pulmonary manifestations. She is also skilled in the management of patients with advanced obstructive lung disease, especially emphysema. Dr. Neptune has extensive expertise in the diagnosis and management of the pulmonary complications of Marfan Syndrome, Loeys-Dietz Syndrome, vascular Ehlers Danlos Syndrome and other genetic matrix disorders and serves on the Professional Advisory Board of the Marfan Foundation. She is also an expert on tobacco dependence and is the Vice-Chair of the Tobacco Action Committee of the American Thoracic Society.

Dr. Neptune has a research focus on regenerative approaches to COPD-emphysema, mucus hypersecretion in COPD, genetic disorders of the matrix and health disparities in tobacco dependence. She has been funded by the NIH, Marfan Foundation, March of Dimes, Maryland Stem Cell Research Foundation. Her active research efforts include a detailed assessment of airway mucus properties that associate with COPD progression, the dissection of the role of TGF-β and angiotensin signaling in lung maintenance and repair, the identification of novel approaches to lung regeneration and the determination of the role of sleep apnea in Marfan aortic disease. Her lab uses a combination of mouse and human reagents, novel genetically targeted animal models and primary lung resident cell based approaches. Her long term goal is to provide therapeutic reagents that can reverse established emphysema in patients with acquired, congenital or genetic disorders. Dr. Neptune is a standing member of the LIRR Study Section in the NHLBI, a member of the Board of Scientific Advisors at the NIH Clinical Center, Scientific Advisory Board of the American Lung Association, Scientific Advisory Board of the Tobacco Related Disease Research Program, Executive Cmte of the American Society of Matrix Biology and the Chair of the Respiratory Cell and Molecular Biology Assembly in the American Thoracic Society.
The Spotlight on Research Talks is a fast-paced session that showcases some of the past year’s best science from the Johns Hopkins Department of Medicine and the Whiting School of Engineering. Each presenter will share highlights of their research findings delivered in a five-minute ‘lightning talk’.

Alexis Battle, PhD
Associate Professor of Biomedical Engineering and Computer Science
“Integrative Data Analysis for Personalized Genomics”

Melanie Dispenza, MD
Assistant Professor of Medicine in Clinical Immunology
“Novel Strategies for the Prevention and Diagnosis of Anaphylaxis”

Jude Phillip, PhD
Assistant Professor of Biomedical Engineering, Chemical & Biomolecular Engineering and a core member in the Institute for Nanobiotechnology (INBT)
“Human Ageing at Cell Resolution”

Elizabeth Luczak, PhD
Assistant Professor of Medicine in Cardiology
“How to Repair a Failing Heart”

Sridevi Sarma, PhD
Associate Professor in the Department of Biomedical Engineering
“Fragility in the Brain: A Biomarker for the Seizure Onset Zone in Epilepsy”

Florin Selaru, MD
Associate Professor of Medicine and Director of the Meyerhoff Inflammatory Bowel Disease Center
“Shape Changing Microdevices for Extended Drug Release”

Suchi Saria, PhD
John C. Malone Associate Professor of Computer Science
Associate Professor of Statistics and Health Policy
“Shape Changing Microdevices for Extended Drug Release”

Janet Siliciano, PhD
Associate Professor of Medicine in Infectious Diseases
“Mechanisms of HIV-1 Persistence”
Co-Chairs:
Claire Hur, PhD
Nicholas Zachos, PhD

Members:
Peter Abadir, MD
Gail Daumit, MD, MHS
Najim Dehak, PhD
D. Brian Foster, PhD
Morgan Grams, MD
Deok-Ho Kim, PhD
Sangwon Kim, PhD
Gregory Lucas, MD, PhD
Alison Moliterno, MD
Larry Nagahara, MD
Enid Neptune, MD
Brian O’Rourke, PhD
Prasanna Santhanam, MD

Event Coordinators:
Kelsey Bennett
Emily Brady
Helen Harrison
Melanie Mossman
Holly Sellers
Alexander Van Horn

(Listed alphabetically)
THURSDAY, MARCH 11TH

9:30 a.m.
Welcome Remarks

9:45 a.m.
“Spotlight on Research”
Moderated by Peter Abadir and Steven Cohen
Alexis Battle, PhD
Associate Professor of Biomedical Engineering and Computer Science
Melanie Dispenza, MD
Assistant Professor of Medicine
Jude Phillip, PhD
Assistant Professor of Biomedical Engineering, Chemical & Biomolecular Engineering
Elizabeth Luczak, PhD
Assistant Professor of Medicine
Sridevi Sarma, PhD
Associate Professor of Biomedical Engineering
Florin Selaru, MD
Associate Professor of Medicine and Director of the Meyerhoff Inflammatory Bowel Disease Center
Suchi Saria, PhD
John C. Malone Associate Professor of Computer Science
Associate Professor of Statistics and Health Policy
Janet Siliciano, PhD
Associate Professor of Medicine, Division of Infectious Diseases

10:45 a.m.
Break

10:55 a.m.
Dr. Mark Anderson will introduce Dr. Ha

11:00 a.m.
Taekjip Ha, PhD
Bloomberg Distinguished Professor
Department of Biophysics and Biophysical Chemistry
Howard Hughes Medical Institution
Johns Hopkins University School of Medicine

11:40 p.m.
Alison Moliterno, MD
Associate Professor of Medicine, Division of Hematology
“Clinical applications of CRISPR technology”

11:55 p.m.
“Clinical application of CRISPR technology” Panel Discussion
Moderator: Alison Moliterno, MD

Panelists:
Ada Hamosh, MD, MPH
Professor of Genetic Medicine
Professor of Pediatrics
Dr. Frank V. Sutland Professor of Pediatric Genetics
Sophie Lanzkron MD, MHS
Professor of Medicine, Division of Hematology
Professor of Oncology
Director, Sickle Cell Center for Adults, JHH
Enid Neptune, MD
Associate Professor of Medicine, Pulmonary & Critical Care

12:15 p.m.
Lunch Break

12:50 p.m.
Dr. Taekjip Ha will introduce Dr. Doudna

1:00 p.m.
Keynote Address
Jennifer Doudna, PhD
Professor of Chemistry
Professor of Biochemistry & Molecular Biology
Li Ka Shing Chancellor's Professor in Biomedical and Health
University of California, Berkeley

1:45 p.m.
Day 1 Retreat Closing

FRIDAY, MARCH 12TH

8:15 a.m.
Opening Remarks from Mark E. Anderson, MD, PhD
William Osler Professor of Medicine
Director, Department of Medicine

8:45 a.m.
Opening Remarks from T.E. “Ed” Schlesinger, PhD
Benjamin T. Rome Dean
Whiting School of Engineering

9:00 a.m.
Poster Session (Gather.Town)

11:00 a.m.
Research Award Finalist Announcements

11:15 a.m.
Research Award Winner Presentations

12:30 p.m.
Retreat Closing
Basic Research Faculty Finalists
(in alphabetical order)

Lama Al-Qusairi, Ph.D.  
Abstract 123

Ho Yee (Bonnie) Yeung, Ph.D.  
Abstract 117

Qinchuan Wang, Ph.D.  
Abstract 54
THE W. LEIGH THOMPSON EXCELLENCE IN RESEARCH AWARD

Basic Research Fellow Finalists
(listed alphabetically)

Carla Freire, M.D.  Abstract 37
Andreas Patsalos, Ph.D.  Abstract 1
Mohanraj Sadasivam, Ph.D.  Abstract 28
THE W. LEIGH THOMPSON EXCELLENCE IN RESEARCH AWARD

Clinical Research Faculty Finalists (in alphabetical order)

Jacek K. Urbanek, Ph.D. Abstract 44
Kristin Bigos, Ph.D. Abstract 42
Ethel D. Weld, M.D., Ph.D. Abstract 131
THE W. LEIGH THOMPSON
EXCELLENCE IN RESEARCH AWARD

Clinical Research Fellow Finalists
(listed alphabetically)

Richard Ferraro, M.D., M.Ed.        Abstract 61
Sonya Krishnan, M.D.                Abstract 17
Anum Minhas, M.D.                   Abstract 98
Erin Spaulding, R.N., Ph.D.         Abstract 62
Trainee Finalists
(listed alphabetically)

Kaustav Bera, M.Tech.  Abstract 118
Andy S. Ding, M.S., M.D.  Abstract 136
Madi Kusmanov, M.S.  Abstract 134
Alexander Trick, B.S.  Abstract 59
Jeff Wang, Ph.D.  
Trainees:
Fan-En Chen, B.S.
Joon Soo Park, M.S.
Alexander Trick, B.S.
Pengfei Zhang, M.S.
**ABSTRACT 1**

**A Growth Factor-Expressing Reparative Macrophage subpopulation orchestrates skeletal muscle regeneration via Growth Differentiation Factor-15**

Andreas Patsalos, Laszlo Halasz, Miguel A. Medina-Serpas, Wilhelm K. Berger, Bence Daniel, Petros Tzerpos, Mate Kiss, Gergely Nagy, Cornelius Fischer, Zoltan Simandi, Tamas Varga, Laszlo Nagy

Muscle regeneration is the result of concerted action of multiple cell types driven by the temporarily controlled phenotype switches of infiltrating monocyte-derived macrophages. The pro-inflammatory macrophages transition into a phenotype that drives tissue repair through the production of effectors such as cytokines and growth factors. This highly orchestrated sequence of events, which we termed Regeneration Promoting Program (RPP), is essential for proper muscle regeneration. We set out to explore how repair-macrophage identity is achieved at the level of transcription, uncover macrophage-secreted factors with multivalent roles in repair, and mechanisms promoting this process. Gene expression kinetics-based clustering, using profiles of blood circulating Ly6Chigh, infiltrating inflammatory Ly6Chigh, and reparative Ly6Clow macrophages, isolated from acutely injured skeletal muscle, revealed the TGF-beta superfamily member, Growth Differentiation Factor-15 (GDF15), as a component of the RPP. We show that myeloid GDF15 is required for proper muscle regeneration following acute injury by utilizing genetic knockouts and gain of function approaches. Functionally, GDF15 acts both on proliferating myoblasts and muscle-infiltrating macrophages. Epigenetic analyses for upstream regulators of Gdf15 expression identified that it is under the control of nuclear receptors Retinoid X Receptor (RXR) and its heterodimeric partner Peroxisome Proliferator-Activated Receptor Gamma (PPARγ). Finally, single-cell RNA-seq immune cell profiling confirmed that Gdf15 expression is largely limited to a unique subpopulation of repair-type macrophages (Growth Factor-Expressing Macrophages, GFEM) and co-expressed with other known muscle regeneration-associated growth factors (e.g., Igf1, Gdf3).

**ABSTRACT 2**

**Does digital health exacerbate existing inequities? Examining sociodemographic predictors of digital health intervention use for readmission reduction post-MI**

Lochan M. Shah MD, Jie Ding PhD, Erin M. Spaulding PhD BSN RN, William E. Yang MD, Matthias A. Lee PhD, Ryan Demo MSE, Francoise A. Marvel MD, Seth S. Martin MD MHS

COVID-19 has ignited rapid implementation of digital health interventions (DHIs). While increasing evidence suggests that DHIs are an effective tool to reduce hospital readmissions by improving adherence to guideline-directed therapy, little is known about whether sociodemographic characteristics influence DHI use and if digital health might deepen existing health inequities. We investigated use of a DHI targeting 30-day readmission reduction after acute myocardial infarction (MI). The DHI, “Corrie”, includes educational videos, vital signs tracking via wearable devices, and medication and appointment reminders. DHI feature use was assessed during hospitalization and through 30 days post-discharge in 133 patients using back-end smartphone app analytics. Age, sex, and race were not significantly associated with DHI use before or after covariate adjustment (adjusted OR 0.98 (95% CI: 0.95-1.01), 0.6 (95% CI: 0.29-1.25), and 1.22 (95% CI: 0.60-2.48) respectively). Being married was associated with high DHI use (OR 2.12; 95% CI 1.02-4.39). Our findings that age, sex, and race are not associated with DHI use supports the use of digital health to reduce, rather than exacerbate, some existing health inequities in cardiovascular patients post-MI. Finally, the presence of a spouse, perhaps a proxy for enhanced caregiver support, may encourage DHI use.
Although physicians are in a unique position to directly see how government policy impacts health, prior studies have shown that they are less likely to vote and are less engaged in civic participation than the general population. In the context of an initiative designed to increase voter registration among patients, this study sought to understand attitudes and beliefs towards physicians helping patients register to vote as well as physician roles in health advocacy. The goal of this study was to 1) determine the success and barriers of a physician led QR code voter registration initiative and 2) determine the current perception of physicians in training in regard to physician advocacy.

In September of 2020, badges with the QR codes for the Maryland online voter registration website were distributed to medical students, residents and fellows. After online voter registration ended and prior to the presidential election, a survey was e-mailed to all medical students, resident, and fellows. The survey, developed using iterative pilot testing, assessed perceptions of the voter registration initiative, perceived roles of physician advocacy, and obtained demographic information including age, political affiliation and specialty. Data was collected and stored in Qualtrics and statistical analysis was done using Stata version 16.2 and group differences were analyzed using Chi square analysis.

The QR code for the voter registration initiative was scanned 130 times by patients prior to the presidential election. From the 1,719 medical students, residents and fellows, a total of 366 (21.3%) responded to the survey. Among respondents, nearly half (47.4%) were aged 26 to 30, 18.9% were medical students while 30.5% were residents or fellows from an internal medicine subspecialty. Over three quarters (76.1%) were Democrats while 4.8% were Republican. Prior to this initiative, only 11.4% of respondents had asked a patient whether they were registered to vote. Since the initiative, 20.9% had asked a patient about their voter registration. The most commonly cited barriers were discomfort asking the patient, time and workflow constraints. Overall, 44% agreed or strongly agreed that physicians have a role registering patients to vote (50.5% among Democrats vs 30% among non-Democrats, p=0.001). In regard to physician advocacy, 259 (86%) respondents agreed with the statement that physicians have an obligation to address health advocacy (92% among Democrats vs. 68% among non-Democrats, p=0.000). Further, 64% agreed or strongly agreed with the statement “COVID-19 has changed my perception of my role in society” (67% among Democrats vs. 54% among non-Democrats, p=0.051). In contrast, 45% agreed that physicians should focus on clinical care rather than health advocacy (16% among Democrats vs. 42% among non-Democrats, p=0.000) and 44% agreed or strongly agreed that “physicians should generally avoid political issues” (17% among Democrats and 42% among non-Democrats, p=0.000).
**ABSTRACT 5**

**Preeclampsia across pregnancies and associated risk factors: Findings from a high-risk US birth cohort**

*S. Michelle Ogunwole, MD, George Mwinnyaa, MSH, Xiaobin Wang, MD, MPH, ScD, Xiumei Hong, MD, PhD, Janice Henderson, MD, MFA, Wendy L. Bennett MD, MPH*

**Background:** Preeclampsia increases women's risk for maternal morbidity and future cardiovascular disease. The aim of this study is to identify opportunities for prevention by examining the association between cardiometabolic risk factors and preeclampsia across two pregnancies among women in a high-risk US birth cohort.

**Methods:** Our sample included 618 women in the Boston Birth Cohort with index and subsequent pregnancy data collected using standard protocols. We conducted log-binomial univariate regression models to examine the association between preeclampsia in the subsequent pregnancy (defined as incident or recurrent preeclampsia) and cardiometabolic risk factors (i.e., obesity, hypertension, diabetes mellitus [DM], preterm birth, low birth weight, and gestational diabetes mellitus) diagnosed before and during the index pregnancy, and between index and subsequent pregnancies.

**Results:** At the subsequent pregnancy, 7% (36/540) had incident preeclampsia and 42% (33/78) had recurrent preeclampsia. Compared to women without obesity, women with obesity had greater risk of incident preeclampsia (unadj RR 2.2 [95% CI: 1.1, 4.5]) and recurrent preeclampsia (unadj RR 3.1 [95% CI: 1.5, 6.7]). Pre-index pregnancy chronic hypertension and DM were associated with incident, but not recurrent preeclampsia (hypertension unadj RR 7.9 [95% CI: 4.1, 15.3]; DM unadj RR 5.2; [95% CI: 2.5, 11.1]. Women with new inter-pregnancy hypertension (vs. those without) had a higher risk of incident and recurrent preeclampsia (incident preeclampsia unadj RR 6.1 [95% CI: 2.9, 13]); recurrent preeclampsia unadj RR 2.4 [95% CI: 1.5, 3.9]).

**Conclusions:** In this diverse sample of high-risk US women, we identified modifiable and treatable risk factors, including obesity and hypertension, for the prevention of preeclampsia.

**ABSTRACT 6**

**Leveraging the Johns Hopkins COVID-19 Precision Medicine Center of Excellence to Evaluate Clinical Outcomes Among Immunocompromised Persons**

*Kathleen M. Andersen, MSc; Hemalkumar B. Mehta, MS, PhD; Natasha Palamuttam, BA; Daniel Ford, MD; Brian T. Garibaldi, MD MEHP; Paul G. Auwaerter, MD; Jodi Segal, MD, MPH; G. Caleb Alexander, MD, MS*

**Background:** There are plausible mechanisms by which chronic immunosuppressive drugs could worsen, or could improve, the severity of coronavirus disease (COVID-19).

**Methods:** We conducted a retrospective cohort study using electronic health record data from the JH-CROWN, a sequential registry of adults hospitalized with COVID-19 in the Johns Hopkins Medicine network. We considered persons hospitalized for COVID-19 from March 4 - August 29, 2020. We defined chronic immunosuppression as immunosuppressive drugs (WHO ATC group L01, L04 or >7.5mg prednisone) current at admission. The primary outcome was time from admission to mechanical ventilation. Secondary outcomes considered mortality, and length of stay. We used Fine and Gray's proportional subdistribution hazards models with stabilized inverse probability of treatment weights and doubly robust adjustment for remaining imbalances between groups. We accounted for the competing risk of death, or discharge, when not the primary outcome.

**Results:** We identified 2,121 COVID-19 patients, with 5% immunosuppressed before COVID-19, most often with prednisone, tacrolimus or mycophenolate mofetil. We did not find statistically significant differences in the risk of mechanical ventilation [hazard ratio [HR] 0.79, 95% confidence interval [CI] 0.46-1.35], in-hospital mortality (HR 0.86, 95% CI 0.38-1.94) or length of stay (HR 1.16, 95% CI 0.92-1.47) between groups.

**Discussion:** Chronic immunosuppression was neither associated with worse nor better clinical outcomes among a diverse cohort of American adults hospitalized with COVID-19. Our results, while reflecting the experience of a single health system, demonstrates the value and importance of a COVID-19 registry within our academic health system.
Health Information Technology Use among Persons with Self-Reported Atherosclerotic Cardiovascular Disease: An analysis of the 2011-2018 National Health Interview Survey

Uchenna Nuokeji BA MS, Erin M. Spaulding PhD BSN RN, Rongzi Shan MD, Ruth-Alma Turkson-Ocran PhD MPH RN FNP-BC, Diana Baptiste DNP RN CNE, Biniu Koirala PhD MGS RN, Timothy B. Plante MD MHS, Seth S. Martin MD MHS, and Yvonne Commodore-Mensah PhD MHS RN

Introduction: Atherosclerotic cardiovascular disease (ASCVD) is the leading cause of morbidity and mortality worldwide. Health-Information Technologies (HIT) have recently emerged as a viable intervention to mitigate the burden of ASCVD. At least 60% of United States (US) adults report searching the internet for health information; however, previous research has not examined the prevalence of general technology or HIT use between adults with and without ASCVD. In addition, social determinants in HIT use among adults with ASCVD are not well understood.

Objective: To evaluate the prevalence and social determinants of HIT use among U.S. adults with vs without self-reported ASCVD.

Methods: We pooled cross-sectional data from the 2011-2018 National Health Interview Survey (NHIS) to examine general technology and HIT use between adults aged ≥18 years with and without self-reported ASCVD (coronary heart disease and/or stroke). General technology use was defined as mobile phone ownership, Internet use, and computer use. HIT use was defined as looking up health information on the Internet, filling a prescription online, scheduling a medical appointment on the Internet, communicating with a healthcare provider by email, or using online group chats to learn about health topics. We evaluated sociodemographic differences in HIT use among respondents using Poisson regression. Analyses were weighted according to NHIS standards.

Results: A total sample of N=256,534 individuals were included, 2,194 (0.9%) reported prior ASCVD. Among adults with prior ASCVD, the mean (±SD) age was 70.6 (11.5) years, and 47% were female. General technology use differed between participants with and without prior ASCVD, with 36% (657/1,826) and 76% (162,500/213,816) indicating internet usage and 25% (394/1,575) and 61% (112,580/184,557) indicating using a computer every day, respectively. Similarly, adults with ASCVD were less likely to use HIT than those without ASCVD (25% vs. 51%, p<0.001). Among adults with prior ASCVD, social determinants that were associated with HIT use included younger age, higher education, higher income, being employed, and being married.

Conclusion: HIT use was low among adults with a history of ASCVD, which may represent a barrier to delivering care via emerging HIT. Given the associations with social determinants such as income, education and employment, targeted strategies and policies are needed to eliminate barriers to impact HIT usage.

Higher Angiotensin II type 1 receptor (AT1R) levels and activity in the postmortem brains of older persons with Alzheimer’s disease

C. Cosarderelioglu1, 2, C. George3, R. Marx3, Q. Xue4, J. Tian5, E. Oh5, L. Ferrucci6, D. Bennett5, J. Walston5, P. Abadir6

1Johns Hopkins University; 2Ankara University, Turkey; 3Albert Einstein College of Medicine/Montefiore Medical Center; 4National Institute on Aging, National Institutes of Health, Baltimore, MD; 5Rush Alzheimer’s Disease Center, Rush University.

Background: Alzheimer’s disease (AD) is the most common cause of dementia. Although multiple potential etiologies have been proposed, no clear aging-related etiological mechanisms have been identified. Renin-angiotensin system (RAS) is a hormonal system that is implicated in blood pressure control and has been suggested as a potential contributor to the development of AD. Here, using postmortem frontal cortex brain samples of age- and sex-matched cognitively normal individuals (n=30) and AD patients (n=30), we sought to examine the brain-specific RAS (b-RAS) differences with AD and how these findings correlate with brain AD pathologies.

Methods: Samples were obtained from the Rush Memory and Aging Project. We measured angiotensigen, renin, and ACE gene expression by qPCR and both gene expression and protein levels of Angiotensin II receptor type 1, 2, and 4 and their downstream signaling pathway (pERK, eNOS, and nNOS) by qPCR and Western blot. Brain cytokines and oxidative stress (OS) markers, as well as average tangle and β-amyloid load, were used as specific markers of AD pathology.

Results: Our results demonstrate an increase in both gene and protein expression (2.47 folds p=0.01, median0.59(range1.99) vs. 0.47(1.95) p=0.03, respectively) and signaling activity (0.35(11.41) vs. 0.04(1.84), p<0.01 of AT1R in AD. We have not observed any significant changes in other RAS components. Our data show that higher AT1R levels correlate with OS (r=0.301 p=0.01) and β-amyloid load (r=0.245 p=0.04), while higher pERK levels negatively correlate with mitochondrial numbers (r=-0.239 p=0.03) and positively correlate with total tangle (r=0.413 p<0.01) and amyloid (r=0.208 p<0.02) scores. Finally, mRNA, protein, and pERK levels of AT1R were negatively correlated with global cognitive function (GCF) scores (r=-0.216 p=0.05, r=0.258 p=0.02, r=0.376 p<0.01, respectively) and were associated with greater decline in GCF (r=0.265 p<0.02, r=0.223 p=0.04, respectively) in all subjects.

Conclusions: This study highlights molecular changes in b-RAS and offers insight into the association of these changes with brain pathology in AD.
MicroRNA-155-5p is an Effector of Endostatin in Pulmonary Endothelial Cells
Rose Yu, Mery Marimoutou, Catherine E. Simpson, Anjira S. Ambade, Paul M. Hassoun, Rachel L. Damico

Rationale: Pulmonary arterial hypertension (PAH) is a disease characterized by pathologic pulmonary vascular remodeling secondary to aberrant angiogenesis. Decreased expression and signaling via bone morphogenetic protein receptor type 2 (BMPR2) is a common feature of PAH. Normal BMPR2 signaling promotes pulmonary endothelial cell (PEC) survival. PEC dysfunction is a hallmark of pathologic vascular remodeling in PAH. PEC apoptosis is a necessary event in the pathogenesis of disease. Loss of BMPR2 expression alters PEC phenotype and promotes PEC apoptosis. While germline BMPR2 mutations are responsible for the majority of heritable PAH, incompletely defined mechanisms are responsible for BMPR2 suppression in the majority of idiopathic PAH. Endostatin (ES) is a potent circulating angiostatic peptide which induces PEC apoptosis. Circulating ES levels are elevated in PAH patients and are associated with adverse hemodynamics and poor survival. We have demonstrated that ES impacts the BMPR2 signaling pathway, yet the molecular mechanisms are not fully known. MicroRNAs (miRs) are important in gene expression, and BMPR2 is known to be a target of several miRs including miR-155. We hypothesize that ES suppresses BMPR2 via miR-155 in PECs.

Methods: Primary human microvascular PECs were exposed to recombinant ES (rES) or its vehicle for increasing durations. MicroRNA expression and function were manipulated via transient transfection of artificial miRs, antagomirs, and their respective controls. Total cellular microRNA, mRNA, and protein were assessed using RT-qPCR and Western blotting.

Results: Recombinant ES increased expression of miR-155 in PECs relative to vehicle treatment (11 +/- 3-fold, p=0.004). This was associated with a 45% +/- 29% (p=0.03) decrease in PEC BMPR2 mRNA expression. Transduction of the miR-155 mimetic was sufficient to suppress BMPR2 mRNA expression by 40% +/- 11% (p=0.01) relative to control miR. In contrast, rES failed to suppress BMPR2 mRNA in PECs transfected with the miR-155-antagomir.

Conclusions: Our studies indicate that miR-155 is an effector of ES in PECs and plays a role in ES-mediated suppression of BMPR2. We predict that miR-155 may represent a novel mediator of PEC dysfunction in PAH and may be a future therapeutic target. Current studies are underway to address the role of miR-155 in ES-mediated angiostasis and its expression in disease.

Outcomes after COVID-19 diagnosis for people with versus without serious mental illness
Karly A. Murphy, Emma E. McGinty, Gail L. Daumit

Background: Persons with serious mental illness (SMI) have a high burden of risk factors for COVID-19 morbidity and mortality including obesity, tobacco use, diabetes, lung diseases, and substance use disorder. However, little is known about clinical outcomes following COVID-19 infection among people with SMI.

Methods: We used data from the TriNetX Research Network, a federated network providing access to deidentified aggregate electronic medical record data across 45 U.S. health systems. We identified adults (age>=18 years) diagnosed with COVID-19 between January 20-November 30, 2020. We used ICD-10 codes to identify diagnoses of COVID-19, SMI (schizophrenia, bipolar disorder), and comorbidity (diabetes, hypertension, ischemic heart disease, obesity, chronic kidney disease, COPD, asthma, cerebrovascular disease, nicotine dependence, substance use disorder). Our outcome measures were inpatient admission for COVID-19, use of mechanical ventilation during inpatient admission, and mortality overall and among those with an inpatient admission within 30 days of a COVID-19 diagnosis. We matched cohorts with versus without SMI on demographics and comorbidities using 1:1 matching and greedy nearest neighbor propensity score approaches. We used logistic regression models to examine the association between SMI and COVID-19 clinical outcomes. Data analysis was performed on the TriNetX platform which utilizes a combination of JAVA, R, and Python programming.

Results: In our unmatched cohorts diagnosed with COVID-19, we identified 67,938 individuals with SMI and 2,013,590 individuals without SMI. Of these, 16,302 (24%) individuals with SMI and 305,969 (15%) without SMI had an inpatient admission linked to a COVID-19 diagnosis. Analyses of the matched sample with COVID-19 showed that people with SMI were more likely than those without SMI to experience hospital admission (21.2% vs 15.7%; OR=1.45 [95% CI 1.41-1.49]) and mortality (1.1% vs 0.9%; OR=1.24 [95% CI: 1.11-1.3]). Of those hospitalized with a COVID-19 diagnosis, we found no difference in likelihood of use of mechanical ventilation (4.9% vs 4.9%; OR=1.00 [95% CI: 0.90-1.10]) or death (2.6% vs 2.5%; OR=1.06 [95% CI: 0.92-1.21]) between persons with or without SMI.

Conclusion: Persons with SMI are at elevated risk of COVID-19 infection leading to hospital admission and death compared with the general population. Possible explanations include vulnerability due to living situations (homelessness, congregate living) and chronic health conditions, and seeking healthcare later in the course of illness due to stigma or difficulty navigating the healthcare system. Once persons with SMI received acute care for COVID-19 infection, mortality rates appear similar to that of the general population.

Recognize persons with serious mental illness are at an at-risk population for worse clinical outcomes after COVID-19.

Identify mechanisms why persons with serious mental illness may be more likely to experience poor clinical outcomes after COVID-19 infection.
**ABSTRACT 11**

**Digital Health Interventions for Cardiac Rehabilitation: A Systematic Literature Review**

Shannon Wongvibulsin, PhD, Evagelia E Habeos, MD, Pauline P Huynh, BA, Helen Sun, BS, Rongzi Shan, BS, Kori Porosnicu Rodriguez, BA, Jane Wang, MD, Yousuf K Gandapur, MD, Ngozi Osuji, MD, MPH, Lochan M Shah, BA, Erin M Spaulding, RN, BSN, George Hung, MD, Kellen Knowles, MD, William E Yang, MD, Francoise A Marvel, MD, Eleanor Levin, MD, FACC, FAHA, David J Maron, MD, FACC, FAHA, Neil F Gordon, MD, PhD, MPH, Seth S Martin, MD, MHS, FACC, FAHA, FASPC

Cardiovascular disease is the leading cause of death worldwide. Despite evidence supporting the benefits of cardiac rehabilitation (CR), over 80% of eligible patients do not participate in CR. Digital health technologies have the potential to address these challenges but little is known about the comprehensiveness of these interventions in delivering core components of CR. To address this gap in knowledge, we conducted an in-depth investigation of the literature on digital interventions for CR.

Our strategy for identifying the primary literature pertaining to CR with digital solutions included consultation with a digital CR expert and searches of PubMed (MEDLINE), Embase, CINAHL, and Cochrane databases for original studies published from January 1990 to October 2018.

Our search returned 31 eligible studies. CR interventions primarily targeted physical activity counseling (31/31, 100%), baseline assessment (30/31, 97%), and exercise training (27/31, 87%). The most commonly used modalities were smartphones or mobile devices (20/31, 65%), web-based portals (18/31, 58%), and email/SMS (11/31, 35%). Approximately one-third of the studies addressed the CR core components of nutrition counseling, psychological management, and weight management. In contrast, &lt;1/3 of studies addressed other CR core components, including the management of lipids, diabetes, smoking cessation, and blood pressure.

In conclusion, although digital technologies have the potential to increase access and participation in CR, previously evaluated interventions primarily focused on physical activity counseling and exercise training. Thus, further research is required with more comprehensive CR interventions and long-term follow-up to understand the clinical impact of digital interventions.

**ABSTRACT 12**

**Induction regimen patterns in ANCA associated vasculitis during the COVID-19 pandemic: A multicenter study**

Sam Kant, Antonio Salas, Adam Morris, Steve McAdoo, Silke Brix, Jennifer Scott, Manisha Shah, Isabelle Ayoub, Purva Sharma, Tingting Li, Andreas Kronbichler, Ulf, Duvuru Geetha

Standard of care for active AAV consists of high doses of glucocorticoids in combination with cyclophosphamide or rituximab. While treatment response and patient survival has improved, infection remains the leading cause of early mortality in AAV. There has been increasing caution with respect to immunosuppressive regimens, especially rituximab, during the COVID-19 pandemic.

We conducted a multi-center study, spanning the US, UK and Europe, to assess patterns of induction treatment of AAV during the pandemic period of January-July 2020.

Of 163 patients, median age was 67 (IQR 58-75), 49% female with majority (87%) being Caucasian. Mean eGFR on diagnosis of AAV was 35 (SD 32). For induction, mean cumulative steroid dose was 2395 mg with highest mean dosing in the US (3660 mg; p&lt;0.0005), with 77% receiving rituximab (p=0.058) and 46% given cyclophosphamide (p&lt;0.0005). Majority received PJP prophylaxis (84%) with highest use in the UK (96%; p&lt;0.0005). With respect to outcomes, mean eGFR at 6 months was 39 in the rituximab group, 35 for cyclophosphamide and 42 for those who received the combination. Ninety percent of patients were in remission at 6 months in both cyclophosphamide and rituximab induction groups. Nine patients were diagnosed with COVID-19 during induction, with similar cyclophosphamide and rituximab exposure.

There was significant deviation in patterns of induction regimen prescription in newly diagnosed AAV patients during the pandemic. AAV outcomes or COVID-19 infection were not influenced by type of induction therapy, suggesting that a change in induction regime may not be warranted during COVID-19 pandemic.
Endostatin Expression in the Progression of Right Ventricular Remodeling and Dysfunction in Pulmonary Arterial Hypertension


Rationale: Right ventricular (RV) remodeling and dysfunction predict adverse outcomes in PAH. Determinants of RV dysfunction are an area of active investigation in both human and experimental models. Previously, we have demonstrated that circulating endostatin (ES), the angiostatic cleavage product of collagen type XVIII, alpha 1 (Col18a1), is significantly elevated in PAH, and predicts increased mortality. Further, we demonstrated ES is a novel positive regulator of thrombospondin-1 (TSP1), an angiostatic protein also implicated in PAH severity.

Thus, we hypothesize that altered ES-TSP1 expression negatively impacts RV remodeling and function in experimental PAH and angiostatic factors are associated with RV dysfunction in clinical PAH. Utilizing complementary human and experimental data sources, we examined relationships between ES-TSP1 and RV morphology in PAH.

Methods: Male Wistar rats were randomized to Sugen 5416 (single dose) and chronic hypoxia (SuHx) versus vehicle-treated rats housed in room air and hemodynamics, RV morphology, histology, and total tissue mRNA were assessed over time. Immunohistochemical analysis for ES/Col18a1 in RV, Left ventricle (LV) and lung were performed, using Image J software, intensity of the selected area were measured and normalized to vehicle-treated control average values and expressed as positive area (%). In parallel, serum ES levels were quantified (ELISA) and cardiac morphology was assessed by cardiac magnetic resonance (CMR) in a cohort of PAH patients. Linear regressions were performed to examine relationships between serum ES and CMR measures of RV function including RV ejection fraction, TAPSE, RV end-diastolic and systolic volumes, and ventricular mass index (RV mass/LV mass, VMI).

Results: ES/Col18a1 expression increased in the vascular beds of the RV, lung, and LV in SuHx model, and the greatest increase (85-fold relative to control, p &lt; 0.0001) in perivascular expression of ES/Col18a1 was observed in the remodeled RV. Increased mRNA expression of both ES/Col18a1 and TSP1 occurred early in the RV during development of experimental PAH. In human PAH, each log-unit higher ES was associated with an increase of greater than 80ml in RV end diastolic and systolic volumes and a 5.3 mm decrease in TAPSE.

Conclusions: In the SuHx PAH model, increased RV expression of ES/Col18a1 and TSP1 are early events during RV remodeling. In human PAH, increased serum ES is associated with pathologic RV remodeling as assessed by CMR. ES/TSP1 signaling may be a direct pathogenic mediator of RV dysfunction in preclinical and clinical PAH making these potentially reliable biomarkers of RV dysfunction with prognostic implication.

Research Funding Source:

This work was supported by ROSE T32, CATHERINES K23, NIH HL114910/R01 (PMH), NIH HL135114/R01 (RLD/ADE/JY), NIH HL132153/R01 (RLD/PMH)

**ABSTRACT 13**

**SPILLOVER IMPACT OF CAREGIVER PARTICIPATION IN THE NATIONAL DIABETES PREVENTION PROGRAM LIFESTYLE INTERVENTION ON CHILDREN’S HEALTH BEHAVIORS: EMERGING THEMES FROM IN-DEPTH INTERVIEWS OF PROGRAM PARTICIPANTS**

Maya Venkataramani MD MPH; Ishmael Williams; Nisa M Maruthur MD MHS; Tina L Cheng MD MPH; Michelle Eakin MA, PhD

**Background:** Adult participation in the Diabetes Prevention Program (DPP) lifestyle intervention could impact dietary and physical activity habits of household members, including children. Children and grandchildren of adults in the DPP may also be at risk for chronic health conditions. Thus, the DPP presents a platform through which to reach high-risk children. Characterizing spillover impacts is an important first step in understanding how to leverage the platform to promote health child behavior change. Via in-depth interviews of DPP participants, we explored emerging themes regarding the impact of caregiver participation on children’s lifestyle behaviors.

**Methods:** Former or current DPP participants from Baltimore were recruited on a rolling basis to participate in a semi-structured, in-depth phone interview in 2020. Participants were eligible if they were a caregiver of a child under 18. The interview covered topics including spillover and mechanisms of spillover on children’s lifestyles.

**Results:** To date, 15 interviews were analyzed. Participants were predominantly female (87%), African American (93%) and grandparents (67%). In terms of emerging themes, participants described positive spillover impacts on children’s diet and physical activity, resulting from changes in the caregiver’s own dietary behaviors, activity levels or awareness of principles of healthier lifestyles.

**Conclusions:** Caregivers described improvements in the diet and activity levels of children resulting from caregiver participation in the DPP. Next steps include quantifying spillover impacts on children’s health behaviors, and augmenting the program to enhance unintentional and intentional beneficial impacts on high-risk children.
**ABSTRACT 16**

**A Novel Source of Endogenous DNA Damage that Requires Repair by the Fanconi Anemia Pathway**

Moonjung Jung, Sunandini Sridhar, Audrey Goldfarb, Ryan White, Danielle Keahi, Raymond Noonan, Tom Wiley, Francis Lach, Agata Smogorzewska

Fanconi anemia (FA) is the most common inherited bone marrow failure (BMF) syndrome, caused by impaired DNA interstrand crosslink repair. FA patients usually develop BMF during the first decade of life, prior to any known exposure to exogenous crosslinking agents. Therefore, endogenous sources of DNA damage are likely to play an important role in the pathogenesis of FA.

To identify novel detoxifying enzymes preventing endogenous DNA damage, we performed a metabolism-focused CRISPR synthetic lethality screen, using wild-type and FANCD2−/− Jurkat. From the screen, we identified ALDH9A1 as the most significantly depleted gene in FANCD2−/− Jurkat compared with wild-type. In vitro fluorescence-based competition assay confirmed synthetic lethal interaction between the two genes, in two independent FANCD2−/− clones. The numbers of 53BP1 and gamma-H2AX foci-markers of DNA damage, and apoptosis were increased in double knockout (dKO) condition, consistent with DNA damage-induced apoptosis.

To determine whether ALDH9A1 deficiency also caused cell death in FA-deficient human hematopoietic stem progenitor cells (HSPC), we edited human umbilical cord blood CD34+ cells and cultured on methylcellulose. dKO condition produced fewest hematopoietic colonies and lowest frequency of multipotent progenitors. We also observed fewer colonies targeted for both genes (biallelic dKO; observed to expected ratio 0.33) as compared to either single gene KO. These results suggest that loss of ALDH9A1 is deleterious in FANCD2-deficient HSPC.

In conclusion, we identified that cells with ALDH9A1 deficiency require the FA pathway for survival. ALDH9A1 may protect human HSPC that are deficient in the FA pathway from DNA damage and cell death.
ABSTRACT 17

SERUM MARKERS AND INTEGRATIVE MULTI-OMICS OF TB DIAGNOSIS IN ADVANCED HIV


Background: Persons living with HIV (PLWH) have high risk of developing active TB, but conventional methods of TB diagnosis in PLWH have lower sensitivity. Novel high-throughput approaches may advance our insights into difficult to diagnose TB, especially in advanced HIV.

Methods: We conducted a case-control study leveraging REMEMBER, a multi-country, open-label RCT comparing 4-drug empiric TB treatment with isoniazid preventive therapy in PLWH initiating ART (CD4 cell counts <50 cells/μL). Active TB was ruled out at baseline. Twenty-three incident TB cases were site-matched with up to 2 controls. We performed miRNA NGS (QIAGEN), LC-MS/MS quantitative metabolomic analysis (Metabolon, Inc.), and multiplex immunoassays (Luminex) on serum obtained at time of TB diagnosis. Multi-omics data were integrated, and a decision tree algorithm was used to identify the best model for TB diagnosis.

Results: Differentially expressed miRNA analysis revealed 11 altered miRNAs with fold change higher than ±1.4 in cases relative to controls (p<0.05). Differentially altered metabolite analysis showed no significant alterations between cases and controls. We found higher TNFα and IP-10/CXCL10 in cases (p=0.011, p=0.0005), and higher MDC/CCL22 in controls (p=0.0072). A decision tree algorithm identified gamma-glutamylthreonine and hsa-miR-215-5p as the optimal variables to classify incident TB cases (AUC 0.965). hsa-miR-215-5p, which targets genes in the TGF-β signaling pathway, was downregulated in cases. Gamma-glutamylthreonine, a breakdown product of protein catabolism, was less abundant in cases.

Conclusions: Use of a machine learning approach in multi-omics data from advanced HIV participants revealed two variables with the ability to accurately discriminate TB cases from controls.
Traditional risk factors have low predictive value for determining diabetes control; a comparative analysis of machine learning methods from the LOOK AHEAD study
Tanmay Nath, Justin Echouffo Tcheugui, Rexford S Ahima and Prasanna Santhanam

Introduction: Diabetes mellitus (DM) is likely to affect more than 425 million adults aged between 20-79 by 2030, contributing significantly to chronic disease-related morbidity and death. However, the early determinants of adequate diabetes control are still unknown. In this study, we used different machine learning methods to predict the probability of achieving DM control in persons with DM 40 years and above by analyzing the LOOK AHEAD study cohort (a large-scale prospective NIH funded study) at baseline and at the 4-year time point. Study Hypothesis: Are commonly associated high-risk baseline markers of poor metabolic health able to predict who achieved DM control after a 4-year time frame, using different commonly employed AI methods? Study Population: We analyzed the data from the LOOK AHEAD study. The LOOK AHEAD (a randomized, open-label, controlled trial) was designed to investigate the effects of an intensive lifestyle intervention (focusing on weight loss achieved through dietary changes and physical activity) compared to controls who received traditional DM counseling and care. We selected vital baseline characteristics (obtained in usual clinical settings) and analyzed their impact on achieving DM control at the 4-year mark. Study Methods: We used the following variables: age (years), gender, ethnicity (white, Hispanic, African-American, and other), baseline serum creatinine (mg/dl), the severity of diabetes (insulin w/wo oral agents compared to oral agents alone), duration of diabetes (in years), baseline BMI (kg/m2), history of smoking (past, present, never), screening systolic blood pressure (mm/Hg), initial A1C (%), and initial waist circumference (in cm) for predicting diabetes control at the 4-year time point. The A1C at the 4-year mark was analyzed as a categorical variable. An A1C ≤7% based on ADA guidelines) was considered the classification model’s cut-off. We used six machine learning methods, specifically Random forest, Gradient boosting, Support vector machine, Multi-layer perceptron, Logistic regression, and Stacking regression for classification of diabetes. The data was randomly split the dataset into 70% training and 30% test dataset. The training dataset was used for a 5-fold cross-validation strategy to tune the parameters of our algorithm. The tuned model was used to make predictions of who would achieve diabetes control. We computed the area under the ROC (Receiver Operating Characteristic) curve to compare the algorithms’ performance in classifying diabetes. The analysis was done using Python 3.6 (packages: scikit-learn, numpy, and pandas were employed).

Results: There was a total of 4187 subjects that were included in the study. We found that none of the algorithms could classify diabetes with substantial accuracy and the AUC values are around chance level. The AUC value for different algorithms is as follows: Random forest classifier (RFC): 0.45, Gradient boosting (GB): 0.49, Logistic regression (LR): 0.50, Support vector machine (SVC): 0.53, Multi-layer perceptron (MLP): 0.53and stacking classifier (SC): 0.46. The recall, precision values and the F1-scores of the different algorithms/predictive models were as follows: (RFC: 0.82, 0.55, 0.66, GBC:1.0,0.56,0.71, LR1.0,0.55,0.71,SVC:1.0,0.55,0.71,MLP: 0.99,0.55,0.70, SC:1.0,0.55,0.71)

Discussion: DM control does not seem to depend heavily on baseline clinical and laboratory characteristics. Self-care support and psychological counseling may significantly impact DM control, especially in the younger population [1]. Prior interventional studies have shown that people attending DM support groups achieve better DM control [2]. Techniques like motivational interviewing and individual perceptions of dietary control are essential factors in achieving reasonable DM control [3, 4]. Cognitive-behavioral therapy was shown to improve depression and, consequently, DM control in a systematic review [5].

Conclusions: Our study shows that baseline clinical risk factors are not predictive of DM control through machine learning methods.
Pasireotide Use for Refractory Hypoglycemia in Two Patients with Endogenous Hyperinsulinemic Hypoglycemia

Hasan Husni, Sarah A. Khan, Buraq Alghaieb, Mohammed Abusamaan, Amir H. Hamrahian

Insulinomas are rare neuroendocrine pancreatic tumors that can be associated with severe episodes of hypoglycemia, leading to significant morbidity and mortality. The localization of these tumors and hypoglycemia control may be challenging since they can be resistant to conventional therapies. Pasireotide is a novel somatostatin analogue with a high affinity to multiple somatostatin receptors. It has up to 30-40 times higher affinity for somatostatin receptor subtype 5 compared with octreotide, leading to inhibition of insulin release from beta cells. There are only a few case reports regarding its use in refractory hyperinsulinemic hypoglycemia.

We describe two challenging cases of endogenous hyperinsulinemic hypoglycemia refractory to standard medical treatment, in which pasireotide was used. The first case was due to an unresectable insulinoma in a 69-year-old gentleman, where treatment with diazoxide, octreotide, and glucocorticoids did not achieve significant improvement in blood glucose levels. The patient required dextrose-containing IV fluids to maintain euglycemia. In the second case, imaging studies and calcium stimulation test failed to localize the disease in an 83-year-old woman. Glucose levels remained low despite treatment with diazoxide, verapamil, and octreotide, necessitating the use of IV dextrose solutions. After starting pasireotide 0.9 mg subcutaneously bid, there was a significant improvement in the hypoglycemic events in both cases, allowing the patients to be discharged from hospital without the need for IV glucose support.

Pasireotide should be considered for patients with endogenous hyperinsulinemic hypoglycemia refractory to conventional therapy. However, further studies regarding its use in this setting are needed.

Evaluating the Longitudinal Association of Marijuana Use and Adverse Kidney Outcomes

Flor Alvarado, MD, MPH; Dingfen Han, PhD; Alan B. Zonderman, PhD; Michele K. Evans, MD; Deidra C. Crews MD, ScM

Background: Marijuana is the most used federally controlled substance in the US. We examined the longitudinal association of marijuana use and adverse kidney outcomes among adults living in Baltimore, MD.

Methods: We used data from the Healthy Aging in Neighborhoods of Diversity Across the Life Span Study. Baseline exposure, defined as self-reported never, former, or current marijuana use, and covariates were obtained between 2004 and 2009. The primary outcome was incident reduced kidney function (eGFR <60 ml/min/1.73m2) at follow-up. Rapid kidney function decline (decline in eGFR of ≥5 ml/min per 1.73 m2 per year between baseline and follow-up) and incident albuminuria (albumin-to-creatinine ratio (ACR) ≥30 mg/g) at follow-up were also assessed. We compared participant characteristic by marijuana use status using ANOVA or chi-square tests. Multivariable-adjusted logistic regression was used to evaluate associations of marijuana use with kidney outcomes.

Results: Among 1,529 participants, 54.5%, 31.8% and 13.7% reported never, former, or current marijuana use, respectively. Participants with current marijuana use were more likely to be younger, male, African American, have lower BMI and concurrently use cigarettes, opiates and/or cocaine. Mean follow-up time was 8.6 years. Marijuana use was not significantly associated with incident reduced kidney function among those with current use (aOR 1.03 [95% CI, 0.47 to 2.28]), or former use (aOR 0.88 [95% 0.49 to 1.59]). Current marijuana use was not significantly associated with rapid kidney function decline or incident albuminuria.

Conclusion: In this Baltimore-based cohort, there was no independent association of marijuana use and longitudinal adverse kidney outcomes.
ABSTRACT 22

Examining Post-Donation Outcomes in Hispanic/Latinx Living Kidney Donors in the United States: A Systematic Review

Flor Alvarado, MD, MPH; C. Elena Cervantes, MD; Deidra C. Crews, MD ScM; Fawaz Al Ammary MD, PhD; Tanjala Purnell PhD

Introduction: Living kidney donation is regarded as a relatively safe procedure in studies primarily involving White donors; however, less is known about Hispanic/Latinx donors. We conducted a systematic review to assess outcomes in Hispanic/Latinx donors.

Methods: We identified eligible studies by searching PubMed, EMBASE, and Scopus through July 2020. Our search strategy included key words pertaining to living donor outcomes and Hispanic/Latinx ethnicity. We included studies with original research evaluating post-donation outcomes in adult Hispanic/Latinx kidney donors living in the US. Two reviewers independently screened study titles, abstracts, and full texts, and qualitatively synthesized results and quality of studies.

Results: 17 studies were included with sample sizes ranging from 4,007 to 143,750 living donors. Mean ages ranged from 37 to 54 years. Median follow-up time ranged from 6 to 12 years. Most study participants were women (54.8% or higher). Hispanic donors ranged between 5.7 to 13.0%. Studies did not show an increased risk of end-stage kidney disease (ESKD) in Hispanic/Latinx compared to White donors. Mixed results were reported for future risk of diabetes, hypertension, and chronic kidney disease (CKD). There were no significant differences in risk for surgical mortality or long-term survival, non-pregnancy related hospitalizations, cardiovascular disease, or gout among Hispanic donors compared to Whites.

Conclusion: Findings suggest there is not a substantial difference in risk of ESKD, mortality, cardiovascular disease, or non-pregnancy related rehospitalizations in Hispanic/Latinx donors as compared to White donors in the US. Mixed results were appreciated in the risk of developing post-donation hypertension and diabetes.

ABSTRACT 23

Accurate Measurement of Neck Flexion Angle during Otolaryngologic Surgery

Zhen Hu B.S., Lekha Yesantharao B.S., Chris Razavi, MD, Russell Taylor, PhD, Deepa Galaiya M.D.

Increasing evidence demonstrates that a surgeon's operating posture can contribute to chronic pain. Specifically, trapezius muscle fatigue has been shown to be highest when neck flexion exceeds 50°. This study sought to accurately measure the surgeon’s neck flexion angle while performing otolaryngologic surgery, comparing the risks of traditional “heads down” surgery to that of “heads up” endoscopic surgery.

Two Inertial Measurement Units (IMUs) were utilized, with one banded to the forehead and the other attached to the back. Neck flexion angle was indicated by the pitch angle between the two IMUs. To confirm accuracy, the IMUs’ pitch angle was calibrated against electromagnetic trackers. Two surgical scenarios were simulated to compare traditional thyroid surgery and endoscopic ear surgery.

Neck flexion angles for traditional thyroid surgery ranged between 60° and 90° (mean = 74.93°, SD = 25.16°). The total time recorded was 38.19 seconds, of which the surgeon spent 32.24 seconds (84.7%) at neck flexion greater than 50° – the threshold angle for harmful trapezius pressure as defined in the literature.

For endoscopic surgery, neck flexion angles ranged between 25° and 35° (mean = 32.11°, SD = 6.68°) over 111.4 seconds. The maximum angle was 48.25°, so the harmful 50° threshold was never achieved.

Our results show that traditional open surgery, such as thyroid surgery, is performed with neck flexion angles above 50° for a majority of the time. Contrastingly, the neck flexion angle remains near 30° for endoscopic cases. The development of “heads up” techniques for surgery using endoscopes and exoscopes can significantly improve surgical ergonomics and decrease harmful trapezius pressure and chronic neck injury.
ABSTRACT 24

Blockade of Trpm7 in the Carotid Body area reversed Obesity-Induced Hypertension


Rationale: Obesity leads to cardiovascular morbidity and mortality acting via multiple mechanisms including hypertension. One of the mechanisms of obesity-induced hypertension is an adipocyte-produced hormone leptin, which activates the sympathetic nervous system. We have recently reported that acute leptin infusion induces hypertension acting via the TRPM7 cation channel in the carotid bodies (CB). Here, we examined the relevance of TRPM7 signaling in CB for the pathogenesis of hypertension in diet-induced obesity (DIO) with high circulating leptin levels. We hypothesize that DIO causes hypertension acting on CB TRPM7.

Methods: DIO male mice (n = 11) were implanted with telemetry in the left femoral artery for continuous blood pressure monitoring. Trpm7 was silenced in the carotid body areas by transfection with Ad-Trpm7 shRNA (n = 6); control mice (N = 5) were transfected with Ad-CON-shRNA. Plasma leptin level were measured by ELISA before and after tranfection; RT-PCR was performed to confirm Trpm7 knockdown.

Result: Trpm7 shRNA group induced a decrease in Trpm7 mRNA expression in the CB compared to control (0.089±0.02 vs 0.416±0.08 relatively to a house-keeping gene Rpl19 respectively, p<0.01). In Trpm7 shRNA treated group, blood pressure decreased from 119.0±2.2 mmHg to 109.6±1.4 mmHg (p<0.01), whereas scrambled shRNA had no effect (from 114.8±0.9 mmHg to 116.3±2.6 mmHg) (Fig. 1). Both groups had similar levels of plasma leptin at baseline, which did not change after transfection.

Conclusion: Our study has shown that downregulation of Trpm7 in carotid bodies abolished hypertension in obese mice.

ABSTRACT 25

Markers of endothelial cell activation are associated with the severity of pulmonary disease in COVID-19.

William Osburn, Lisa Yanek, Nuria Amat-Codina, David Thiemann, Andrea Cox, Thorsten Leucker, Charles Lowenstein

Objective: Severe coronavirus disease-19 (COVID-19) is characterized by vascular inflammation and thrombosis. We and others have proposed that the host response to coronavirus infection activates endothelial cells, leading to endothelial release of pro-thrombotic and pro-inflammatory signals. These mediators can mediate obstruction of the pulmonary microvasculature, leading to worsening oxygenation, acute respiratory distress syndrome, and death. In the current study we tested the hypothesis that higher levels of biomarkers released from endothelial cells are associated with worse oxygenation in patients with COVID-19.

Approach and Results: We studied 83 participants aged 18 – 70 years who were admitted to a single center with a COVID-19 positive nucleic acid test. The severity of pulmonary disease in these patients with COVID-19 was classified by oxygen requirement, ranging from no oxygen requirement to low-flow oxygen to high-flow nasal cannula oxygen to mechanical ventilation to death. We measured plasma levels of two proteins released by activated endothelial cells, von Willebrand Factor (VWF) antigen and soluble P-selectin. We found that elevated plasma levels of VWF antigen and soluble P-selectin are associated with increased oxygen requirements and mortality in patients with COVID-19. Additionally, levels of VWF were associated with D-dimer; and levels of soluble P-selectin were associated with levels of C-reactive peptide.

Conclusions: Increased levels of VWF antigen and soluble P-selectin are linked to the severity of lung disease in COVID-19, suggesting that COVID-19 is a vascular disease which may involve endothelial injury.
**ABSTRACT 26**

**miR-200 Family and Diabetic Cardiomyopathy**

M. Cristina Florio, Laura Montenefro, Melissa Krawczyk, Adrian Santana, Sunayana Syed, Bruce Ziman, Alessandra Magenta, Edward G. Lakatta, Maurizio C. Capogrossi

Reactive oxygen species (ROS) play a pivotal role in diabetes and markedly enhance miR-200 family members (co-transcribed miR-200c/miR-141 and co-transcribed miR-200a/miR-200b/miR-429) expression in numerous cell types, with miR-200c/miR-141 exhibiting the most pronounced increase. The effect of diabetes on miR-200 family expression in the diabetic heart is still unknown.

In streptozotocin-induced diabetic mice miR-200c and miR-141 expression increased in cardiomyocytes (CMs) and the increase positively correlated with glycemia. Since diabetic cardiomyopathy modulates myocardial function, we examined the effect of miR-200c overexpression on excitation-contraction coupling of adult rats left ventricular CMs infected with lenti-miR-200c or lenti-scramble. Action potentials (APs) were recorded in current-clamp mode, cytosolic Ca²⁺ transients (Cai) were evoked by field stimulation (0.5 Hz), and caffeine pulse in another subset of cells loaded with Fluo4. Although AP amplitude was unchanged, time to AP peak, upstroke velocity and AP duration, measured at 50% and 90% of the repolarization, were significantly (p<0.05) prolonged in miR-200c overexpressing CMs (n=11) vs control (n=8). These effects on the APs were associated with reduced Cai transient amplitude and prolonged duration measured at 50% and 90% of the transient and a prolongation of the tau velocity (miR-200c n=25; control n=18; p<0.01). Similar effects were observed in the caffeine-induced Ca²⁺ transients (miR-200c n=28; control n=22; p<0.05).

These data suggest that the increase of miR-200c induced by diabetes in the diabetic heart may impair myocardial EC coupling and play a role in the clinical features of diabetic cardiomyopathy.

**ABSTRACT 27**

**Impact of Local Food Environments on Lifestyle Change Efforts of Diabetes Prevention Program Participants**

Ishmael Williams BS, Nisa Maruthur MD, MHS; Raquel Greer MD, MHS; Marissa Alert PhD; May Thu Thu Maw MBBS, MPH, Kimberly A. Gudzune MD, MPH; Carolyn Bramante MD, MPH; Geetanjali Chander MD, MPH; Maya Venkataramani MD, MPH

**Background:** Successful community-based translation of lifestyle interventions, such as the Diabetes Prevention Program (DPP), requires understanding how food environments impact participants’ dietary change efforts. Among DPP groups held in an urban setting, we explored local food environment interactions and examined how the food environment impacted lifestyle change efforts.

**Methods:** We conducted a phone-based survey along with in-person focus groups with DPP participants in Baltimore in 2018. Initial recruitment was via in-person outreach; participants were contacted by phone for final recruitment for the survey or focus groups. Survey topics included where participants shopped for food. The focus groups explored food environment-related barriers, and facilitators; focus groups were recorded, transcribed, and double-coded into emerging themes.

**Results:** 27 DPP participants expressed initial interest in survey participation; 16 were reached and completed the survey. Respondents were majority female (87.5%), African American (93.8%), and had an annual income less than $40,000 (63%). All respondents frequented at least one supermarket; 87.5% reported changing shopping habits to buy healthier items. From the 2 focus groups (with 6 and 4 participants each), emerging themes regarding barriers included the cost of healthier options and food wastage; participants requested more information about food preservation. Access to farmers’ markets and restaurants with healthier menu options were considered facilitators.

**Conclusion:** While DPP participants reported access to healthier food items, cost was a barrier; providing food preservation information may help address this. Further characterizing food environment-related barriers and facilitators will be essential to inform how the program can best support lifestyle change efforts.
Renal tubular epithelial cells drive continuous proliferation of TCR+CD4-CD8- (double negative) T cells through MHC-independent and IL-7 dependent mechanisms

Mohanraj Sadasivam, Somayeh Gharaei Fathabad, Kyungho Lee, Sanjeev Noel, Abdel Rahim Hamad*, Hamid Rabb*

Double-negative (DN) T cells are unconventional lymphocytes that are extremely rare in peripheral lymphoid organs, but are highly enriched in mouse and human kidneys. Kidney DN T cells are actively dividing in the steady state but the cell types and signals driving their activation are unknown. Here, we show that renal tubular epithelial cells (RTEC) play an active role in driving proliferation and reducing apoptosis of DN T cells. Co-culture of DN T cells with RTEC significantly increased the frequency [DN; 1.2%± 0.6 vs RTEC+DN; 13.8%± 3.5, p≤0.001] and absolute cell number [DN; 0.2 ± 0.1x10^4 vs RTEC+DN; 2.9 ± 0.9 x10^4, p<0.001] of DN T cells in in-vitro. RTEC mediates proliferation of DN T cells by TCR/MHC independent mechanisms, as shown by the ability of RTEC from class I and class II MHC KO in promoting proliferation of DN T cells in in-vitro co-culture [DN; 2.1%±0.8 vs RTEC(WT)+DN; 17.0% ±1.5 vs RTEC(MHC I KO)+DN; 21.2% ±1.9 vs RTEC(MHC II KO)+DN; 17.9% ±1.6, p<0.001], as well as TCR-tg DN T cells. Furthermore, the proliferation of DN T cells is also mediated by IL-7 dependent mechanisms, as blocking of IL-7 in the coculture leads to significant loss of DN T cell expansion [DN; 0.6%± 1.6 vs RTEC+DN; 4.6%± 2.5 vs RTEC+DN+anti-IL-7; 2.4%± 0.5, p<0.001]. Kidney DN T-cells depends on IL-7 for its optimal homeostasis and remain functionally responsive to external stimuli as indicated by their rapid activation and expansion in response to kidney IRI, whereas the proliferation are reduced in IL-7r KO mice [WT; 0.4 ± 0.1x104 vs WT-IRI; 1.2 ± 0.9 x104 vs IL-7r KO; 0.3 ± 0.1 x104 vs IL-7r KO-IRI; 0.2 ± 0.7 x104, p<0.05]. Reciprocally, DN T cells are also increased the survival of kidney epithelial cells in vitro. These findings demonstrate a previously unknown functional relationship between RTEC and DN T cells that may explain the selective accumulation of DN T cells in the kidney and its beneficiary effect to the host. The results have important implications for developing strategies to ameliorate AKI and kidney transplants by promoting the survival of local DN T cells.

Role of Leptin-TRPM7 Signaling in Carotid bodies in the Pathogenesis of Sleep-Disordered Breathing in Obesity

Lenise J. Kim, Mi-Kyung Shin, Huy Pho, Nishitha Hosamane, Frederick Anokye-Danso, Rexford S. Ahima, Luu Pham, and Vsevolod Y. Polotsky

Introduction: Sleep-disordered breathing (SDB) affects 50% of obese individuals. Augmented hypoxic ventilatory response (HVR) is a trait of obesity-induced SDB. We have shown that leptin acts in the carotid bodies (CB) to increase HVR possibly through the activation of transient receptor potential melastatin 7 (TRPM7). However, the relevance of these findings to diet-induced obese (DIO) mice and the effect of leptin-TRPM7 axis in CB on SDB remain unknown. We hypothesized that leptin acts via TRPM7 in the CB to increase the hypoxic chemoreflex leading to obesity.

Methods: Male C57BL/6j mice, 12 weeks-old, received high-fat diet for 8 weeks and underwent a full-polysomnography. HVR (10% O2+3% CO2) was measured while awake. Mice were transfected with Trpm7 shRNA (n=9) or control shRNA (n=8) in the CB area bilaterally and HVR/sleep studies were repeated. Another subset of mice (n=5/group) underwent 24-h metabolic studies.

Results: Trpm7 knockdown in CB decreased hypoxic minute ventilation and suppressed HVR during wakefulness compared to baseline (P<0.01) and to control group (P<0.05). Trpm7 shRNA in the CB increased inspiratory flow, tidal volume and minute ventilation (0.69±0.1 vs 0.81±0.1 mL/min/g) during NREM sleep (P<0.05) with no significant effects on sleep architecture. Consumed O2, produced CO2, and respiratory exchange ratio did not change with Trpm7 knockdown in the CB.

Conclusions: Trpm7 knockdown in the CB attenuates the hypoxic chemoreflex during wakefulness and improves the obesity-induced hypoventilation of DIO mice during sleep. Thus, leptin-TRPM7 signaling in the CB could be a potential therapeutic target for the treatment of obesity-related SDB.
Abstract 30

High Mobility Group A1 Chromatin Remodeling Proteins Drive Progression in Myeloproliferative Neoplasms and Induce GATA2 through Epigenetic Rewiring

Liping Li, Jung-Hyun Kim, Wenyan Lu, Leslie Cope, Raajit Rampal, Richard Koche, Karen Reddy, Donna Williams, Joseph Kim, Li Luo, Lionel Chia, Marija Vasiljevic, Lingling Xian, Daniel Matson, Ophelia Rogers, Joey Zhao, Jerry Spivak, Alison Moliterno, Linda M. S. Resar

Myeloproliferative neoplasms (MPN) are clonal hematopoietic stem cell disorders characterized by hyperactive JAK/STAT signaling and an increased risk of transformation to myelofibrosis (MF) and acute myeloid leukemia (AML). However, mechanisms driving progression have remained elusive and clinical outcomes are abysmal once progression occurs. The High Mobility Group A1 (HMGA1) gene encodes chromatin regulators which are enriched in normal stem cells and aberrantly overexpressed in aggressive tumors. Here, we uncover a novel epigenetic program whereby HMGA1 recruits activating histone marks to induce transcriptional networks required for progression. Silencing HMGA1 in cell lines derived from MPN patients after transformation to leukemia disrupts proliferation and clonogenicity in vitro while preventing leukemia engraftment in mice. Surprisingly, loss of just a single Hmga1 allele prevents progression to MF in transgenic mouse models of MPN. RNA sequencing revealed genes induced by HMG1 that govern cell fate decisions and cell cycle progression, including the GATA2 master regulator. Silencing GATA2 recapitulates anti-leukemia phenotypes observed with HMG1 deficiency, whereas restoration of GATA2 partially rescues leukemogenic properties in MPN leukemia cells with HMG1 silencing, reducing apoptosis, increasing clonogenicity and leukemia engraftment. Mechanistically, HMG1 occupies a GATA2 developmental enhancer (+9.5kb) and recruits active histone marks (H3K4me1, H3K4me3) to induce GATA2 expression. In primary human MPN cells, both HMGA1 and GATA2 are up-regulated with progression from MF to AML and HMGA1 transcriptional networks correlate positively with progression. Together, our studies reveal a new paradigm whereby HMGA1 up-regulates GATA2 to drive progression in MPN and illuminate HMGA1 as a novel therapeutic target.

Abstract 31

Productive Partnerships Between Engineers and Physicians: The CBID Model

Youseph Yazdi

The Johns Hopkins Center for Biomedical Innovation & Design (CBID) is a center within the department of Biomedical Engineering and is a part of both WSE and SOM. Over the past 12 years, we have refined a model for partnership between faculty, students, clinicians, and outside stakeholders to design and develop healthcare solutions. In this talk, we will present an update on overall progress of the center, highlight 4 recent projects, and discuss challenges. Some specifics we will present are: our TL1 Fellowship program allowing medical students and residents at JHSOM to engage in the CBID MSE degree program, our partnership model with major medtech companies, and recent successes in translating US and Global Health innovations into use and impact.

Abstract 32

Alternative complement pathway activation in COVID-19 patients

Jia Yu, Hang Chen, Gloria Gerber, Xuan Yuan, Shruti Chaturvedi, Evan M. Braunstein, Robert A. Brodsky

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) can lead to thrombosis, stroke, renal failure, myocardial infarction, and thrombocytopenia, reminiscent of other complement-mediated diseases. Dysregulation in the alternative complement pathway (APC) can lead to uncontrolled complement attack against the host cell and end-organ damage. We previously showed that the SARS-CoV-2 spike protein activates the APC. We demonstrated that the spike protein has high affinity for heparan sulfate and interfered with the binding of factor H, a critical negative regulator of APC on host cells. APC inhibition with C5 or factor D inhibitors effectively blocked complement activation induced by SARS-CoV-2 spike proteins.

To further understand whether complement activation is inherent to the pathogenesis of COVID-19, we analyzed serum samples from 59 hospitalized patients with varying degrees of illness severity. Serum from patients who required intubation induced a 127% increase in complement-mediated cell death in the modified Ham assay as compared to patients on minimal oxygen support (p=0.008). This correlated with increased C5b-9 deposition on TF1PIGAnull target cells and was mitigated by addition of APC inhibitors. Utilizing standard ELISA for factor Bb, a biomarker of APC activation, we found that patients with severe COVID-19, as defined by WHO score, had significantly higher Bb levels. Lastly, serum samples from individuals who experienced symptoms of fever and fatigue after receiving the Pfizer COVID-19 vaccine showed elevation in serum Bb levels from baseline.

Taken together, our study suggests that overwhelming APC activation could be a major driver of severe COVID-19 infection.
The High Mobility Group A1 Chromatin Remodeling Protein Induces FGF19 and Recruits Cancer-Associated Fibroblasts to Drive Tumor Progression in Pancreatic Ductal Adenocarcinoma

Lionel Chia, Shuai Shuai, Lingling Xian, Jung-Hyun Kim, Woo Jung Sung, Ruitao Zhang, Tait Huso, David Huso, Leslie Cope, Karen L. Reddy and Linda M. S. Resar

Pancreatic ductal adenocarcinomas (PDACs) are highly lethal tumors characterized by dense desmoplastic stroma comprised of cancer associated fibroblasts (CAFs) and fibrotic scar tissue. However, the role of CAFs in PDAC is controversial. While they provide a barrier to therapy and release tumorigenic signals, CAFs restrict tumor growth in transgenic mouse models. High Mobility Group A1 (HMGA1) proteins are oncofetal proteins and epigenetic regulators that amplify signals from the microenvironment to foster stem cell properties within intestinal epithelium. HMGA1 is silenced in most differentiated tissues, but becomes aberrantly re-expressed in PDAC and other tumors where high levels predict poor clinical outcomes. Here, we discovered an epigenetic program whereby HMGA1 recruits CAFs to drive tumor progression. Silencing HMGA1 disrupts oncogenic properties and depletes tumor-initiator cells. RNA sequencing revealed FGF19 among HMGA1 transcriptional networks regulating proliferation and tumor-stromal interactions. HMGA1 binds directly to the promoter, recruits active histone marks, and induces FGF-19 expression and secretion from PDAC cells. Silencing FGF19 disrupts HMGA1-mediated oncogenic properties. In co-culture experiments, FGF19 is required for CAF migration towards PDAC cells. Silencing HMGA1, FGF19, or treatment with FGF19 inhibitors prevent CAF recruitment and decrease desmoplastic stroma formation in xenograft tumors. Hmga1 deficiency impairs tumor and stroma formation in transgenic PDAC mouse models. Moreover, co-expression of HMGA1 and FGF19 predict decreased survival in human PDAC. Together, our results reveal a new paradigm whereby cancer cells collaborate with CAFs via HMGA1 and FGF-19 to drive progression, thus illuminating FGF19 as a rational therapeutic target for PDACs overexpressing HMGA1 and FGF19.

Overnight sleep architecture and heart rate predict morning glucose tolerance during CPAP withdrawal

Chenjuan Gu, Daisy Duan, Mudiaga Sowho, Shannon Fonti-Bevans, Vsevolod Y. Polotsky, Luu V. Pham, Jonathan C. Jun

Rationale: Obstructive sleep apnea (OSA) is associated with impaired glucose tolerance, but causal mechanisms are not well-established. Objectives: To examine morning glucose tolerance after acute exposure to OSA elicited by continuous positive airway pressure (CPAP) withdrawal.

Methods: Patients with OSA acclimated to CPAP were recruited in a randomized crossover study. Patients underwent polysomnography with therapeutic CPAP or without CPAP after 2 days of withdrawal, in random order. Venous blood was sampled at 30 min intervals on both nights and a 75g oral glucose tolerance test (OGTT) was performed in the morning after each study.

Measurements and Main Results: Data from 72 participants (53 males and 19 females) aged 50.9 ± 1.3 years, with a BMI of 35.5 ± 0.8 kg/m2 were included in the analysis. CPAP withdrawal elicited severe OSA; the apnea hypopnea index (AHI) on CPAP and withdrawal (OSA) nights was 5.4 ± 0.6 and 58.9 ± 3.6 events/h, respectively. CPAP withdrawal did not affect overall OGTT, and changes in glucose tolerance were normally distributed. We performed a subgroup analysis of patients whose OGTT values were higher during the OSA visit compared to the CPAP visit, whom we termed glucose responders. Compared to non-responders, glucose responders exhibited decreased rapid eye movement sleep, and increased sleep fragmentation, heart rate, and overnight glucose and free fatty acids. Of note, AHI and measures of oxygen saturation did not distinguish responders from non-responders.

Conclusions: CPAP withdrawal impairs nocturnal and next-morning metabolism in patients with disrupted sleep architecture and heart rate elevation.
Leveraging Big Data and Machine Learning for Clinical Risk Prediction in Patients Hospitalized with COVID-19

Shannon Wongvibulsin, PhD, Brian T. Garibaldi, MD, MEHP, Annukka A. R. Antar, MD, PhD, Jiyang Wen, BS, Mei-Cheng Wang, PhD, Amita Gupta, MD, MHS, Robert Bollinger, MD, MPH, Yaxun Xu, PhD, Kunbo Wang, MS, Joshua F. Betz, MS, John Muschelli, PhD, Karen Bandeen-Roche, PhD, Scott L. Zeger PhD, Matthew L. Robinson MD

Globally, COVID-19 cases continue to increase and clinicians urgently need reliable tools to determine which patients are high risk for deterioration. To address clinical demands for individualized predictions of COVID-19 trajectories, we employed a registry of hospitalized COVID-19 patients and used a dynamic random forest machine learning (ML) approach to develop the Severe COVID-19 Adaptive Risk Predictor (SCARP).

Our algorithm achieved high predictive performance for both 1-day and 7-day risk predictions of progression to severe disease or death. The areas under the receiver operating characteristic curve (AUCs) for 1-day risk prediction were 0.89 (95% CI: 0.88 - 0.90) and 0.89 (95% CI: 0.87 - 0.91), during the first and second weeks of admission, respectively. The AUCs for 7-day risk were 0.83 (95% CI: 0.83 - 0.84) and 0.88 (95% CI: 0.86 - 0.89), during the first and second weeks of admission, respectively.

Using ML and longitudinal data from >3,500 hospitalized COVID-19 patients, we created tools for individual-level risk predictions of COVID-19 trajectories in hospitalized patients. Furthermore, we translated these tools to clinical use in record time at Johns Hopkins. First, we developed SCARP (https://rsconnect.biostat.jhsph.edu/covid_trajectory/), an interactive tool for predictions of progression to severe illness or death in patients hospitalized with COVID-19. Additionally, we integrated our risk calculator into Epic to facilitate use within the clinical workflow and incorporation of the risk score as part of the electronic medical record. This work fills an important gap in clinical care of COVID-19 patients through the use of ML, big data, and informatics.

Choosing Wisely: Avoiding Unnecessary Preoperative Testing

Nina M. D’Amiano, BA, C. Matthew Stewart, MD, PhD, Rosalyn W. Stewart, MD, MS, MBA

Background: Some studies suggest that routine preoperative assessment includes more testing than is beneficial. The ABIM’s Choosing Wisely campaign opposes excessive testing, which imposes unnecessary burdens on individual patients and the U.S. healthcare system. The goal of this project was to assess the degree to which professional guidelines for preoperative evaluations are followed in the context of head and neck surgery.

Methods: We retrospectively reviewed medical records of patients who were able to obtain an outpatient preoperative assessment and underwent surgery in the Johns Hopkins Department of Otolaryngology Head and Neck Surgery (OHNS) during the first two weeks of January 2019 (N=99). We used the NSQIP Risk Calculator to compute the preoperative risk of a major adverse cardiac event (MACE score), which helped determine what preoperative testing was indicated according to professional guidelines. Standard descriptive statistics were used to determine the appropriateness of the preoperative evaluations. The departmental OHNS recommendations to PCPs were compared to professional guidelines.

Results: In 44.3% of the preoperative evaluations, tests were ordered in excess of professional guidelines. We discovered that the departmental OHNS recommendations conflicted with professional guidelines; OHNS advises obtaining an ECG on any patient over the age of 50, although guidelines do not endorse routine age-based preoperative ECG testing.

Conclusions: Preliminary evidence demonstrates that preoperative testing exceeds professional guidelines. Next steps include reconciling departmental OHNS recommendations with professional guidelines, identifying the reason(s) for guideline discordance, and then intervening accordingly in order to prevent patient harm and reduce healthcare costs.
Intranasal Leptin Reduces Mortality from Opioid Overdose in Mice
Carla Freire, Huy Pho, Shannon Fonti, Luiz U Sennes, Vsevolod Y Polotsky

North America is currently facing an opioid epidemic. Over 130 Americans die every day from an opioid overdose and the COVID-19 pandemic has increased opioid usage and opioid-related deaths. Respiratory depression is the main cause of morbidity and mortality from opioids. We have previously shown that leptin acts as respiratory stimulant and prevents opioid-induced respiratory depression and upper airway obstruction without reducing analgesia. In this study, we aimed to examine if intranasal (IN) administration of leptin effectively delivers leptin to the brain and prevents opioid-related deaths.

To determine leptin delivery to the brain male C57BL/6J mice, were treated with IN leptin at 1.2mg/kg (n=5) or vehicle (n=5) and sacrificed 20 min later. Brains were harvested, the olfactory bulbs, medullas and hypothalami were isolated, quick frozen and stored at -80°C. For leptin level measurements, brain tissue was homogenized, protease inhibitors and protein concentrations were determined and ELISA was performed. To determine the survival probability, male C57BL/6J mice, received IN leptin (n=26) or vehicle (n=25) at 1.2mg/kg and 30 minutes later received a bolus of intraperitoneal morphine (400mg/kg). Mice survival time after morphine injection was recorded for 24h.

IN leptin significantly increased leptin levels in the olfactory bulb and medulla and presented a trend in the hypothalamus. Mice that received IN leptin had a higher survival rate when compared to mice that received vehicle (69% vs 92%, p = 0.044).

We demonstrated that the intranasal route is effective for the delivery of leptin to the brain and that leptin reduced opioid-induced mortality.

Visit Completion During the Telemedicine Transition in Early Months of the Pandemic

Background: Due to the COVID-19 pandemic, on March 16th, 2020, the John G. Bartlett Specialty Practice converted from exclusively in-person visits to mostly telemedicine visits. We studied the impact of this transition on visit completion.

Methods: We analyzed 1,580 patients scheduled for visits in the 14 weeks before the transition and 1,598 patients scheduled the 14 weeks after. For each period, we calculated the percentage who completed ≥1 visit. We calculated odds ratios (OR) for completing ≥1 visit, associated with demographic and clinical factors in each period.

Results: Pre-transition, 79% of patients completed ≥1 visit. Post-transition, 1,315 patients (82%) were scheduled for telemedicine and 283 were scheduled for in-person. Post-transition, 84% of all patients completed ≥1 visit, while 98% of telemedicine patients completed ≥1 visit. Telemedicine visits were conducted 70% by phone, 30% by video. A History of IDU was associated with lower odds of visit completion, pre-transition OR=0.84 [95% confidence interval (CI):0.64,1.11], post-transition OR=0.74 [CI:0.55,0.99]. Heroin [OR=0.39 [CI:0.24,0.62]] and cocaine use [OR=0.57 [CI:0.37,0.86]] were also associated with lower odds. OR for visit completion associated with tobacco use pre-transition was 0.64 [CI:0.50,0.82] and post-transition was 0.86 [CI:0.66,1.14]. Age 60+ was associated with higher odds of visit completion pre-transition [OR=1.67 [CI:1.16,2.41]] but not post-transition [OR=0.87 [CI:0.57,1.35]].

Conclusions: Telemedicine improved visit completion, but many patients only completed visits by phone. The impact of telemedicine on probability of visit completion and the differential effects on subsets of the population should be explored more once data for longer time periods are available.
Genomic datasets from traditional murine models of AKI and AKI-lung cross-talk reveal molecular pathways relevant to COVID-19 infection

Dmitry N. Grigoryev & Hamid Rabb

**Background:** COVID-19 leads to acute respiratory distress syndrome (ARDS) and acute kidney injury (AKI), with likely mechanistic links between AKI and ARDS. Using publicly available genomics datasets of ischemic and sepsis induced AKI in mice, we searched for molecular pathways of AKI and AKI-lung cross-talk relevant to COVID-19.

**Methods:** Microarray datasets GSE6730 and GSE60088 were downloaded from Gene Expression Omnibus (GEO) and analyzed to identify differentially expressed genes using GEO-built in GEO2R tool. GSE6730: lungs after moderate (30 min) and severe (60 min) ischemic AKI at 6h and 36h. GSE60088: kidneys after 6h of pneumonia+mechanical ventilation (PMV).

**Results:** AKI downregulated kidney angiotensin-converting enzyme 2 (ACE2) at both 6h (fold change (FC)=-2.41) and 36h (FC=-3.23) after severe but not moderate ischemia (6 h: FC=-1.3, 36h: FC=-1.16). In lungs from AKI mice, ACE2 was significantly downregulated (FC=-2.89, P<0.0001). Ischemic AKI and PMV led to a decrease in lung transmembrane protease, serine 2 (TMPRSS2) FC=-1.83, P=0.01 and FC=-1.68, P<0.0001, respectively. The filtering for known genes with P-value<0.01 and FC>4 identified 53 kidney genes upregulated by PMV and 254 lung genes upregulated by AKI, of which 9 genes were common to both organs. 3 of 9 genes were previously linked to AKI-lung cross-talk: Lcn2, Socs3 & Inhbb.

**Conclusions:** We identified changes in COVID-19 related genes ACE2 and TMPRSS2 in ischemic and sepsis mouse models of AKI and lung cross-talk. We also found new candidate genes activated in kidney during PMV and in lung during AKI, which could be important in combined kidney-lung injury during COVID-19.

Keap1 gene edited T cells using CRISPR/Cas9 improve outcomes from experimental ischemic acute kidney injury

Johanna T. Kurzhagen, Sanjeev Noel, Sepideh Gharai, Mohanraj Sadasivam, Sul A Lee, Jing Gong, Lois J. Arend, Abdel Hamad & Hamid Rabb

**Background:** Keap1/Nrf2 pathway regulates T cell functions and modulates ischemic AKI. We hypothesized that Keap1 editing using CRISPR/Cas9 protect from ischemic AKI to set the stage for T cell therapy for human AKI.

**Methods:** Keap1 gene was edited in mouse primary CD4 T cells using CRISPR/Cas9 and functional effects evaluated by sequencing, qRT PCR and flow cytometry. Keap1 edited CD4 T cells were studied under hypoxic/normoxic conditions in-vitro. Keap1 edited or control CD4 T cells were adoptively transferred into nu/nu mice before inducing AKI and functional and structural effects assessed.

**Results:** Keap1 editing significantly increased Nrf2 targets Nqo1 (8.5-fold), Hmox1 (4.4-fold) and Gclc (2-fold) mRNA. Keap1 edited CD4 T cells displayed higher Hif1a mRNA expression than control cells under hypoxic conditions (1.5-fold, p=0.02). Ifng mRNA expression was significantly decreased in Keap1 edited cells compared to control cells under normoxic (0.4-fold, p=0.04) or hypoxic (0.6-fold, p=0.01) conditions. nu/nu mice that received Keap1 edited CD4 T cells showed significantly reduced serum creatinine (0.55±0.05 vs 1.14±0.19mg/dl, p=0.05) at 24h and reduced percentage of necrotic tubules (40±16% vs 63±15%, p=0.05) compared to mice that received control cells. Kidney Keap1 edited CD4 T cells had significant reduction in TNFa expression (43±9% vs 54±7%, p=0.03) compared to control cells post IRI.

**Conclusions:** CRISPR/Cas9 mediated Keap1 editing increases Nrf2 regulated antioxidant gene expression and modified responses to in-vitro hypoxia. Adoptive transfer of ex-vivo Keap1 edited CD4 T cells ameliorated ischemic AKI in mice. These results set the stage for T cell-based therapy for human AKI.
ABSTRACT 41

COVID-19 HOSPITALIZATION AMONG HIV OR SOLID ORGAN TRANSPLANT PATIENTS IN THE U.S.
Jing Sun and Gregory D. Kirk for National COVID Cohort Collaborative (N3C)

Background: The role of immunosuppression/compromise (ISC) in risk of severe COVID-19 is unknown. While ISC could reduce control of SARS-CoV-2 viremia, it might also dampen the severe immune response; data comparing ISC groups is limited.

Methods: Using patient-level data from 34 sites in the U.S. National COVID Cohort Collaborative (N3C), we compared risk of COVID-19 hospitalization amongst COVID-19 patients in 3 ISC groups (1,300 persons with HIV [PWH]; 2,142 solid organ transplant [SOT] patients; 41 PWH with SOT) to 288,743 COVID-19 patients without HIV or SOT(HIV-/SOT-).

Results: Of 292,226 COVID-19+ patients, the median age was 41 years (IQR: 25-58), 46% male, 47% non-Hispanic white (NHW), and 17% non-Hispanic black (NHB). PWH and SOT patients, respectively, were more likely to be older, male, minorities, and have ≥ 3 comorbidities than overall N3C patients. Overall, 26% of HIV-/SOT- COVID-19 patients were hospitalized. In crude analyses with HIV-/SOT- as the referent group, COVID-19 patients with HIV, SOT or both had a 2.3, 4.4, or 6.9-fold increased odds of hospitalization, respectively. After adjustment for demographics and site, the risk was attenuated but remained statistically significant. Adjustment for comorbidities obviated the estimated risk among PWH, while SOT patients had persistently increased odds of hospitalization.

Conclusion: ISC patients are more likely to be hospitalized with COVID-19 independent of sociodemographics. Risk was driven mainly by the high burden of comorbidities in both groups, although SOT still had increased hospitalization independently.

ABSTRACT 42

The Role of FOLH1/GCPII and NAAG in Cognitive Dysfunction
Caroline F. Zink, Peter B. Barker, Akira Sawa, Daniel R. Weinberger, Min Wang, Henry Quillian, William S. Ulrich, Qiang Chen, Andrew E. Jaffe, Joel E. Kleinman, Thomas M. Hyde, Greer E. Prettyman, Mellissa Giegerich, Kayla Carta, Marcus van Ginkel, Kristin L. Bigos

Objective: Altering the metabotropic glutamate receptor 3 (mGluR3) by pharmacology or genetics is associated with differences in learning and memory in animals and humans. The neurotransmitter N-acetyl-aspartyl-glutamate (NAAG) is the selective endogenous agonist of mGluR3, and increasing NAAG may improve cognition. Glutamate carboxypeptidase II (GCPII), coded by the gene folate hydrolase 1 (FOLH1), regulates the amount of NAAG in brain. The goal of this study was to determine the relationship between FOLH1/GCPII, NAAG levels, cognition, and neural activity associated with cognition.

Methods: We measured the effects of genetic variation in FOLH1 on mRNA expression in human brain and NAAG levels using magnetic resonance spectroscopy. We then correlated NAAG levels and FOLH1 genotypes with measures of cognition in healthy and psychotic subjects. We also correlated FOLH1 genetics and neural activity during memory, as measured by functional MRI.

Results: We found that a missense mutation in FOLH1 (rs202676 G allele) is associated with increased FOLH1 mRNA in the prefrontal cortex of normal and schizophrenia brains. This FOLH1 variant is associated with decreased NAAG levels in healthy and psychotic patients. NAAG levels were positively correlated with visual memory performance. Carriers of the FOLH1 variant had lower IQ scores and poorer cortical activity during memory tasks.

Conclusions: Higher NAAG levels are associated with better cognition, suggesting increasing NAAG levels through FOLH1/GCPII inhibition may improve cognition. Furthermore, NAAG levels and cortical activity during memory may be useful neuroimaging biomarkers for future clinical trials.
TRANSMISSION OF ATTENUATED HIV-1 FROM A CHRONIC PROGRESSOR TO A VIREMIC CONTROLLER
Bezawit A. Woldemeskel, Caroline C. Garliss, Joseph Cofrancesco Jr, Joel N. Blankson

Background: Viremic controllers (VC) are individuals who maintain low viral loads without anti-retroviral therapy and are considered models of functional cure. Characterizing the mechanisms that lead to the natural control of HIV-1 is needed to better understand and inform cure strategies. In this study, we analyze an HIV-1 transmission pair, where a chronic progressor (TP5M) transmitted HIV-1 to a recipient who became a VC (TP5F).

Methods: We sequenced replication competent virus to assess genome integrity. Virus replication was tested in-vitro by a growth kinetics assay utilizing a p24 ELISA. In-vivo replication was assessed by infecting CD4 T cell engrafted Nod SCID Gamma humanized mice. Further, the ability of viruses to downregulate HLA-A2 and CD4 in infected cells was assessed by flow cytometry.

Results: The chronic progressor (TP5M) had a CD4 count of 76 cells/µL and a viral load of 673,000 copies/mL, while the VC (TP5F) had a CD4 count of 964 cells/µL and a viral load of 62 copies/ml. Neither patient had protective HLA alleles. Replication-competent isolates from both subjects were obtained with a viral outgrowth assay. The isolate from TP5M replicated slightly better than an isolate from TP5F with p24 concentrations of 1441 ng/ml versus 655 ng/ml at day 10 compared to 44 ng/ml versus 23 ng/ml at day 0. The viral isolate from TP5M replicated better than the isolate from TP5F in humanized mice with viral loads of 1.4e8 and 3.7e6 copies/ml versus 9.6e6 and 2.5e4 copies/ml respectively at 7 days post-infection. Sequencing of LTR, gag, pol, integrase and the accessory genes confirmed that TP5F and TP5M were a transmission pair. All four TP5F replication-competent viral isolates had numerous mutations and deletions in nef, including a 16 base pair deletion that resulted in a frame shift and premature stop codon. These mutations affected nef function as primary CD4+ T cells infected with TP5F virus had 2-to-4-fold lower downregulation of CD4 and HLA-A2 (mean fluorescence intensity [MFI] of 9664 and 3083) compared to TP5M virus infected cells (MFI of 3634 and 782 respectively).

Conclusions: Together, these data suggest that unlike TP5M, TP5F viral isolates are attenuated due to non-functional nef. This attenuation probably contributed to the natural control of viral replication in TP5F. Our data suggests that transmission of attenuated minor variants can explain discordant outcomes in some transmission pairs.

Free-living gait cadence measured by wearable accelerometers: a promising alternative to traditional measures of mobility for assessing fall risk
k K. Urbanek, David L. Roth, Marta Karas, Amal A. Wanigatunga, Christine M. Mitchell, Stephen P. Juraschek, Yurun Cai, Lawrence J. Appel, Jennifer A. Schrack

Wearable devices that collect biosignals have experienced widespread growth in research applications, yet evidence on whether such devices are superior to structured laboratory-and-clinic-based tests is sparse. Given substantial limitations to the duration and content of in-person healthcare visits, especially for older adults, the need for remote clinical diagnostics is imperative. We compared traditional in-clinic measures of mobility (6-minute walk cadence, 6-minute walk gait speed, 4-meter gait speed, and 6-minute walk distance) with estimates of free-living gait cadence derived from wearable accelerometers in predicting fall rates in 432 community-dwelling older adults. Across all participants, baseline free-living cadence was significantly related to fall rates; each 10 steps/min. higher cadence was associated with a 13.2% lower fall rate. These findings were stronger among higher-functioning participants (cadence >100 steps/min.), with a 27.7% lower fall rate for every 10 steps/min. higher free-living cadence. Among older adults with slow baseline gait (4-meter gait speed <0.8 m/s), all clinic-based and free-living metrics were significantly associated with fall rates. In conclusion, data collected from biosensors in the free-living environment provide a more sensitive indicator of fall risk than structured in-clinic tests, especially among higher functioning older adults who may be more responsive to intervention.
ABSTRACT 45

Comparison of Clinical Improvement of Treatment With or Without Remdesivir Among Hospitalized Patients With COVID-19


Clinical effectiveness data on remdesivir is urgently needed, especially in diverse populations and in combination with other therapies. To examine the effectiveness of remdesivir with or without corticosteroids for COVID-19, we conducted a retrospective cohort study, from March 4 to August 29, 2020, under 5-hospital health system in Baltimore/DC area.

The primary outcome was clinical improvement (discharge or decrease of 2 points on the WHO severity score) and the secondary outcome was 28-day mortality. An additional outcome was the combined effectiveness of remdesivir and corticosteroids.

Individuals with confirmed SARS-CoV-2 infection by polymerase chain reaction (PCR) who received remdesivir were matched to infected individuals who did not receive remdesivir using both time invariant and time-dependent covariates. An individual in the remdesivir group with k days of treatment was matched to a control who stayed in the hospital at least k days (5 days maximum) beyond the matching day.

Remdesivir recipients had a faster time to clinical improvement (median 5.0 days [IQR 4.0, 8.0], remdesivir vs. 7.0 days [IQR 4.0, 10.0], control; adjusted hazard ratio (aHR) 1.47; 95% confidence interval [1.22, 1.79]) among a cohort of predominantly non-white patients hospitalized with COVID-19. Remdesivir plus corticosteroids did not result in a significantly reduced hazard of death at 28 days (aHR 1.94, [0.67 to 5.57]) compared to remdesivir alone.

ABSTRACT 46

Meal intervals and weight trajectories in adults using the Daily24 mobile application: A prospective cohort study

Di Zhao, Thomas B. Woolf, Lindsay Martin, Eliseo Guallar, Harold Lehmann, Janelle Coughlin, Shanshan Song, Katherine Holzhauer, Jeanne M. Clark, Kathleen M. McTigue, Michelle R. Lent, Wendy L. Bennett

Background: Small pilot and randomized controlled studies suggest that time-restricted feeding may decrease body weight. However, the role of meal timing and intervals, measured using mobile applications, has not been examined in larger population-based studies. The objective of this study is to evaluate the association between meal intervals and weight trajectories among adults from a population-based clinical cohort.

Methods: Multi-site prospective cohort study of adults recruited from three health systems. Over the 6-month study period, 547 participants downloaded and used the Daily24 mobile application to record the timing of meals and sleep for at least one day. Intervals were calculated as the average of all available daily entries for each participant. We obtained information on weight and comorbidities at each outpatient visit from electronic health records available for up to 10 years prior to until 10 months after baseline. We used mixed linear regression to model weight trajectories.

Results: The mean (SD) baseline (at consent) age was 51.1 (15.0) years and body mass index (BMI) 30.8 (7.8) kg/m2; 77.9% were women and 77.5% were White. Average time in the cohort was 5.9 years prior to and 0.3 years after baseline. The mean interval from first to last meal was 11.5 (2.3) hours. The associations between meal intervals and weight trajectories are shown in the Table. The number of meals per day was positively associated with weight change before baseline, and number of snacks and drinks per day was inversely associated with weight change after baseline. Each additional occasion of snacks and drinks was associated with a 3.20 kg weight decrease (95% CI 1.41 to 4.99). None of the other associations were statistically significant.

Conclusions: Number of daily meals was positively associated with weight change in previous periods, while the number of daily snacks and drinks was inversely associated with weight trajectory. The intervals from first to last meal was not associated with weight change.
Prognostic Significance of Urinary Biomarkers in Patients Hospitalized With COVID-19

Steven Menez, MD, MHS; F. Dennis G. Moledina, MBBS, PhD; Heather Thiessen-Philbrook, MMath; F. Perry Wilson, MD, MSCE; Wassim Obeid, PhD; Michael Simonov, MD; Yu Yamamoto, MS; Celia P. Corona-Villalobos, MD, MS; Crystal Chang, BS1; Brian T. Garibaldi, MD; William Clarke, PhD; Shelli Farhadian, MD, PhD; Charles Dela Cruz, MD, PhD; Steven G. Coca, DO, MS; Chirag R. Parikh, MD, PhD

Acute kidney injury (AKI) is common in patients with COVID-19 and associated with poor outcomes. Urinary biomarkers have been associated with adverse kidney outcomes in other settings and may provide additional prognostic information in patients with COVID-19.

We evaluated 19 urinary biomarkers of injury, inflammation, and repair in patients hospitalized with COVID-19 at 2 academic medical centers between April and June 2020. We associated biomarkers with a primary composite outcome of KDIGO stage 3 AKI, requirement for dialysis, or death within 60 days of admission. We also compared various kidney biomarker levels in the setting of COVID-19 versus other common AKI settings.

Of the 157 patients in the cohort, 24 (14.6%) experienced the primary outcome. Among the 10 biomarkers that were associated with the primary outcome, higher levels of neutrophil gelatinase-associated lipocalin (NGAL) (HR: 1.53; 95% CI: 1.33-1.76), monocyte chemoattractant protein (MCP-1) (HR: 1.86; 95% CI: 1.48-2.33), and kidney injury molecule-1 (KIM-1) (HR: 2.32; 95% CI: 1.69-3.18) were associated with highest risk. Higher epidermal growth factor (EGF) levels were associated with a lower risk of the primary outcome (0.52; 95% CI: 0.40-0.69). Compared to other clinical settings, COVID-19 was associated with higher biomarker levels of injury to the proximal and distal tubules in patients with AKI, and with evidence of subclinical AKI in patients without AKI.

Urinary biomarkers can prognosticate development of severe kidney complications in patients with COVID-19 and provide valuable information in identifying those who require close follow-up to monitor kidney disease recovery and progression.

Use of Chronic Therapies for Cystic Fibrosis (CF) in Patients on Elexacaftor/Tezacaftor/Ivacaftor at a Single Center

Alexandra Toporek, MD, Kristin Reikert, PhD, Christian Merlo, MD, Natalie West, MD

Objective: Elexacaftor/tezacaftor/ivacaftor (ETI) was approved for use in 90% of the CF population in 2019, resulting in vastly improved clinical outcomes. Anecdotally, our patients on ETI report using chronic maintenance therapies less frequently since starting ETI; we sought to quantify changes in use of chronic maintenance therapies in patients on ETI.

Methods: In November 2020, we surveyed patients seen in our CF center via email listserv. We queried patients on their adherence to chronic therapies after starting ETI.

Results: 95 of 299 patients (32%) completed the survey. 76 of 95 patients (80%) were prescribed ETI. Of those prescribed ETI, mean age (SD) of respondents was 37.9 (11.5); 45 (59%) were female. Before starting ETI, 48 patients reported taking prescribed chronic therapies (dornase alfa, hypertonic saline, inhaled antibiotics, azithromycin, airway clearance, pancreatic enzymes) at least 3-5 days per week. 50 patients reported decreasing the frequency of use of chronic therapies after starting ETI: these patients now never use or rarely use (less than 1 day per week) dornase alfa (n=22), hypertonic saline (22), inhaled antibiotics (24), and airway clearance therapies (17). While there was no association between current reported baseline FEV1 and frequency of therapy discontinuation (p=0.27), patients were more likely to simplify regimens if their FEV1 improved by ≥5% in after starting ETI (p<0.01).

Conclusion: A majority of patients within our center report taking less of their maintenance therapy regimens since starting ETI. Further research regarding the effect of changes in chronic maintenance therapies on clinical outcomes is necessary.
ADAPTATION OF A 5 A’S OBESITY COUNSELING IN PRIMARY CARE CASE FOR A LIFESTYLE BEHAVIOR CHANGE LEARNING EXERCISE FOR FIRST-YEAR MEDICAL STUDENTS

Marci Laudenslager, Zoobia Chaudhry, Selvi Rajagopal, Kimberly Gudzune

Aim: Improve understanding of lifestyle counseling measures among medical student trainees.

Description: Participants are first-year medical students (n=121) at the Johns Hopkins University School of Medicine completing the “Topics in Interdisciplinary Medicine: Obesity, Nutrition and Behavior Change” course. We adapted a 5 A’s (Assess, Advise, Agree, Assist, Arrange) obesity counseling case and expanded the focus to address multiple lifestyle changes central to chronic disease management (nutrition, exercise, sleep, tobacco use, stress, and medication adherence). Students were assigned to small groups with each group led by a facilitator with expertise in lifestyle counseling. The case-based learning activity was 90 minutes in duration and began with a case presentation and review of the 5 A’s framework. Students were then divided into discussion pods. Each pod was assigned a behavior change topic and asked to use the 5 A’s approach to develop an evidence-based action plan. Pod discussions were supported by facilitators. Students then rejoined their small group to present their pods’ findings and together composed a comprehensive lifestyle behavior change plan for the patient. The activity concluded with a debriefing session.

Evaluation: We will obtain evaluations from medical students via survey and faculty via focus group discussions.

Discussion: The 5 A’s tool is an evidence-based approach used for weight management counseling in the primary care setting. We sought to introduce this tool in a case-based format to first-year medical students. Our goal is to establish a foundation for future physicians to comprehensively address lifestyle behavior change to manage chronic disease.

The Effects of Four Doses of Vitamin D Supplements on Falls in Older Adults: A Response-Adaptive, Randomized Clinical Trial

Lawrence J. Appel, MD, MPH; Erin D. Michos, MD, MHS; Christine M. Mitchell, ScM; Amanda L. Blackford, ScM; Alice L. Sternberg, ScM; Edgar R. Miller III, MD, PhD; Stephen P. Juraschek, MD, PhD; Jennifer A. Schrack, PhD, MS; Sarah L. Szanton, PhD, ANP; Jeannie Charleston, BSN, RN; Melissa Minotti, MPH; Sheriza N. Baksh, PhD, MPH; Robert H. Christenson, PhD; Josef Coresh, MD, PhD; Lea T. Drye, PhD; Jack M. Guralnik, MD, PhD; Rita R. Kalyani, MD, MHS; Timothy B. Plante, MD, MHS; David M. Shade, JD; David L. Roth, PhD; James Tonascia, PhD; for the STURDY Collaborative Research Group

Background: Vitamin D supplementation may prevent falls in older persons, but evidence is inconsistent, possibly because of dosage differences.

Objective: To compare the effects of 4 doses of vitamin D3 supplements on falls.

Design: 2-stage Bayesian, response-adaptive, randomized trial. (ClinicalTrials.gov: NCT02166333)

Setting: 2 community-based research units.

Participants: 688 participants, aged 70 years and older, with elevated fall risk and a serum 25-hydroxyvitamin D [25-(OH)D] level of 25 to 72.5 nmol/L.

Intervention: 200 (control), 1000, 2000, or 4000 IU of vitamin D3 per day. During the dose-finding stage, participants were randomly assigned to 1 of the 4 vitamin D3 doses, and the best noncontrol dose for preventing falls was determined. After dose finding, participants previously assigned to receive noncontrol doses received the best dose, and new enrollees were randomly assigned to receive 200 IU/d or the best dose.

Measurements: Time to first fall or death over 2 years (primary outcome).

Results: During the dose-finding stage, the primary outcome rates were higher for the 2000- and 4000-IU/d doses than for the 1000-IU/d dose, which was selected as the best dose (posterior probability of being best, 0.90). In the confirmatory stage, event rates were not significantly different between participants with experience receiving the best dose (events and observation time limited to the period they were receiving 1000 IU/d; n = 308) and those randomly assigned to receive 200 IU/d (n = 339) [hazard ratio [HR], 0.94 [95% CI, 0.76 to 1.15]; P = 0.54]. Analysis of falls with adverse outcomes suggested greater risk in the experience-with-best-dose group versus the 200-IU/d group (serious fall: HR, 1.87 [CI, 1.03 to 3.41]; fall with hospitalization: HR, 2.48 [CI, 1.13 to 5.46]).

Limitations: The control group received 200 IU of vitamin D3 per day, not a placebo. Dose finding ended before the prespecified thresholds for dose suspension and dose selection were reached.

Conclusion: In older persons with elevated fall risk and low serum 25-(OH)D levels, vitamin D3 supplementation at doses of 1000 IU/d or higher did not prevent falls compared with 200 IU/d. Several analyses raised safety concerns about vitamin D3 doses of 1000 IU/d or higher.
EMERGING IMMUNE CHECKPOINT INHIBITOR TIGIT MEDIATES ISCHEMIC ACUTE KIDNEY INJURY

Sanjeev Noel, Kyungho Lee, Sepideh Gharaei, Johanna T Kurzhagen, Mohanraj Sadasivam, Abdel R A Hamad, Philip Pierorazio & Hamid Rabb

**Background:** T cells play important roles in acute kidney injury (AKI), but the molecular mechanisms are not known. We discovered increased kidney T cell immunoreceptor with Ig and ITIM domains (TIGIT) expression during AKI, and studied its pathophysiologic role in mice.

**Methods:** C57BL/6J mice underwent bilateral ischemia reperfusion (IR). Kidney T cells were studied by RNA sequencing (RNA-Seq) and flow cytometry. Mice were treated with TIGIT blocking (1B4) or control antibodies and kidney outcomes measured. Sections from human kidneys undergoing IR were also evaluated.

**Results:** RNA-seq analysis revealed significant increase in Tigit expression (63.0±12.6 vs 21.8±2.6; p≤0.03) in CD4 T cells from post-IR mouse kidneys compared to controls. Flow analysis showed significantly (p≤0.001) increased TIGIT expression in CD4 (8.9±1.2 vs 1.8±0.4 & 2.7±0.3), CD8 (10.1±0.9 vs 3.0±0.6 & 4.4±1.1) and double negative (DN) T cells (7.7±1.4 vs 0.7±0.2 & 2.0±0.3) from post-IR kidneys compared to control and sham kidneys. 1B4 mediated TIGIT blocking significantly increased SCr compared to isotype control, 24h post-IR (2.6±0.4 vs 1.3±0.3 mg/dL; p=0.03). Furthermore, 1B4 treated mice had worse survival (4/7) compared to isotype control mice (7/7; p=0.06). TIGIT+ CD8 (7.4±22.7 vs 14.4±5.1; p≤0.04) and DN T cells (18.1±4.9 vs 1.3±0.9; p≤0.01) were significantly increased in post-clamp versus pre-clamp human kidney tissue.

**Conclusions:** TIGIT expression increased in kidney T cells after IR in mouse and ischemic human kidney. Blocking TIGIT co-inhibitory signaling increases IR injury to kidneys. Thus, TIGIT is a novel mechanistic and therapeutic target for AKI.

GEOGRAPHIC DISPARITIES AND COMMUNITY CONTEXT: ASSOCIATIONS OF ABANDONED COAL MINE LANDS WITH NEW ONSET TYPE 2 DIABETES IN PENNSYLVANIA

Evans K. H. Brown, Jonathan S. Pollak, Annemarie G. Hirsch, Brian S. Schwartz

Differences in community features could help explain the strong geographic disparities found in type 2 diabetes (T2D). A growing literature documents that communities with abandoned industrial infrastructure, including coal mine lands with hazards such as spoil piles, rusting structures, open pits, and acid mine drainage, can adversely affect health through impacts on health-related behaviors and stress. We investigated the association between living near abandoned coal mine lands (AML) and new onset T2D through a case-control analysis using electronic health records from a health system in Pennsylvania. We identified cases as persons with new onset T2D (n = 15,888) and frequency-matched controls (5:1, n = 79,435) on sex, age and year of encounter. Residential addresses were geocoded using ArcGIS. We used the Abandoned Mine Land Inventory System database to obtain AML data in Pennsylvania and created variables to represent metrics of AML type and density. We performed logistic regression for each AML variable, adjusting for individual level covariates of age, sex, race (white vs non-white), ethnicity (Hispanic vs non-Hispanic), and Medical Assistance (≥ 50% of time). The highest quartile (vs. the lowest) of multiple AML variables were associated (odds ratio, 95% confidence interval) with new onset T2D, including the density of physical hazards (1.20, 1.11–1.29), toxic contamination sites (1.17, 1.09–1.26), acid mine drainage impacted streams (1.12, 1.04–1.22), and the density of total mined area (1.18, 1.11–1.25). These findings contribute to the growing literature on geographic disparities in T2D. Further research is needed to investigate the physiologic mechanisms through which these associations may occur.
Body fat distribution and reduced coronary endothelial function in people living with HIV

Erin Goerlich, MD; Michael Schär, PhD; Shashwatee Bagchi, MD; Patricia Barditch-Crovo, MD; Gabriele Bonanno PhD; Yohannes Afework; Valerie Streeb, MS; Gary Gerstenblith MD; Robert G. Weiss, MD; Allison G. Hays, MD

Introduction: Cardiovascular disease is the primary cause of morbidity and mortality in people living with HIV (PLWH) on antiretroviral therapy (ART). Coronary artery endothelial function (CEF) is impaired in early atherosclerosis and in PLWH compared to CEF in controls. Body fat distribution abnormalities are common in PLWH and associated with increased mortality. Visceral fat stores are thought to be capable of secreting inflammatory molecules that promote atherosclerosis. However, the relationship between fat distribution and coronary health in PLWH is unknown. We aimed to investigate associations between body fat distribution and CEF in virally-suppressed PLWH.

Methods: Fifty-eight PLWH on ART underwent non-contrast MRI to quantify CEF, measured as coronary artery cross-sectional area (CSA) change from rest to that during isometric handgrip exercise, a known endothelial-dependent stressor. Abdominal visceral and subcutaneous fat area and liver fat fraction were quantified with MRI. Associations between independent variables and CEF were determined using linear regression.

Results: Among 58 PLWH (51±11 years; 22% women), mean CSA change was 0.9±12.6%, suggesting impaired CEF (as previously reported in PLWH). On univariate regression, CSA changes were inversely related to BMI (R=-0.30, p=0.023), waist circumference (R=-0.39, p=0.005), and subcutaneous fat area (R=-0.34, p=0.012). No significant relationships were observed between CEF and liver fat fraction, waist/hip ratio, or visceral fat area.

Conclusions: In stable PLWH, BMI, waist circumference, and subcutaneous, but not visceral fat, are associated with impaired CEF, a metric of vascular health. These fat indices may contribute to higher rates of heart disease in PLWH. Additional studies are needed to confirm these observations.

Abstract 54

A Critical Performance/Disease Trade-off at the Dawn of Vertebrate Evolution


Antagonistic pleiotropy is a foundational theory that predicts aging-related diseases are the result of evolved genetic traits conferring advantages early in life. We examined CaMKII, a pluripotent signaling molecule that contributes to common aging-related diseases, and found that its activation by reactive oxygen species (ROS) was acquired more than half-a-billion years ago along the vertebrate stem lineage. Functional experiments using genetically engineered mice and flies reveal ancestral vertebrates were poised to benefit from the union of ROS and CaMKII, which conferred physiological advantage by allowing ROS to increase intracellular Ca2+ and activate transcriptional programs important for exercise and immunity. Enhanced sensitivity to the adverse effects of ROS is thus a trade-off for positive traits that facilitated the early and continued evolutionary success of vertebrates.
ABSTRACT 55

Angiotensin receptor blocker treatment upregulates the memory protective angiotensin type 4 receptor (AT4R) in the postmortem brains of cognitively intact individuals but not in Alzheimer’s Dementia

C. Cosarderelioglu1, 2, C. George3, R. Marx3, Q. Xue1,4, J. Tian5, E. Oh1, L. Ferrucci6, D. Bennett1, J. Walston1, P. Abadir1

1Johns Hopkins University School of Medicine, Division of Geriatric Medicine and Gerontology, Baltimore, Maryland, USA; 2Ankara University School of Medicine, Department of Internal Medicine, Division of Geriatrics, Ankara, Turkey; 3Albert Einstein College of Medicine/Montefiore Medical Center, Department of Medicine, Division of Geriatrics, Bronx, New York, USA; 4Johns Hopkins University Center on Aging and Health; 5Department of Biostatistics, Bloomberg School of Public Health; 6National Institute on Aging, National Institutes of Health, Baltimore, MD; 7Rush Alzheimer’s Disease Center, Rush University Medical Center, Chicago, Illinois, USA

The primary dementia-protective benefits of Angiotensin receptor type1 (AT1R) blockers are believed to arise from systemic effects on blood pressure. However, there is a brain-specific renin-angiotensin system (b-RAS) that acts mainly through three receptor subtypes: AT1R, AT2R, and AT4R. AT1R promotes inflammation and oxidative stress (OS). AT2R increases nitric oxide. AT4R is essential for dopamine and acetylcholine release and mediates memory consolidation. This prompts the question: Does brain AT1R blockade contribute to the salutary effects of Angiotensin receptor blockers (ARBs)? Here, we aimed to investigate the effects of ARBs in terms of changes to b-RAS, OS, inflammation, tau, beta-amyloid load, and cognition. Postmortem frontal-cortex brain samples of age- and sex-matched cognitively normal (CN) individuals using (n=30) and not using ARBs (n=30) and Alzheimer’s disease (AD) patients using (n=30) and not using ARBs (n=30) were studied. Protein levels of receptors were measured by Western blot. Protein carbonyl (PC) and cytokine levels were measured by ELISA. Average tau, β-amyloid load, global cognitive function (GCF) score, and rate of decline in GCF were analyzed. In cognitively intact individuals (non-AD), our data show that ARB-treatment was associated with higher protein levels of AT4R (median(range)0.69(1.92)vs0.17(1.18), CN+ARBs vs CN, p=0.02), but there was no significant difference in brain AT1R or AT2R. Our data also shows lower level of OS marker PC (10.60(8.32)vs11.26(7.44), CN+ARBs vs CN, p=0.03) and lower overall and hippocampal amyloid scores (0(5.45)vs1.15(4.21) p=0.03, 0.79(12.75)vs3.41(13.36) p=0.04, CN+ARBs vs CN, respectively). In AD group, ARB-treatment was associated with lower AT1R protein level (0.47(1.15)vs0.59(1.99), AD+ARBs vs AD, p=0.02). No significant changes observed in OS, inflammation, tau, and amyloid load in AD brains treated with ARBs. Our results suggest a role for AT4R in ARBs-mediated protective effects in cognitively intact individuals. Our data also may suggest the absence of such salutary effects in AD individuals.

ABSTRACT 56

Antigen-driven clonal selection shapes the persistence of HIV-1 infected CD4+ T cells

Francesco R. Simonetti, Hao Zhang, Garshasb P. Soroosh, Jiayi Duan, Kyle Rhodehouse, Alison L. Hill, Subul A. Beg, Kevin McCormick, Christopher L. Nobles, Jennifer A. White, Jun Lai, Joseph B. Margolick, Rebecca Hoh, Steven G. Deeks, Frederic D. Bushman, Janet D. Siliciano, Robert F. Siliciano.

Clonal expansion of infected CD4+ T cells is a major mechanism of HIV-1 persistence and a barrier to cure. Potential causes are homeostatic proliferation, effects of HIV-1 integration, and interaction with antigens. Here we show that it is possible to link antigen responsiveness, full proviral sequence, integration site, and T cell receptor β-chain (TCRβ) sequence to examine the role of recurrent antigenic exposure in maintaining the HIV-1 reservoir. We isolated Cytomegalovirus (CMV)- and Gag-responding CD4+ T cells from 10 treated individuals. Proviral populations in CMV-responding cells were dominated by large clones, including clones harboring replication-competent proviruses. TCRβ repertoires showed high clonality driven by converging adaptive responses. Although some proviruses were in genes linked to HIV-1 persistence (BACH2, STAT5B, MKL1), proliferation of infected cells under antigenic stimulation occurred regardless of the site of integration. Paired TCRβ-integration site analysis showed that infection could occur early or late in the course of a clone’s response to antigen and could generate infected cell populations too large to be explained solely by homeostatic proliferation. Together these findings implicate antigen-driven clonal selection as a major factor in HIV-1 persistence, a finding that will be a difficult challenge to eradication efforts.
Sensorimotor cortical mechanisms of somatosensory attenuation
Teresa George, Mohit Singhal, Agostina Casamento-Moran, Jeremy Brown, Gabriela Cantarero

Somatosensory attenuation is a phenomenon where a self-generated touch feels weaker than an externally generated touch of the same intensity, thereby increasing the saliency of external signals. It has been purported that this occurs because a copy of the motor command (efference copy) is sent to other cortical areas such as the cerebellum and primary somatosensory cortex (S1) allowing the brain to anticipate the sensory consequences of our own movements and attenuate them. However, whether S1 plays a direct role in the perceptual attenuation of self-generated movements remains speculative. Here we aim to understand the S1 neurophysiological correlate of sensory motor attenuation and how it relates with perceptual behavior. We hypothesize that S1 activity relating to afferent sensory feedback will be inhibited during self-generated movements and this reduction in activity will correlate with the reduced percept of a self-generated movement. To test this, we will record perceptual and sensorimotor (M1 and S1) multi-unit neural activity (MUA), in a chronically implanted human spinal cord injury participant, during self-generated and externally generated somatosensory feedback. Using a two-alternative force choice (2AFC) paradigm, the participant will receive two taps on his index finger and be asked to report which tap felt stronger. The first tap (i.e. test tap) will be either self-generated by the participant or externally generated by a motor at a fixed force value, the second tap (i.e. comparison tap) will be exclusively externally generated by a motor and will consist of a range of force values. This allows us to systematically compare the behavioral percept of self-generated and externally generated movements and record their respective S1 MUA responses. We predict that S1 MUA for a self-generated tap as compared to an externally generated tap of the same force will be associated with a reduction in S1 firing rate that will correlate with a reduced behavioral percept of force. In contrast, at the point of subjective equality (PSE), i.e. the tap forces at which the self-generated tap and externally generated tap are perceived to be equal by the participant, the firing rate in S1 will look similar between the two taps despite their absolute difference in force values. Our results will reveal a new understanding of the neurophysiological underpinnings of somatosensory attenuation.

Complement activation drives progression of pre-eclampsia to HELLP syndrome
Hridaya Shah, Theresa Boyer, Arthur Vaught, Hang Chen, Evan Braunstein

Pre-eclampsia is a hypertensive disorder of pregnancy that can progress to HELLP syndrome (hemolysis, elevated liver enzymes, and low platelet counts) in 1% of cases (Vaught et al. 2018). We have previously shown that HELLP syndrome is due to abnormal complement activation in a subset of patients harboring a germline variant in a complement regulatory gene (Vaught et al. 2016). However, the factors driving progression of pre-eclampsia to HELLP syndrome are unknown. We hypothesized that patients with pre-eclampsia harboring complement gene variants are at increased risk for progression to HELLP syndrome and worse maternal and fetal outcomes due to dysregulation of the complement system. In the present study, targeted sequencing of 13 genes known to regulate the alternative complement pathway was performed on HELLP and pre-eclampsia patients compared to a cohort of healthy normotensive pregnant females. Clinical outcomes were also collected to correlate with patient genotypes. In total, 26 cases of HELLP, 14 cases of pre-eclampsia, and 43 controls were enrolled. Heterozygous germline variants with a MAF < 0.01 were isolated, followed by stratification by predicted deleteriousness via four independent algorithms. We found that the frequency of germline variants in the HELLP cohort was 42% with 31% predicted to be harmful. By comparison, the frequency of germline variants in the pre-eclampsia and control cohort was 36% and 26% respectively. The frequency of harmful germline variants in both pre-eclampsia and control cohorts was 21%. In addition, patients with HELLP syndrome that harbored a complement gene variant were 30% more likely to present with earlier onset disease and suffer from intra-uterine fetal death than those without complement mutations. Additional samples will be needed to further these conclusions, however preliminary results indicate that pre-eclampsia is a pre-cursor to HELLP, and patients with complement-associated variants are more susceptible to severe disease.
PROMPT multiplexed point-of-care detection of SARS-CoV-2 and other respiratory pathogens

As the COVID-19 pandemic rages around the world, the ability to accurately screen for the virus and other respiratory pathogens with rapid results remains critically important to identify and control the spread. Current options for highly sensitive point-of-need nucleic acid tests lack short turnaround times or are prohibitively expensive for widespread use. To address the need for fast, affordable testing, we have developed a Portable, Rapid, On-Cartridge, Magnetofluidic Purification and Testing (PROMPT) platform that integrates sample-to-answer readout from saliva or nasopharyngeal swabs in less than 30 minutes with a ~$3 cost per assay. The PROMPT platform uses transfer of functionalized magnetic particles within a simple 3-layer thermoplastic cartridge to automate RNA purification and transfer into PCR reactions for multiplexed detection of SARS-CoV-2, Influenza A, Influenza B, and an internal control. Cartridges are loaded into a compact instrument (8.5”x5.7”x5.7”) where a magnetic arm automates sample preparation and a miniaturized thermocycler mounts onto the cartridge for rapid RT-PCR. The PROMPT platform was evaluated with both clinical nasopharyngeal swabs (n=116) and saliva samples (n=14) with overall sensitivities/specificities for SARS-CoV-2, Flu A, and Flu B of 98.4%/95.7%, 85.7%/100%, and 100%/98.4% respectively. This study demonstrates the PROMPT platform’s potential as a user-friendly and affordable option for rapid decentralized testing in the effort to triage patients, guide treatment, and curb the pandemic.

Depression and anxiety among healthcare workers at Johns Hopkins Bayview Medical Center during the COVID-19 pandemic: a cross-sectional survey.
Sonal Gandhi, MD; Michael Van Wert, LCSW-C, MPH; Mansoor Malik, MD; Amtshwar Singh, MD, FACP; Haroon Burhanullah, MD; Ishaan Gupta, MD.

Background: Severe acute respiratory syndrome coronavirus 2 has continued to spread in Unites States with largest number of deaths in the world. While there are concerns about the mental health of healthcare workers (HCWs) around the globe during this pandemic, there is paucity of literature from US.

Objective: To determine the prevalence of depression, anxiety and attitudes among healthcare workers (HCWs) during COVID-19 pandemic and their relationship with demographics and work role.
Setting: Johns Hopkins Bayview Medical Center
Participants: 1198 HCWs were invited for the survey, 605 (52%) started the survey. Of those, 542 (88%) completed the survey.

Measurements: Patient Health Questionnaire-2 (PHQ-2); Generalized Anxiety Disorder-7 (GAD-7).

Results: Approximately 14% of healthcare workers screened positive for depression, and 43% for anxiety. 16% of participants reported moderate to severe anxiety. During the pandemic, more than 80% of HCWs reported fear for their health, 55.8% reported work-related stress and 37.3% reported social avoidance due to nature their work. HCWs who spent most of their time providing in-person care to patients with COVID-19 reported higher frequency of depression (OR 2.9) and anxiety (OR 2.7) than those who spent none of their time doing so.

Conclusion: The current pandemic has adversely affected the mental health of HCWs. The time spent taking care of patients with COVID-19 patients is consistently associated with adverse impact on mental health. Additionally, the HCWs are worried about their safety, institutional isolation practices, job stress and social avoidance due to the nature of their work.
**ABSTRACT 61**

**Hepatocyte Growth Factor and 10-year Change in Left Ventricular Structure: The Multi-Ethnic Study of Atherosclerosis**


**Introduction:** Hepatocyte Growth Factor (HGF) is a mesenchymal cytokine linked to incident heart failure (HF), with recent data from our group showing highlighting a strong and independent association with HF with preserved ejection fraction (HFpEF). Cardiac MRI (cMRI) allows for precise analysis of morphologic changes in left ventricular (LV) structure. Increased LV mass and concentric remodeling (defined by an increased mass:volume ratio) are imaging markers of HFpEF risk. Whether HGF is associated with a pattern of adverse LV remodeling is unknown. Hypothesis: Higher HGF at baseline will be associated with increased LV mass, decreased volume, and increased mass:volume ratio over 10 years.  

**Methods:** We studied 4762 participants of the MESA cohort, free of CVD and HF at baseline, who had completed both HGF measurement and cMRI at the baseline exam. Of these, 2855 completed a 2nd cMRI at 10-years. We examined the cross-sectional and longitudinal associations of HGF and LV parameters using multivariableadjusted linear mixed effect models. Results: The mean (SD) for age was 61 (10) years. Median (IQR) for HGF level was 888 pg/mL (745-1066); 53% were women. In cross-sectional analysis, the highest HGF tertile, compared to the lowest, was associated with greater mass:volume ratio (1.66 (0.43, 2.89)) and lower LV end diastolic volume (-1.87 mL (-3.45, -0.28)), after full covariate adjustment. In longitudinal analysis, the highest HGF tertile was also associated with increased mass:volume ratio ((4.79 (2.73, 6.85)) and reduced LV end diastolic volume (-4.97 (-7.10, -2.85)) over 10-year follow-up. Conclusions: In a community cohort, higher HGF levels were independently associated with a concentric LV remodeling pattern of increased mass:volume ratio and decreased LV end diastolic volume over 10 years. This association may be an intermediate phenotype explaining the association of HGF with HFpEF risk.

**ABSTRACT 62**

**A Digital Health Intervention in Acute Myocardial Infarction**

Francoise A. Marvel, MD; Erin M. Spaulding, PhD, BSN, (co-first author); Vinayak Bhardwaj, BS, MS, MPH; Matthias A. Lee, PhD; William E. Yang, MD; Ryan Demo, BS, MS; Jie Ding, PhD; Jane Wang, MD; Helen Xun, BS; Lochan M. Shah, MD; Daniel Weng, BS; Jocelyn Carter, MD, MPH; Maulik Majmudar, MD; Eric Elgin, MD; William Padula, PhD; Jerilyn K. Allen, ScD, RN, FAAN; and Seth S. Martin, MD, MHS

**Background:** Hospital readmissions among acute myocardial infarction (AMI) patients contribute to preventable healthcare complications and costs. We aimed to (1) determine if AMI patients using a digital health intervention (DHI) have lower 30-day all-cause readmissions than a historical control and (2) establish cost-effectiveness of the DHI.  

**Methods:** This nonrandomized controlled trial, conducted at four hospitals from 2015-2019, included 1,064 AMI patients (DHI n=200, historical control n=864). The DHI consisted of a smartphone application, smartwatch, and blood pressure monitor to engage patients in guideline-directed care during hospitalization and 30 days post-discharge. Propensity score-adjusted Cox proportional hazard models estimated hazards for all-cause 30-day readmissions than control patients (6.5% vs. 16.8%). Adjusting for multiple confounding factors, DHI patients had a 52% lower risk for all-cause 30-day readmissions (HR: 0.48; 95% CI: 0.26-0.88) and $7,319 cost savings per patient compared to standard care.  

**Conclusions:** In AMI patients, DHI use was associated with lower risk of all-cause unplanned 30-day readmissions and shows promise as a cost-effective management approach.
oped large particle fluorescence-activated cell sorting approach to
in the isolation of single CMs. Here, we used our previously devel-

PSC-CM maturation arrest, are unknown. The advent of single cell
ation differs from endogenous development, leading to consequent
trajectory reconstruction methods, we identified a perinatal matu-
scRNA-seq reference of PSC-CM directed differentiation. Through
generate an scRNA-seq reference of mouse in vivo CM maturation

ABSTRACT 63

Sensitive and portable sensing platform for exosome analysis
Sangmoo Jeong

Exosomes are &lt;100-nm phospholipid vesicles, actively secreted
from cells. As they are abundant in blood and carry molecular infor-
mation from their parental cells, exosomes have emerged as a prom-
ising biomarker for diagnosis. However, previous techniques were
inappropriate for exosome analysis in clinical settings due to low
sensitivity and complicated procedures required. To address these
limitations, I have developed sensitive and easy-to-use electrochem-
ical sensors to identify exosome biomarkers directly from biological
fluids. The first device was termed Integrated Magneto-electrochem-
ical EXosome sensor (iMEX). With a size of smaller than an iPhone,
it achieved a &gt;1000-fold higher sensitivity than ELISA without any
filtration and centrifugation steps. With the iMEX system, I profiled
four different surface markers in cancer exosomes directly from 100
ul of plasma samples from patients within one hour. The second de-
vice, termed integrated Kidney Exosome Analysis (iKEA) sensor, was
designed to detect T-cell exosomes in urine for monitoring kidney
transplant rejection. During acute cellular rejection of kidney al-
lografts, T-cells infiltrate the kidney tubular cells, and thus it is likely
that T-cell specific exosomes exist in urine. With the iKEA platform,
we found a high level of CD3-positive exosomes in urine from kidney
rejection patients, and the detection accuracy was &gt;90%. My lab
at JHU will continue developing technologies for rapid and cost-
effective analysis of exosomes and identify sensitive and non-invasive
biomarkers.

ABSTRACT 64

Trajectory reconstruction identifies dysregulation of perinatal maturation programs in pluripotent
stem cell-derived cardiomyocytes
Suraj Kannan, Matthew Miyamoto, Brian L. Lin, Chulan Kwon

A primary limitation in the clinical application of pluripotent stem
cell derived cardiomyocytes (PSC-CMs) is the failure of these cells
to achieve full functional maturity. In vivo, cardiomyocytes undergo
numerous adaptive changes during perinatal maturation. By con-
trast, PSC-CMs fail to fully undergo these developmental processes,
instead remaining arrested at an embryonic stage of maturation. To
date, however, the precise mechanisms by which directed differenti-
ation differs from endogenous development, leading to consequent
PSC-CM maturation arrest, are unknown. The advent of single cell
RNA-sequencing (scRNA-seq) has offered great opportunities for
studying CM maturation at single cell resolution. However, perinatal
cardiac scRNA-seq has been limited owing to technical difficulties
in the isolation of single CMs. Here, we used our previously de-
veloped large particle fluorescence-activated cell sorting approach to

generate an scRNA-seq reference of mouse in vivo CM maturation
with extensive sampling of perinatal time periods. We subsequently
generated isogenic embryonic stem cells and created an in vitro
scRNA-seq reference of PSC-CM directed differentiation. Through
trajectory reconstruction methods, we identified a perinatal matu-
ration program in endogenous CMs that is poorly recapitulated in
vitro. By comparison of our trajectories with previously published
human datasets, we identified a network of nine transcription
factors (TFs) whose targets are consistently dysregulated in PSC-CMs
across species. Notably, we demonstrated that these TFs are only
partially activated in common ex vivo approaches to engineer PSC-
CM maturation. Our study represents the first direct comparison of
CM maturation in vivo and in vitro at the single cell level, and can be
leveraged towards improving the clinical viability of PSC-CMs.

ABSTRACT 65

Antibiotic modulation of gut microbiome influences recovery from experimental acute kidney injury
Sepideh Gharraie, Sanjeev Noel, Kyungho Lee & Hamid Rabb

Background: Repair from acute kidney injury (AKI) can be complete
or maladaptive with progression to chronic kidney disease. There is
no treatment to enhance recovery. We therefore hypothesized that
changing the gut microbiome would modify repair after AKI.

Methods and Results: C57BL6 mice underwent bilateral 30 minute
(moderate) or unilateral 50 minute (severe) ischemia then reperfu-
sion (IR). Mice received either water, amoxicillin, metronidazole,
or combination antibiotics (ampicillin, metronidazole, neomycin &
vancomycin) after IR (n=5/group). Glomerular filtration rate (GFR)
with inulin clearances was measured at baseline, 24h, 1 week, and
2 weeks after IR. Kidneys were evaluated for fibrosis with Masson's
trichrome, immunofluorescence staining and mRNA expression for
Col1α1, TGF-β, aSMA, and P21, and immunophenotyped using flow
cytometry. Stool were taken for 16s rRNA taxonomic sequencing
analysis. Preliminary data demonstrate that amoxicillin improved
GFR (98.0 ± 15.6%) 2 weeks after severe IR, whereas GFR was de-
creased in controls (82.3 ± 20%), metronidazole alone (69.2 ± 18.3 %,
P = 0.01) or combination antibiotics (71.8 ± 14.3%, P =0.02). Germ
free mice at baseline had decreased kidney TCR+ cells (10051 ± 2869)
compared to controls (32383 ± 13689, P = 0.001) and combined
antibiotic treated mice (19079 ± 6643).

Conclusion: Preliminary results demonstrate that changing the
microbiome with antibiotics can improve or worsen recovery from
AKI depending on type of antibiotic. Kidney T cells are a potential
mediator of this. Modifying the microbiome could be a novel therapy
to accelerate recovery from AKI.
**ABSTRACT 66**

**Autologous IgG antibodies block outgrowth of a substantial but variable fraction of viruses in the latent reservoir for HIV-1**

Joseph Varriale, Lynn Bertagnolli, Sarah Sweet, Jacqueline Brockhurst, Francesco R. Simonetti, Jennifer White, Subul Beg, Kenneth Lynn, Karam Mounzer, Ian Frank, Pablo Tebas, Katharine J. Bar, Luis J. Montaner, Robert F. Siliciano, and Janet D. Siliciano

In untreated HIV-1 infection, rapid viral evolution allows escape from immune responses. Viral replication can be blocked by antiretroviral therapy. However, HIV-1 persists in a latent reservoir in resting CD4+ T cells, and rebound viremia occurs following treatment interruption. The reservoir, which is maintained in part by clonal expansion, can be measured using quantitative viral outgrowth assays (QVOAs) in which latency is reversed with T cell activation to allow viral outgrowth. Recent studies have shown that viruses detected in QVOAs prior to treatment interruption often differ from rebound viruses. We hypothesized that autologous neutralizing antibodies directed at the HIV-1 envelope (Env) protein might block outgrowth of some reservoir viruses. We modified the QVOA to reflect pressure from low concentrations of autologous antibodies and showed that outgrowth of a substantial but variable fraction of reservoir viruses is blocked by autologous contemporaneous immunoglobulin G (IgG). A reduction in outgrowth of >80% was seen in 6 of 15 individuals. This effect was due to direct neutralization. We established a phylogenetic relationship between rebound viruses and viruses growing out in vitro in the presence of autologous antibodies. Some large infected cell clones detected by QVOA carried neutralization-sensitive viruses, providing a cogent explanation for differences between rebound virus and viruses detected in standard QVOAs. Measurement of the frequency of reservoir viruses capable of outgrowth in the presence of autologous IgG might allow more accurate prediction of time to viral rebound. Ultimately, therapeutic immunization targeting the subset of variants resistant to autologous IgG might contribute to a functional cure.

**ABSTRACT 67**

**FoxM1: at the nexus of β-cell proliferation and function**

Elham Mosleh, Andrew Yuhas, Maria Golson

The forkhead box transcription factor FoxM1 is widely known for its role in driving proliferation, including in β cells. FoxM1 is upregulated in β cells exposed to proliferative stimuli, such as obesity or high fat diet, during pregnancy, and in response to injury such as partial pancreatectomy or streptozotocin treatment. Moreover, Foxm1 declines with age, which correlates with a decrease in both basal and stimulated β-cell replication. Mice lacking Foxm1 in β cells display diminished β-cell proliferation in conjunction with 40% reduction of β-cell mass by nine weeks of age. In males, this reduction results in glucose intolerance or diabetes. On the other hand, females lacking Foxm1 have normal glucose tolerance unless challenged further.

My previous work demonstrated that young male mice expressing an activated form of FoxM1 (FoxM1*) have increased insulin secretion and improved glucose tolerance with no increase in β-cell mass or proliferation; conversely, insulin secretion was reduced from size-matched islets lacking FoxM1, confirming a novel role for FoxM1 outside of proliferation. Intriguingly, female mice expressing FoxM1* displayed normal glucose tolerance. These findings were confirmed in human islets transduced with an adenovirus encoding FoxM1*; islets from males transduced with activated FoxM1* displayed higher glucose-stimulated insulin secretion than controls, while human female islets showed no difference. Surprisingly, while FoxM1 expression is high in proliferating β cells, we also detect low levels of FoxM1 in quiescent cells. We are currently investigating what mediates the difference between male and female islets and how FoxM1 distinguishes its proliferative and β-cell functional targets.

**ABSTRACT 68**

**Germline ERBB2 coding variants are associated with increased risk of myeloproliferative neoplasms**

Lindsay Tao, Hang Chen, Anna Yang, Donna M. Williams, Shruti Chaturvedi, Aparna Pallavajjala, Theodoros Karantanos, Alison Moltierno, Evan M. Braunstein

Familial cases of myeloproliferative neoplasms (MPN) are relatively common, yet few inherited risk factors have been identified. Exome sequencing of a kindred with a familial cancer syndrome characterized by both MPN and melanoma produced a germline variant in the ERBB2/HER2 gene that co-segregates with disease. To further investigate whether germline ERBB2 variants contribute to MPN predisposition, the frequency of ERBB2 variants was analyzed in 1606 cases that underwent evaluation for hematologic malignancy, including 238 cases of MPN. MPN cases had a higher frequency of rare germline ERBB2 coding variants compared to non-MPN hematologic malignancies (9.2% vs. 4.1%, OR 2.4, 95% CI: 1.4 to 4.0, p = 0.0015) as well as cases without a blood cancer diagnosis that served as an internal control (9.2% vs. 2.7%, OR 3.6, 95% CI: 1.5 to 8.6, p = 0.0037). This finding was validated via comparison to an independent control cohort of 1587 cases without selection for hematologic malignancy (9.2% in MPN cases vs 5.2% in controls, p = 0.014). The most frequent variant identified, ERBB2 c.1960A>G; p.I654V, was present in MPN cases at more than twice its expected frequency. These data indicate that rare germline coding variants in ERBB2 are associated with an increased risk for development of MPN. The ERBB2 gene is novel susceptibility locus which likely contributes to cancer risk in combination with additional risk alleles.
Inhibition of the O-GlcNAc transferase (OGT) activates a p38-stress response pathway in cardiac myocytes

Kyriakos N. Papanicolaou, Natasha E. Zachara, Deepthi Ashok, Nina Nguyen, Mellissa Picker, Eddie Avila, D. Brian Foster & Brian O’Rourke

Cardiomyocyte hypertrophy is a tightly regulated form of cellular growth that allows the heart to cope with increased hemodynamic demands such as those occurring during development and endurance training (physiologic hypertrophy) but also in hypertension, or after a myocardial infarction (pathologic hypertrophy). Key features of cardiomyocyte hypertrophy include enhanced protein synthesis, rearrangement of the sarcomeres and increase of myocyte diameter. Multiple signaling pathways coordinate cardiomyocyte hypertrophy, including the phosphorylation signaling cascade of mitogen activated protein kinases (MAPK). Ample evidence implicates MAPKs in both physiologic and pathologic cardiomyocyte hypertrophy via the effector kinases Erk1/2 and p38 respectively. β-O-linked N-Acetylglucosamine (O-GlcNAc) on S or T residues, is a glyco-PTM that is catalyzed by the O-GlcNAc transferase (OGT) and removed by the O-GlcNAcase (OGA). Apart from targeting S and T via dedicated enzymes, O-GlcNAcylation exhibits several other parallels with phosphorylation, including dynamic nature, inducible responsiveness to environmental cues and widespread intracellular occurrence. Mounting evidence implicates protein O-GlcNAcylation in cardiovascular physiology and pathology, including cardiomyocyte hypertrophy. Here we investigated the intersections between O-GlcNAc, Erk1/2 and p38 kinases in neonatal rat ventricular myocytes (NRVM) utilizing the OGT and OGA inhibitors OSMI1 and TMG respectively. Cardiomyocyte hypertrophy was kept in check by serum withdrawal and induced with the Gaq agonist phenylephrine (PE). Treating NRVMs with 25 μM OSMI1 for 6 hrs increased by nearly 3.5-fold the baseline phosphorylation of p38 (T180 Y182). On the other hand, treatment for 6 hrs with 200 nM TMG did not have a significant impact on p38 phosphorylation. Exposure of NRVMs to 5 μM PE for 30 minutes had a mild effect in inducing p38 phosphorylation by almost 2.0-fold and a stronger effect in inducing Erk1/2 phosphorylation (4-fold). Moreover, pre-treatment of NRVMs with 25 μM OSMI1 for 6 hrs proportionally increased the PE-induced phosphorylation of the two kinases (p38 and Erk1/2) following a 30-minute stimulation. Further investigation in OSMI1-treated NRVMs revealed increased phosphorylation of the p38-downstream target Hsp27 and the transcription factor Creb. Lastly, we found that treating NRVMs with OSMI1 restricts basal and PE-induced protein synthesis and also appears to disrupt the growth response of these cells to PE, as assessed by phalloidin staining of filamentous actin. Our data indicate that treatment with OSMI1, and by extent acute inhibition of OGT and O-GlcNAcylatation, activates a p38-mediated stress-response pathway in NRVMs. Ongoing efforts seek to identify potential signaling mediators that are differentially O-GlcNAcylated and could serve as upstream activators of p38 in this system.

Costal Cartilage Calcification; a Marker of Short- and Long-term Glycemic Status in Female Population; The Multi-Ethnic Study of Atherosclerosis

Mahsima Shabani; Farhad Pishgar; Sepehr Akhtarkhavari; Thiago Quinaglia A. C. Silva; Matthew Budoff; David A. Bluemke; Graham R. Barr; Wendy S. Post; Colin O. Wu; João A. C. Lima; Shadpour Demehri

Background: High fasting blood glucose (FBG) is a global leading risk factor for increased morbidity in current medicine. Glycemia is known to be associated with vascular calcifications. Costal cartilage calcification (CCC) is reliably obtainable from non-contrast cardiac CT scans, acquired for Coronary Artery Calcium (CAC); but its association with glycemic status is unknown.

Hypothesis: CCC is associated with glycemic status at the time of CT and cumulative glycemia prior to the CT exam in both sexes and subjects with zero CAC score.

Method: This is a retrospective analysis in the Multi-Ethnic Study of Atherosclerosis. CCC was defined as the volume of hyperdense tissue (HU > 180) within the borders of costal cartilage observed in the non-contrast cardiac CT image FOV. We used multivariable linear regression models adjusted for age, race, and BMI, and stratified for sex to assess the association between CCC and: 1) serum FBG, HbA1c, insulin, and insulin resistance at the time of CT; 2) cumulative exposure to glycemia (area under FBG-time curve over 10 years); 3) categories of repeated FBG based on FBG levels under or above 100 mg/dL at repeated measures in 4.6-, 6.3-, and 7.8-year intervals.

Results: 2,143 participants with a mean age of 68.7 and a female: male ratio of 1.3 were included. The mean (SD) of the log-transformed CCC (CC Ct) was 7.0 (1.0) in females and 8.0 (0.9) in males (p<0.001). Adjusted CC Ct was associated with FBG, HbA1c, serum insulin, and HOMA-IR. Higher cumulative glycemia (p=0.004) was associated with CCC in females. Also, females with repeated high FBG had higher CC Ct values compared to sustained normal FBG values in the same intervals. All associations were consistently present in females with zero CAC score.

Conclusion: This is the first study to report the association between CCC and glycemic status at the time of CT or over 10 years prior to the CT in women, particularly in subjects with zero CAC score. CCC quantification may be a stand-alone marker of cumulative glycemia in women.
**ABSTRACT 71**

The identity of the interferon signature in SLE

Eduardo Gomez-Banuelos¹, Jessica Li², Daniel Goldman¹, Erika Darrah¹, Kevin S Cashman², Ignacio Sanz², Michelle Petri¹, Felipe Andrade¹

¹Division of Rheumatology, The Johns Hopkins University School of Medicine, Baltimore, Maryland, US.
²Department of Medicine, Division of Rheumatology, Lowance Center for Human Immunology, Emory University, Atlanta, GA, US

Systemic lupus erythematosus (SLE) patients display unique blood transcriptional profiles, including a hallmark interferon (IFN) signature linked to type I IFN (IFN-I). The contribution of IFN-II and IFN-III to SLE pathogenesis, however, is still a debate. Here, using a new approach to quantify the activity of IFN-I, IFN-II and IFN-III in serum/plasma from SLE subjects of SPARE (n=158) and Emory (n=86) cohorts, we distinguished several SLE subsets according to their sole or combined type of IFN activity, which associate with distinctive SLE blood-transcriptional signatures. Highest levels of IFN-I were strongly associated with the IFN signature, but IFN-I was only elevated in 14% of SLE patients. Interestingly, increased levels of IFN-II dominate in SLE (52%), followed by IFN-III (25%). Surprisingly, the expression of IFN induced genes (ISG) was independent of the interferon activity in plasma in subset of subjects with SLE. The expression of ISG in subjects without high IFN-I activity of was associated to anti-DNA and anti-Ro52 antibodies. Further, the type of plasma IFN activity was associated with clinical phenotypes and disease markers. IFN-II and IFN-III were associated with features of secondary antiphospholipid syndrome, whereas IFN-I was associated mostly with autoantibodies (i.e. anti-DNA, anti-RNP, anti-Sm), and low complement. Neither IFN alone was associated with disease activity. Instead, higher disease activity was significantly associated with co-elevation of all types of IFNs. This study provides an explanation for the failure of clinical trials targeting IFN-I and offers an approach to improve trial design.

**ABSTRACT 72**

Rare Genetic Variants Associated with Myocardial Fibrosis; Multi-Ethnic Study of Atherosclerosis (MESA)


**Background:** Rare pathogenic variants in cardiomyopathy genes can predispose to myocardial remodeling or fibrosis. We studied the carrier status for such variants in adults without cardiovascular disease who had completed cardiac MRI (CMR)-derived measures of myocardial fibrosis in Multi-Ethnic Study of Atherosclerosis (MESA).

**Objectives:** To identify rare myocardial fibrosis-related pathogenic variants and assess their prevalence in subjects with CMR-based myocardial fibrosis versus matched controls.

**Methods:** The MESA whole-genome sequencing data (2000-02) was evaluated to capture variants in 55-genes known to cause cardiomyopathy in gnomAD, 1000genomes, and TOPMed databases and damaging/deleterious effects based on PolyPhen, SIFT, FATHMM, and CADD scoring tools were assessed by ClinVar database and ACMG curation guidelines for evidence of pathogenicity. Cases were individuals with high myocardial fibrosis defined as either Late Gadolinium Enhancement (LGE)-based scar, or highest quartile of ECV in T1 mapping in the MESA CMR exam (2010-11). Age, sex, and race-matched subjects with no LGE and lower ECV were used as controls. Results: A total of 1,563 MESA participants had available genetic data and either of the two phenotypic measures. We have found 246 rare variants, 32 of which were pathogenic/likely pathogenic. The prevalence of pathogenic/likely pathogenic rare variants in the case group was higher than the control group (2.33% vs. 0.002%, p=0.023), wherein 8/342 cases and 1/424 were carriers. The missense variants in the case group were MYBC3 c.2450G>T (p.R817L), MYH7 c.2609G>A (p.R870H) and c.1325G>A (p.R442H), TNNI3 c.497C>T (p.S166F), TNNT2 c.341C>T (p.A104V), SCN5A c.673C>T (p.R225W), ABCC9 c.2815C>T (p.R939W), and RAF1 c.770C>G (p.S257W).

**Conclusions:** We observed a higher prevalence of pathogenic/likely pathogenic rare variants in subjects with significant myocardial fibrosis in CMR. Further longitudinal assessment of carriers of these variants and validation in other cardiovascular cohorts may set the foundation for the integration of gene sequencing in preventive cardiology.
Cardiac progenitors auto-regulate second heart field cell fate via Wnt secretion
Matthew Miyamoto, Suraj Kannan, Hideki Uosaki, Tejasvi Kakani, Sean Murphy, Peter Andersen, Chulan Kwon

Proper heart formation requires coordinated development of two anatomically distinct groups of cells - the first and second heart fields (FHF and SHF). Given that congenital heart defects are often restricted to derivatives of the FHF or SHF, it is crucial to understand the mechanisms controlling their development. Wnt signaling has previously been implicated in SHF proliferation; however, the source of Wnts remains unknown. Through comparative gene analysis, we found upregulation of Wnts and Wnt receptor/target genes in the FHF and SHF, respectively, raising the possibility that early cardiac progenitors may secrete Wnts to influence SHF cell fate. To probe this further, we deleted Wntless (Wls), a gene required for Wnt ligand secretion, in various populations of precardiac cells. Deletion of Wls in Mesp1+ cells resulted in formation of a single chamber heart with left ventricle identity, implying compromised SHF development. This phenotype was recapitulated by deleting Wls in cells expressing Islet1, a pan-cardiac marker. Similarly, Wls deletion in cells expressing Nkx2.5, a later-expressed pan-cardiac marker, resulted in hypoplastic right ventricle, a structure derived from the SHF. However, no developmental defects were observed when deleting Wls in SHF progenitors. To gain mechanistic insights, we isolated Mesp1-lineage cells from developing embryos and performed single-cell RNA-seq. Our comprehensive single cell transcriptome analysis revealed that Wls deletion dysregulates developmental trajectories of both anterior and posterior SHF cells, marked by impaired proliferation and premature differentiation. Together, these results demonstrate a critical role of local precardiac mesodermal Wnts in SHF fate decision, providing fundamental insights into understanding heart field development and chamber formation.

Higher Angiotensin II type 1 receptor (AT1R) levels and activity in the postmortem brains of older persons with Alzheimer’s disease
C. Cosardereligolu1, 2, C. George3, R. Marx3, Q. Xue1, 4, J. Tian5, E. Oh1, L. Ferrucci6, D. Bennett7, J. Walston1, P. Abadir1

1Johns Hopkins University School of Medicine, Division of Geriatric Medicine and Gerontology, Baltimore, Maryland, USA; 2Ankara University School of Medicine, Department of Internal Medicine, Division of Geriatrics, Ankara, Turkey; 3Albert Einstein College of Medicine/Montefiore Medical Center, Department of Medicine, Division of Geriatrics, Bronx, New York, USA; 4Johns Hopkins University Center on Aging and Health; 5Department of Biostatistics, Bloomberg School of Public Health; 6National Institute on Aging, National Institutes of Health, Baltimore, MD; 7Rush Alzheimer’s Disease Center, Rush University Medical Center, Chicago, Illinois, USA

Background: Alzheimer’s disease (AD) is the most common cause of dementia. Although multiple potential etiologies have been proposed, no clear aging-related etiological mechanisms have been identified. The Renin-Angiotensin System (RAS) is a hormonal system that is implicated in blood pressure control and has been suggested as a potential contributor to the development of AD. Here, using postmortem frontal cortex brain samples of age- and sex-matched cognitively normal individuals (n=30) and AD patients (n=30), we sought to examine the brain-specific RAS (b-RAS) differences with AD and how these findings correlate with brain AD pathologies.

Methods: Samples were obtained from the Rush Memory and Aging Project. We measured angiotensinogen, renin, and ACE gene expression by qPCR and both gene expression and protein levels of Angiotensin II receptor type 1, 2, and 4 and their downstream signaling pathway (pERK, eNOS, and nNOS) by qPCR and Western blot. Brain cytokines and oxidative stress (OS) markers as well as average paired helical filaments-tau and β-amyloid load were used as specific markers of AD pathology. Our results demonstrate an increase in both gene and protein expression (2.47-folds p=0.01, median 0.59 [range1.99] vs. 0.47 [1.95] p=0.03, respectively) and signaling activity (0.35(11.41) vs. 0.04(1.84), p<0.01) of AT1R in AD. We have not observed any significant changes in other RAS components. Our data show that higher AT1R levels correlate with OS (r=0.301 p=0.01) and β-amyloid load (r=0.245, p=0.04), while higher pERK levels negatively correlate with mitochondrial numbers (r=-0.239, p=0.03) and positively correlate with total tangle (r=0.413, p<0.01) and amyloid (r=0.208, p=0.02) scores. Finally, mRNA, protein, and pERK levels of AT1R were negatively correlated with global cognitive function (GCF) scores (r=0.216 p=0.05, r=0.258 p=0.02, r=0.376 p<0.01, respectively) and were associated with greater decline in GCF (r=0.265 p=0.02, r=0.223 p=0.04, respectively) in all subjects.

Conclusions: This study highlights molecular changes in b-RAS and offers insight into the association of these changes with brain pathology in AD.”
RESUBMITTED FINAL VERSION: Paracrine-Mediated Rejuvenation of Aged Mesenchymal Stem Cells Involves Broad Transcriptional Modulation of Angiogenic Factors

George Hung, Andreas S. Barth, Gary Gerstenblith, Peter V. Johnston

Introduction: Angiogenesis induced by bone marrow mesenchymal stem cells (MSCs) obtained from aged mice is inferior to those obtained from young mice, but is improved following exposure to conditioned media (CM) from young MSCs. To define alterations in gene expression and signaling pathways underlying the observed angiogenic improvement, we characterized differences in cellular mRNA expression between “non-rejuvenated” and “rejuvenated” (i.e. exposed to CM from young MSCs) old MSCs.

Methods: Replicates of 105 MSCs isolated from old (18-24 months) C57BL mice (n=6) were cultured separately, or in co-culture with MSCs from young (4-6 weeks, n=6) mice using 0.4µm Transwell plates that allow transfer of soluble factors, but not of cells. After 7d in culture, mRNA from non-rejuvenated old and rejuvenated old MSCs was isolated and sequenced. Analysis was performed using the open source Galaxy pipeline. Transcription factor (TF) and miRNA target enrichment analyses were performed using ChEA3 and MIENTURNET.

Results: Of the 529 unique transcripts involved in angiogenesis (GO-ID 0001525), 57 transcripts with an expression pattern mimicking that of young MSCs were identified in rejuvenated MSCs (Bonferroni p &lt; 0.0001). Of these, 42 transcripts showed significantly decreased expression, while 15 transcripts were upregulated. Transcriptional changes involved both canonical and non-canonical angiogenic pathways. Top enriched TFs and miRNAs associated with the 42 downregulated genes included MEOX2, BCL6B, FOXC2, miR-140-3p.2, miR-29a-3p, and miR-148a-3p, and those associated with the 15 upregulated genes included HIC1, JUN, and SOX18 (FDR < 0.05).

Conclusion: Improved angiogenesis by old MSCs exposed to CM from young MSCs is accompanied by significant modulation of angiogenic mediators, which may involve regulation by transcription factor and miRNA regulatory pathways. These changes suggest targets for transcriptional modification to improve stem cell mediated angiogenesis and tissue repair in aged patients.

Delayed colonic transit in systemic sclerosis: an objective assessment of delayed transit, risk factors, and clinical phenotype

Jenice X. Cheah, BSc, Jamie Perin, PhD, MS, Elizabeth R. Volkmann, MD, MS, Laura K. Hummers, MD, ScM, Pankaj J. Pasricha, MBBS, MD, Fredrick M. Wigley, MD, Zsuzsanna H. McMahan, MD, MHS

Introduction: Up to 50% of patients with systemic sclerosis (SSc) experience delayed colonic transit, which may be associated with severe outcomes. We sought to determine the clinical features associated with delayed colonic transit in SSc by comparing patients with and without colonic transit delays as measured by whole gut scintigraphy.

Methods: SSc patients with gastrointestinal symptoms (n=100) were prospectively enrolled and completed a scintigraphy-based whole gut transit study. Logistic regression was used to examine the associations between clinical features and delayed colonic transit in univariate and multivariable models.

Results: In the univariate analysis, delayed colonic transit was positively associated with female sex (OR 12.61, 95% CI 1.56-101.9; p=0.02), telangiectasia (OR 4.00, 95% CI 1.32-12.1; p=0.01), anti-centromere antibodies (OR 3.25, 95% CI 1.25-8.44; p=0.02), prior or current smoking (OR 2.56, 95% CI 1.06-6.21; p=0.04), and a Medsger GI severity score of ≥3 (OR 3.94, 95% CI 1.16-13.36; p=0.03). Patients were less likely to have significant restriction on pulmonary function tests (OR 0.23, 95% CI 0.09-0.63; p&lt;0.01). In our multivariable model, female sex (OR 90.02, 95% CI 5.31-1510.20; p&lt;0.01), telangiectasia (OR 5.31, 95% CI 1.34-21.12; p=0.02), and smoking (OR 3.25, 95% CI 1.00-10.59; p=0.05) remained positively associated with delayed colonic transit.

Conclusions: The identification of distinct clinical features associated with delayed colonic transit is important in the diagnostic evaluation and risk stratification of SSc. Further studies examining the temporal relationship between smoking and delayed colonic transit are needed to determine whether smoking is a potentially modifiable risk factor.
**ABSTRACT 77**

**Computational Modeling of Drug Dissolution in Human Stomach**  
Jae Ho Lee, Jung-Hee Seo, Rajat Mittal

The oral route is used most frequently for drug administration in humans but it is also the most complex way for an active pharmaceutical ingredient (API) to enter the body. This complexity is because drug absorption via the gastrointestinal tract depends not only on factors related to the drug, but also the fluid dynamics and stomach motility. The current approach to quantify drug dissolution relies primarily on in-vitro models, but a variety of studies have shown significant shortcomings of in-vitro devices in providing the environment similar to that of the stomach. Computational modeling of drug dissolution in biomimetic models of the stomach have the potential to overcome the limitations of in-vitro models. In this study, we present fluid-structure interaction model of drug dissolution in the MR image-based model of stomach using the immersed boundary method. The pill dissolution and release of API are resolved by solving an additional convection-diffusion equation. We analyze the effect of posture and different types of gastroparesis in local API concentration and characterize its interaction with gastric fluid dynamics.

**ABSTRACT 78**

**Income Disparity and Association with Patient-Reported Outcomes Among U.S. Adults with Atherosclerotic Cardiovascular Disease**  
Andi Shahu, MD, MHS, Victor Okunrintemi, MD, MPH, Martin Tibuakuu, MD, MPH, Anandita Agarwala, MD, Martha Gulati, MD, MS, Safi U. Khan, MD, Oluseye Ogumoroti, MD, MPH, Erica S. Spatz, MD, MHS, Erin D. Michos, MD, MHS

**Background:** Income disparity is associated with atherosclerotic cardiovascular disease (ASCVD). However, the relationship between income & patient-reported outcomes (PROs) in populations with ASCVD is unclear.

**Methods:** We used data from the nationally representative Medical Expenditure Panel Survey, 2006-2015. We studied adults with ASCVD confirmed by ICD9 codes and/or self-reported data. PROs were recorded by telephone survey & medication use by pharmacy data. We used logistic & linear regression to compare healthcare experience, medication use, health resource utilization, perceived health status & health-related quality of life by income group, adjusting for demographics, comorbidities & other socioeconomic factors.

**Results:** We included 21,353 participants with ASCVD: 6,855 high income (32.1%, weighted), 6,235 middle income (29.2%), 3,651 low income (17.1%) & 4,612 poor/very low income (21.6%). Compared with high income, adults with poor/very low income were more likely to report poor patient-provider communication, patient satisfaction, perceived health status, physical & mental quality of life, to visit the Emergency Department or not be treated with statins or aspirin.

**Conclusion:** Poor/very low income adults with ASCVD reported worse healthcare experience, greater healthcare utilization & lower secondary prevention therapies than high income adults. Future work must improve healthcare experience, PROs and medication access in adults with ASCVD regardless of income.

**ABSTRACT 79**

**Myeloproliferative Neoplasms in Pediatrics and Young Adults: Genetic Lesions, Diagnostic Features, and Clinical Outcomes**  
Zoey I. Harris, Linda Resar, Donna M. Williams, Ophelia Rogers, Alison R. Moliterno, Evan Braunstein

Philadelphia chromosome-negative myeloproliferative neoplasms (MPN) comprise a group of hematological malignancies, consisting of essential thrombocytosis (ET), polycythemia vera (PV), and primary myelofibrosis (MF). These are primarily diagnosed in older adults (OA), while those <39 make up an under-recognized subgroup, called the pediatric and young adult (PAYA) cohort. Because optimal management and long-term outcomes for PAYA patients remains unclear, it is crucial to gain better understanding of this cohort. In this study, we compared clinical features, complications, and survival between both PAYA and OA groups. We utilized The Hopkins MPN cohort, which enrolled 630 patients with an MPN between 2005-2015. All had quantitative driver mutation genotyping for JAK2V617F, CALR, and MPL. In total, 171 cases (27%) were diagnosed at <39 years (PAYA) while 459 (73%) were diagnosed >40 (OA). The mean age of diagnosis was 31 in PAYA versus 57 years in OA. At time of diagnosis, ET was more prevalent in PAYA (n=115; 67%), while PV (n=12; 7%) and MF (n=44; 26%) were less common. This is in comparison to OA, which included ET (n=177; 32%), PV (n=74; 16%), MF (n=208; 45%). JAK2V617F was the most common driver mutation in PAYA (64%) and OA (80%). There was a longer latency period to disease progression in PAYA compared to OA. Overall survival was longer in PAYA v. OA (28 v. 11 years). Leukemia developed in 5 (1.8%) PAYA and 25 (5.4%) OA. These findings illustrate the need to better understand PAYA patients to guide management of younger patients with MPN.
Improving Wait times to GI follow-up appointments in Inflammatory Bowel Disease: A Quality Improvement Project

Monica Y. Choe, Brigit VanGraafeiland, Alyssa Parian

Purpose: The purpose of this quality improvement project was to implement and evaluate a clinic appointment scheduling protocol to decrease wait times, time intervals from IBD referrals and/or hospital discharge to clinic appointments with gastroenterologists, among IBD patients at two GI clinics in Baltimore regions.

Methods: An evidence-based clinic appointment scheduling protocol for IBD patients was developed based on a thorough literature review and incorporation of Knowledge Transfer Framework. Inclusion criteria were adult patients with a diagnosis of IBD who were hospitalized and/or were newly referred to the GI clinic for evaluation and management of IBD. The protocol involved urgent scheduling slots, and a dedicated IBD scheduler. Under this protocol, gastroenterology fellows and central scheduling staff were instructed to email a dedicated IBD clinic scheduler, an IBD specialist, and a DNP student for IBD patients who are recently hospitalized or newly referred whose wait times for GI follow-up appointments exceeded 14 days.

A DNP student sent reminders and check-in emails regarding the protocol to gastroenterology fellows, central scheduling staff, and an IBD clinic scheduler every 2 weeks and as needed.

The primary outcome was wait time from the date of discharge or referral to clinic appointments with gastroenterologists per retrospective chart review. The secondary outcome was patient satisfaction as measured by pre-/post-test Qualtrics surveys. Post-intervention data from August – November 2020 will be compared with baseline data from February – July 2020.

Quest2Learn: A Gamification & Augmented Reality Approach to Advance Education in Response to the COVID-19 Pandemic

Chinat Yu, Siddharth Ananth, Jeffery Ji Zhou, Rahul Swaminathan, Cyrus Irani, Ariel Bao, Nikki Ucheyia, Shannon Wongvibulsin

The impact of the COVID-19 pandemic on education has been profound both at Johns Hopkins University (JHU) and worldwide. It has highlighted the shortcomings of remote learning, especially for hands-on laboratory classes which are not easily transitioned to online platforms.

To improve hands-on education of biomedical sciences in a virtual setting, we formed cross-disciplinary and cross-divisional collaborations between the Schools of Engineering, Arts and Sciences, Medicine, and Public Health to develop open-source pedagogy to enhance both online and on-campus education. Specifically, we have created a Quest2Learn “gamification” framework for education through the development of an augmented reality application. The goal is to seamlessly integrate the educational application into the daily lives of learners while creating relatable, personalized experiences to enhance engagement, content acquisition, and long-term retention.

We foresee our app continuing to play an important role in student’s educational experiences post-COVID.

Our team formed in Spring of 2020 and have quickly made progress, first with a prototype (https://marvelapp.com/prototype/eb648f7/screen/69691260), then with a test lab experience in pipetting (https://tinyurl.com/quest2video), and now with a beta version of the app available for testing (https://tinyurl.com/25h3z6s3). We anticipate that Quest2Learn can benefit both faculty and students as this educational platform is integrated into course offerings at JHU. Even post-COVID, the platform may play a role in providing pre-lab learning components to prepare students for the in-person laboratory sessions. Overall, this educational strategy combining gamification, augmented reality, and cross-disciplinary collaboration has the potential to transform how students learn at JHU and around the world.
Clinicians for CARE: A Systematic Review of Interventions to Support Caregivers of Patients with Heart Disease

Kellen A Knowles, MD, MS,* Helen Xun, BS,* Sunyoung Jang, BS, Sharon Pang, BA, Charles Ng, MBBS, Apurva Sharma MD, Erin M. Spaulding, PhD, BSN, RN, Rohanit Singh, BSPH, Alaa Diaib, BS, Ngozi Osuji, MD, MPH, Shannon Wongvibulsin, PhD, Daniel Weng, BS, Pauline Huynh, BS, Julie Nanavati, MLS, MA, Jennifer Wolff, PhD, Francoise A. Marvel MD, Seth S. Martin, MD, MHS

*These authors contributed equally to this work

Background: Caregivers provide critical support for patients with chronic diseases, including heart disease, but often experience caregiver stress that negatively impacts their health, quality of life, and patient outcomes. We aimed to inform healthcare teams on an evidence-based approach to supporting the caregivers of patients with heart disease.

Methods and Results: We performed a systematic review of randomized controlled trials (RCTs) written in the English language that evaluated interventions to support caregivers of patients with heart disease, and that reported caregiver outcomes. We identified 15,562 articles as of April 2nd, 2020 from six databases. We identified 20 unique RCTs that evaluated interventions for these caregivers, representing a total of 1,570 patients and 1,776 caregivers. Most interventions focused on improving knowledge, satisfaction, quality of life, and reducing burden; 85% (17/20) of the RCTs provided psychoeducation for caregivers. Interventions had mixed results, with some studies reporting improvement in caregiver burden (4/9), anxiety (5/10), depression (3/9), physical health and exertion scores (2/5), and quality of life (2/6). Given heterogeneity in outcomes, we conducted a qualitative synthesis of the results to provide clinical recommendations as represented with the acronym “CARE” (Caregiver centered, Active engagement, Reinforcement, Education).

Conclusions: This systematic review highlights the need for greater understanding of the challenges faced by caregivers and the development of guidelines to help clinicians address those challenges. More research is necessary to develop clinical interventions that consistently improve caregiver outcomes.

The Effect of Time-Restricted Feeding on 24-hour Ambulatory Blood Pressure: Results from The Time-Restricted Intake of Meals (TRIM) Study

Ruth-Alma N Turkson-Ocran, PhD, MPH, RN, FNP-BC; Edgar R Miller II, MD, PhD; Di Zhao, PhD; Scott Piila, MD, MHS; Daisy Duan, MD; Byron Jaeger, PhD; Paul Muntner, PhD; Jeanne Clark, MD, MPH; Nisa Maruthur, MD, MHS

Introduction: Some studies suggest that time-restricted feeding may decrease blood pressure (BP), but the current evidence is inconclusive.

Objective: To determine the effect of a time-restricted feeding pattern compared to a usual feeding pattern on ambulatory 24-hour BP in adults.

Hypothesis: An isocaloric, time-restricted feeding pattern will lower 24-hour BP more than an isocaloric usual feeding pattern over 12 weeks.

Methods: Forty-one persons with prediabetes (HbA1c 5.7-6.9%) and obesity (BMI 30-50 kg/m2) were randomized to consume 80% of their total calories before 1 pm (i.e., time-restricted feeding) or more than 50% of their calories after 5 pm (i.e., usual feeding) with identical macronutrient content. We used ambulatory BP monitoring to measure BP over 24-hours at baseline and 12-weeks. Outcomes of interest were mean systolic and diastolic 24-hr, daytime (7 am – 11 pm), and nighttime (11 pm – 7 am) BP.

To examine the difference in BP patterns between time-restricted feeding and usual feeding pattern groups in change in BP outcomes from baseline to 12 weeks, we used linear mixed-effects regression models with participant-specific random intercepts and fixed effects for visit and intervention group. To assess whether feeding patterns affected BP levels over the 12 week intervention period, we tested whether there was an intervention-by-time interaction.

Results: Thirty-five adults (mean age 60.4 years; 91% female, 91% African American) had sufficient data. We found reductions from baseline to 12 weeks, we used linear mixed-effects regression models with participant-specific random intercepts and fixed effects for visit and intervention group. To assess whether feeding patterns affected BP levels over the 12 week intervention period, we tested whether there was an intervention-by-time interaction.

Conclusion: Time-restricted feeding may attenuate the effect of a healthy isocaloric diet on BP compared to typical feeding patterns, and at this time, should not be recommended as a way to lower BP more than simply adopting a healthy diet.
Accuracy of Rapid Antigen Test in Asymptomatic and Symptomatic Population in a High-Volume Self-Referred Testing Site

Zishan K Siddiqui MD, Mihir Chaudhary MD, Mathew Robinson MD, Anna McCall MD, Ria Peralta BS, Rolette Esteve MD, Charles Callahan DO, Mindy Kantsiper MD, CONQUER COVID Consortium, James Ficke MD

Objective: Point-of-care rapid lateral flow antigen tests are simple to perform, often do not require specialized equipment, low cost and provide results within 15 minutes. Many have received Emergency Use Authorization to be use within first 5-7 days of symptom onset—none of the tests are approved for use in asymptomatic populations. We conducted a field study at a high-volume public testing site to assess accuracy of the Abbott BinaxNOW test amongst symptomatic and asymptomatic patients. We also assessed the accuracy among patients with high risk, low risk and no exposure.

Data Source and Study Design: From December 23, 2020-January 11, 2021, 9305 individuals were tested at the Baltimore City Convention Center; 65.5% were recruited and enrolled. A clinician-collected bilateral anterior nares swab was immediately tested on site using the rapid antigen test, BinaxNOW (Abbott); an NP swab in viral transport media was collected and RT-PCR tested in a reference lab (modified CDC assay). Symptomatic and asymptomatic participants were recruited. High risk exposure was defined as living or having been within 6 feet for more than 15 minutes with someone with confirmed or suspected COVID-19, and ‘low-risk’ was defined as any self-reported “other exposure”. Date of symptom onset also was confirmed or suspected COVID-19, and ‘low-risk’ was defined as any self-reported “other exposure”. Date of symptom onset also was confirmed or suspected COVID-19, and ‘low-risk’ was defined as any self-reported “other exposure”. Date of symptom onset also was obtained. RT-PCR cycle threshold count was obtained as well.

Major Findings: 6099 participants &gt;18 years were enrolled; the majority (88.7%; 5407) were asymptomatic, 10.5% (n=639) reported high-risk exposure, 3.5% (n=214) low risk exposure, and 70.7% (p-value= 0.005). Sensitivity of the test was 76.0% (95% CI 56.6%-88.5%) for high risk exposure, 100% (95% CI 100%-70.1%) for low risk exposure, 64.2% (95% CI 50.7% to 75.7%) for no exposure. Median CT count was 24.5 for symptomatic and 27 for asymptomatic. Specificity was 98.8% for symptomatic and over 99% for asymptomatic and all the exposure groups.

Implications: Our results show superior performance characteristics of rapid antigen testing in both symptomatic and asymptomatic patients compared to results recently reported elsewhere. Rapid antigen testing may quickly identify positive symptomatic cases for immediate referral for monoclonal antibody infusion to avoid delays which reduce its efficacy. The favorable performance in the asymptomatic population and improving sensitivity at lower Ct values met the requirements (≥70% sensitivity) of many models showing that antigen tests could impact public health particularly in congregate setting through frequent testing with early identification and isolation.

Characterization of a new mouse model of inducible frailty: the humanized IL-6 mouse

LS Nidadavolu, R Marx-Rattner, Y Wu, H Yang, C Antonescu, L Florea, CC Talbot, FB Foster, JE Fairman, G Yenokyan, A Le, JD Walston, PM Abadir

The cytokine interleukin-6 (IL-6) has pleiotropic effects in aging and is elevated in frail older adults. However, direct effects of age-related increases in IL-6 on altered physical function and mitochondrial function with age is not known. The human IL-6 (hIL-6) knock-in mouse (TetO-hIL6mitoQC) was developed utilizing CRISPR/Cas9 technology with transgene donor vector containing a tetracycline response element (TRE) promoter driving expression of hIL-6 cDNA as well as mitoQC promoter. Male TetO-hIL6mitoQC mice were treated with doxycycline-containing water for six weeks at 8 months old. RNAseq analysis of whole blood demonstrated significant upregulation of pro-inflammatory related markers at 6 weeks compared to baseline and upregulated cell proliferation and metabolism pathways. Physical testing of TetO-hIL6mitoQC mice before and after hIL-6 induction demonstrated decreased grip strength, decreased running capacity, and 40% increase in falls off of the treadmill. Induced mice also demonstrated decreased basal body temperature and impaired response to cold stress. Finally, hIL-6 expression led to changes in serum metabolites, including reduced circulating adenosine triphosphate. The novel TetO-hIL6mitoQC mouse model allows for induction of hIL-6 at various timepoints across the lifespan and demonstrates key features of a frailty phenotype including chronic inflammation physical measures such as decreased grip strength. The TetO-hIL6mitoQC mouse can be a useful model in which to initially identify frailty interventions for humans by demonstrating feasibility and safety in a pre-clinical model.
Universal Modes of Cell Motility: Applications in Ageing
Debonil Maity, Hasini Jayatilaka, Anjil Giri, Denis Wirtz, Jude M. Phillip

As integrators of molecular signals, cells offer a highly sensitive mesoscale view, with cellular dysfunctions likely occurring prior to the manifestation of disorders and diseases at the clinical level. Populations of cells typically display dynamic and heterogeneous phenotypes in the context of health and disease. In this study, to investigate cellular heterogeneity, we use ~14k single cell trajectories to unveil any universal patterns in cell motility. Using Machine Learning, we show that cells can be classified into various motility states based on spatial and temporal patterns. We use multiple cell types, migrating in 2D/3D and under various pharmacological perturbations. We then demonstrate that the heterogeneity in overall cell motility was linked to the fractional distribution of cells among the identified motility states. As expected, Actin polymerization inhibitors resulted in a motility state which shows dramatically reduced MSD, Persistence, Diffusivity and Anisotropy, while contractility inhibitor, Y-27632/Blebbistatin showed a state with high levels of Diffusivity and Lower Persistence. Lastly, using a panel of dermal fibroblasts derived from healthy donors spanning a wide age range, we observe an age-associated decrease in cell motility. We show that the age-dependent decrease in cell motility is not due to the reduced motility of all cells but results from the fractional re-distribution among motility states. Furthermore, these results point to a mechanistic framework of ageing, with the potential to identify cellular states and phenotypic patterns that could have applications in the development of proxies of ageing in the context of health and disease.

An Ultrasensitive Genetically Encoded CaMKII Activity Biosensor
Oscar E. Reyes Gaido, Mark E. Anderson

Ca2+/Calmodulin-dependent protein kinase II (CaMKII) is a critical inducer of cardiomyopathy and premature death. Despite its physiological role in fine-tuning heart rate and contractility, excessive CaMKII activity causes intracellular Ca2+ dysregulation, inflammation, and myocardial death. Our understanding of CaMKII signaling is limited by an inability to sensitively track CaMKII activity in living cells with precise temporal and subcellular resolution. To address this need, I have engineered a novel genetically encoded fluorescent biosensor that reports CaMKII activity in real time. The sensor, named CaMKAR, contains a CaMKII-specific substrate peptide that becomes phosphorylated when CaMKII is active. This phosphorylation triggers a robust increase in fluorescence that is measurable by microscopy, plate reader, and flow cytometry. I have shown that CaMKAR sensitively reports CaMKII activity using pharmacological (Ionomycin-mediated Ca2+ overload, Hydrogen peroxide-induced oxidative stress) and genetic (CaMKII T287D, constitutively active mutation) activators of CaMKII. My results also show that candidate off-target kinases, PKA and PKC, fail to trigger the sensor, suggesting that CaMKAR is specific to CaMKII. This novel tool will allow me to address the fundamental question of whether known CaMKII upstream activating signals (ischemia, β-adrenergic signaling, and Angiotensin II) lead to differential temporal or subcellular patterns of CaMKII activation. This could help explain the mechanisms by which CaMKII is physiological in some contexts and detrimental in others. Furthermore, this biosensor can serve the broader cardiovascular and CaMKII fields as a foundation for signaling mapping, genetic screens, and drug discovery.
**ABSTRACT 89**

**CROHN’S DISEASE-ASSOCIATED GENETIC VARIATION IN METAL TRANSPORTER ZIP8 (A391T) REDUCES STABILITY OF PLASMA MEMBRANE ZIP8 IN HEK293 CELLS**

Laxmi Sunuwar, Varsha Singh, Joanna Melia

**Introduction:** A nonsynonymous single nucleotide polymorphism in SLC39A8 ranks in the top 10 of variants associated with human diseases, including Crohn’s disease, obesity and schizophrenia. SLC39A8 encodes the protein, ZIP8, a ubiquitously expressed divalent cation (Mn, Zn, Fe, and Cd) transporter. Loss-of-function mutations of SLC39A8 in humans and mouse models result in severely depleted Mn levels without alteration in Zn and Fe levels demonstrating ZIP8 is a key regulator of Mn homeostasis. We and others have found ZIP8 391T results in hypomorphic protein function with reduced Mn transport in vitro and in vivo but the molecular mechanisms of reduced function have not been elucidated. The plasma membrane is hypothesized to be the site of active metal transport where ZIP8 appears to form an oligomeric protein. Loss-of-function mutations in SLC39A8 have been shown to prevent membrane trafficking. Moreover, as a glycoprotein, it is suggested that N-glycosylation may play an important role for trafficking of ZIP8, and the alanine to threonine change creates a possible new N-glycosylation site by consensus sequence. In this study, we determined whether ZIP8 391T affects the trafficking or stability of plasma membrane ZIP8.

**Methods:** The human embryonic kidney (HEK293) cells were transfected with the pcDNA vector harboring the HA-tagged wildtype (WT)/391A or mutant 391T alleles of hZIP8 using Lipofectamine 2000 reagent. Stably transfected cells were established by antibiotic selection. Half-life of plasma membrane ZIP8 was determined by applying surface biotinylation method and studied by western blot. ZIP8 localization was carried out by immunofluorescence and confocal microscopy. To measure the Mn transport function, the cells were treated with 500µM MnCl2 for 4h before western blot.

**Results:** ZIP8 WT/391A and 391T were both expressed on the plasma membrane and within intracellular vesicles in HEK293 cells consistent with published literature. Using surface biotinylation, the proportion of intracellular and plasma membrane ZIP8 was not different between WT/391A and 391T. However, tracking biotinylated ZIP8 protein over 24 hours, membrane ZIP8 391T was reduced by 50% at 6h whereas 391A did not reach 50% reduction until 12h. This suggests that 391T induces early degradation of plasma membrane ZIP8. Moreover, on exposure to MnCl2, Mn-sensitive golgi phosphoprotein,GPP130, significantly reduced more in WT/391A cells compared to 391T suggesting the hypomorphic transport function of ZIP8 in 391T.

**Conclusion:** We conclude that ZIP8 391T does not affect localization of ZIP8 to the plasma membrane in an overexpression model system in HEK293 cells. However, ZIP8 391T significantly reduces the stability of membrane ZIP8, potentially contributing to the hypomorphic phenotype. We prioritize further resolution of the molecular mechanisms, including contribution of N-glycosylation, and replication in primary cells to include intestinal epithelial cells to explore the association with risk of Crohn’s disease.

---

**ABSTRACT 90**

**Combination of tryptophan-kynurenine metabolism inhibition and NAM supplementation improves physical performance in an age-specific manner and increases life span in Drosophila melanogaster**

Mariann M. Gabrawy, Reyhan Westbrook, Austin King, Nick Khosravian, Neeraj Ochaney, Anne Le, Fernando Vonhoff, Jeremy Walston, Peter M. Abadir

The tryptophan degradation pathway (TDP) has been implicated as a contributor to frailty and morbidity in humans and is highly conserved across species, including Drosophila. In mammals, the TDP produces kynurenines such as 3-hydroxykynurenine (3-HK) and 3-hydroxyanthranilic acid (3-HAA) that, when elevated, are neurotoxic or cytotoxic. In frail older adults, levels are elevated and reduce grip strength. However, the etiological role of altered kynurenine levels in reduced physical performance is not known. Here, we used DGRP_229 Drosophila to elucidate the role of altered levels of kynurenines in physical performance. To test the effects of 3-HK and 3-HAA on age-related decline in physical performance, flies were chronically fed each metabolite and assayed at young, middle, and old age. We tested the effect of blockade of the TDP, via chronic treatment with α-methyl-tryptophan (α-MT) supplemented with either nicotinamide (NAM) or nicotinamide riboside (NR), on physical performance. Further, we measured the effects of the aforementioned metabolites on life span and dendrite density. Our results show that flies treated with 3-HK or 3-HAA have reduced climbing speed, endurance, and life span. Flies treated with a combination of α-MT plus NAM or NR have greater speed, endurance, and life span than those treated with each metabolite alone. Motor neuron density is commensurate with the aforementioned treatments. We conclude that promotion of the TDP accelerates functional decline and reduces life span while blockade of the TDP, with supplementation, attenuates the effect of age on functional decline and increases life span in an age-specific, synergistic manner.
Factors Associated with Lower Measured than A1C-Estimated Glucose Values in Hospitalized Patients with Diabetes

Sara Wallam, BS; Mohammed S. Abusamaan, MD, MPH; William Clarke, PhD; Nestoras Mathioudakis, MD, MSH

Objective: We sought to explore the association between measured average glucose (mAG) and values estimated from A1C (eAG) in hospitalized patients with diabetes mellitus (DM) and describe factors associated with discordance.

Method: This was a retrospective analysis of 17,903 unique adult patients within the Johns Hopkins Health System over five years. Difference between mAG and eAG (Δ) was used to evaluate A1C concordance and discordance. Concordance was defined as Δ within one standard deviation of the mean, and discordance (mAG<p<eAG) was defined as Δ in the ≤2.5th percentile. Multivariable logistic regressions were used to evaluate factors associated with discordance.

Results: Factors associated with higher odds of mAG<p>eAG include: Black race, Type 1 DM, Type 2 DM, biguanide treatment, carbohydrate-controlled diet, and NPO/liquid diet with aOR [95% CI] of 2.44 [1.94–3.08], 3.43 [2.38–4.93], 1.72 [1.32–2.24], 1.80 [1.30–2.48], 4.05 [2.97–5.54], and 2.08 [1.39–3.13], respectively. Factors associated with lower odds of mAG<p>eAG include: ICU treatment, systolic BP ≥140, higher albumin, stage 5 CKD, anemia, higher hydrocortisone dose, and home steroid treatment with aOR [95% CI] of 0.63 [0.43–0.91], 0.72 [0.55–0.96], 0.65 [0.54–0.77], 0.25 [0.08–0.82], 0.70 [0.54–0.90], 0.35 [0.18–0.69], and 0.37 [0.16–0.88], respectively.

Conclusion: A1C may over-estimate BG values at the transition from home-to-hospital in patients with certain factors, including Black race, and with more aggressive inpatient treatment with antihyperglycemic medications and restrictive diets. Failure to recognize that A1C does not always accurately reflect outpatient glycemic control and may be falsely elevated in some patients may lead to overtreatment, which can precipitate hypoglycemia and negatively impact patient safety.

Point-of-Care CRISPR-Cas-Assisted SARS-CoV-2 Detection in an Automated and Mobile Droplet Magnetofluidic Device

Fan-En Chen, Pei-Wei Lee, Joon Soo Park, Alexander Y. Trick, Kuangwen Hsieh, Tza-Huei Wang

In the fight against COVID-19, there remains unmet needs in developing point-of-care (POC) diagnostic testing tools that can rapidly and sensitively detect the causative SARS-CoV-2 virus to control disease transmission and improve patient management. Although recent CRISPR-Cas-assisted SARS-CoV-2 detection assays (such as DETECTR and SHERLOCK) are viewed as transformative solutions for POC diagnostic testing, their lack of simple sample processing and full integration within an automated and portable device hamper their potential for POC use. We develop herein POC-CRISPR – a new single-step CRISPR-Cas-assisted assay that is coupled to droplet magnetofluidics (DM) – that leverages simple magnetic concentration and transport of nucleic acid-binding magnetic beads to accomplish sample preparation and assay automation. By further adapting the assay into a fully integrated thermoplastic cartridge within a palm-sized mobile device, POC-CRISPR was able to detect 1 genome equivalent (GE)/µL SARS-CoV-2 RNA from a sample volume of 100 µL in 30 min. Moreover, when evaluated with unprocessed clinical nasopharyngeal (NP) swab eluates, POC-CRISPR identified SARS-CoV-2 positive samples in as short as 20 min and achieved full concordance with standard RT-qPCR.

Discrete Stochastic Optimization for Public Health Interventions with Constraints

Zewei Li, James C. Spall

In today’s society, medical professionals are facing high priority topics, such as various public health threats and working to find optimal intervention strategies. Given the stochastic nature of many health problems (e.g., the spread of pandemic influenza, the occurrence of drug overdoses, and the urgent need for health care), deterministic optimization approaches are insufficient. In this paper, we set up stochastic optimization problems concerning the 2009 H1N1 and the COVID-19 pandemic, with the spread of influenza modeled by Monte Carlo simulations with the open-source software FluTE and Covasim, respectively. Without testing every possible option, the objective of this project is to determine the best combination of intervention strategies for each type of influenza so as to result in minimal economic loss to society. To reach our objective, we use the discrete simultaneous perturbation stochastic approximation method (DSPSA), a recursive simulation-based optimization algorithm, to update input parameters in the simulation software so that the output iteratively approaches minimal economic loss. Assuming that the simulation models for the spread of disease (FluTE for H1N1 and Covasim for COVID-19) are accurate representations of society, the simulation-based strategy we present provides decision makers a powerful tool for mitigating potential human and economic losses from any epidemic. Similar techniques from this paper can also be implemented to formulate and solve other medical problems such as the efficient scheduling of operating rooms, the optimal allocation of healthcare facilities, etc.
Breakfast Consumption is Associated with Weight Loss during an Intensive Lifestyle Intervention for Adults with Overweight/Obesity and Type 2 Diabetes: Results from the Look AHEAD Trial

Daisy Duan, Scott J. Pilla, Jeanne M. Clark, Nisa M. Maruthur

Background: Breakfast skipping is linked to obesity and related cardiometabolic outcomes in observational studies, but the association between breakfast eating and weight loss is not well-established. We examined if weight loss outcomes in Look AHEAD were related to breakfast consumption frequency (BCF).

Methods: We included a subset of participants (n=3862) from the public access dataset of Look AHEAD, an RCT that compared intensive lifestyle intervention (ILI) to diabetes support and education (DSE) control in adults with overweight/obesity and type 2 diabetes. A self-reported questionnaire collected BCF over a 7-day week annually. This value (0-7) was averaged across 4 years of the intervention to calculate an average BCF. We used robust multivariable linear regression analysis to estimate the association between % weight change and 4-year average BCF controlling for baseline sociodemographics, BMI, and diabetes-related variables. In separate models, we adjusted for self-reported caloric intake (n=880) and self-reported physical activity level (n=735) among those with data.

Results: 4-year average BCF was similar in DSE (n=1914) and ILI (n=1948) arms, with a median of 7 days (IQR 6-7) for both arms (p=0.11). Each 1 day increase in average BCF was associated with an additional 0.43% weight loss in the ILI arm (p=0.002) but not in the DSE arm (β-coefficient 0.04% weight loss; p=0.73; p-interaction for arm x BCF=0.01). This association in the ILI arm remained significant after adjustment for daily caloric intake (p=0.04) but not after adjustment for physical activity (p=0.16).

Conclusions: Breakfast consumption was associated with greater weight loss in subjects who received ILI, which was attenuated after adjustment for caloric intake and physical activity. To optimize weight loss interventions, the relationship between breakfast consumption and other weight loss behaviors should be further explored.

Digital CRISPR/Cas-Assisted Assay for Rapid and Sensitive Detection of SARS-CoV-2

Joon Soo Park, Kuangwen Hsieh, and Tza-Huei Wang

The unprecedented demand for rapid diagnostics in response to the COVID-19 pandemic has brought the spotlight onto Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/CRISPR-associated systems (Cas)-assisted nucleic acid detection assays. Already benefitting from an elegant detection mechanism, fast assay time, and low reaction temperature, these assays can be further advanced via integration with powerful, digital-based detection. Thus motivated, the first digital CRISPR/Cas-assisted assay – coined digitization-enhanced CRISPR/Cas-assisted one-pot virus detection (deCOViD) – is developed and applied toward SARS-CoV-2 detection. deCOViD is realized through tuning and discretizing a one-step, fluorescence-based, CRISPR/Cas12a-assisted reverse transcription recombinase polymerase amplification assay into sub-nanoliter reaction wells within commercially available microfluidic digital chips. The uniformly elevated digital concentrations enable deCOViD to achieve qualitative detection in <15 min and quantitative detection in 30 min with high signal-to-background ratio, broad dynamic range, and high sensitivity – down to 1 genome equivalent (GE)/µL of SARS-CoV-2 RNA and 20 GE/µL of heat-inactivated SARS-CoV-2, which outstrips its benchtop-based counterpart and represents one of the fastest and most sensitive CRISPR/Cas-assisted SARS-CoV-2 detection to date. Moreover, deCOViD can detect RNA extracts from clinical samples. Taken together, deCOViD opens a new avenue for advancing CRISPR/Cas-assisted assays and combating the COVID-19 pandemic and beyond.
Male sex is associated with worse microbiological and clinical outcomes following tuberculosis treatment: A retrospective cohort study, systematic review of the literature and meta-analysis.

Vignesh Chidambaram, Nyan Lynn Tun, Marie Gilbert Majella, Jennie Ruelas Castillo, Samuel K. Ayeh, Amudha Kumar, Pranita Neupane, Akshay Gupte, Jann-Yuan Wang, Petros C. Karakousis

**Background:** Though the incidence of tuberculosis (TB) is shown to be higher in males compared to females, the relationship of sex with tuberculosis outcomes has not been adequately studied.

**Methods:** We performed a retrospective cohort study followed by a systematic review and meta-analysis of observational studies during the last 10 years to assess the sex differences in clinical and microbiological outcomes in tuberculosis.

**Findings:** In our cohort of 2894 patients with drug-susceptible pulmonary TB (1975 males and 919 females), males had higher adjusted hazards of all-cause (HR 1·43, 95%CI 1·03-1·98) and infection-related mortality (HR 1·70, 95%CI 1·09-2·64) at 9 months and higher adjusted odds ratio for sputum culture (OR 1·56, 95%CI 1·05-2·33) and similar odds ratio for smear positivity (OR 1·27, 0·71-2·27) at 2 months compared to females. Among 7896 articles retrieved, 398 articles were included in our systematic review with a total of 3,957,216 patients. The odds of all-cause mortality were higher in males compared to females in the pooled unadjusted (OR 1·26, 95%CI 1·19-1·34) and adjusted (OR 1·31, 95%CI 1·18-1·45) analyses. Relative to females, males had higher pooled odds of sputum culture (OR 1·44, 95%CI 1·14-1·81) and sputum smear (OR 1·58, 95%CI 1·41-1·77) positivity at the end of the intensive phase, and also at the end of treatment.

**Interpretation:** Among patients receiving treatment for TB, males have higher all-cause, infection- and TB-related mortality, as well as higher rates of sputum smear and culture positivity both after the intensive phase and at the completion of TB treatment after adjusting for confounding factors.

The associations between symptoms and chronic conditions among Covid-19 patients

Natalie Flaks-Manov, PhD1, Jiawei Bai, PhD2, Cindy Zhang BS3, Dina Demner-Fushman, MD3, PhD, Anand Malpani PhD4, Casey Overby Taylor, PhD1

1Johns Hopkins School of Medicine, Baltimore, MD, USA; 2Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA; 3National Institute of Health, National Library of Medicine, Bethesda, MD, USA; 4Johns Hopkins University, Whiting School of Engineering, Baltimore, MD, USA

**Background:** Coronavirus disease (COVID-19) represents a global public health concern. Infected people have had a wide range of symptoms reported. There is no evidence yet as to whether the symptoms differ between people with different chronic conditions. The aim of this study is to explore the associations between chronic conditions and COVID-19 symptoms.

**Methods:** This is a cross-sectional study. We developed a 26-question Web-based survey using Qualtrics, and published it on Amazon Mechanical Turk (MTurk) between August-December 2020. The participants were USA residents, adults (18+ years old), and MTurk workers with a self-reported COVID-19 positive test. We collected information about Covid-19 symptoms, chronic conditions, and demographic characteristics. Hierarchical cluster analysis was conducted to search for patterns based on Covid-19 symptoms and chronic conditions.

**Results:** We collected data from 1254 Covid-19 positive eligible participants. We identified four clusters: (1) population with flu-like symptoms - loss of smell/taste/appetite and diarrhea without any relation to chronic conditions; (2) abdominal and bladder pain symptoms with the chronic conditions as anemia, asthma, alcohol disorder and lung disease; (3) seizure symptoms with seizures/convulsions, psychoses, heart pain, diabetes complicated conditions; (4) skin, eyes, body aches or dryness, cramps and vomiting symptoms with the chronic conditions as ulcer, hypertension, obesity, diabetes, cancer and depression.

**Conclusions:** This study describes symptoms and chronic conditions that cluster together among individuals with a self-reported COVID-19 positive test. Such finding could potentially facilitate early detection of disease by focusing on specific symptoms for certain groups of individuals.
Adverse Cardiovascular and Obstetric Delivery Complications in Pregnant Women with Valvular Heart Disease

Anum S Minhas, Faisal Rahman, Nicole Gavin, Ari Cedars, Arthur Jason Vaught, Sammy Zakaria, Jon Resar, Stefano Schena, Steven Schulman, Di Zhao, Allison G Hays, Erin D Michos

Background: Women with valvular heart disease may be more likely to have adverse obstetric and cardiovascular complications during pregnancy. Most current recommendations focus on stenotic lesions with less guidance regarding regurgitant lesions.

Objective: We aimed to compare adverse events at delivery for women with various stenotic and regurgitant valvular diseases.

Methods: We used the 2016-2018 National Inpatient Sample data to compare demographics, comorbidities, and obstetric and cardiovascular complications during delivery hospitalizations. After adjusting for clinical and socioeconomic factors, logistic regression was performed to investigate associations between valvular heart disease and outcomes.

Results: Among >11.2 million deliveries, 20,349 were in women with valvular disease. Women with valvular disease were older, had longer length of stay, and greater costs associated with delivery. They had greater prevalence of underlying cardiovascular comorbidities compared to women without valvular disease (hypertension: 5.1 vs 0.25%; pulmonary hypertension: 7.0 vs &lt;0.1%). At delivery, they also had higher adjusted odds of obstetric events including pre-eclampsia/eclampsia (aOR 1.9 [1.8-2.2]) and intrapartum/postpartum hemorrhage (aOR 1.4 [1.2-1.6]), and cardiovascular events including peripartum cardiomyopathy (aOR 65 [53-78]), pulmonary edema (aOR 17 [13-22]), acute ischemic heart disease (aOR 19 [12-30]) and arrhythmias (aOR 22 [19-27]). There were valve lesion-specific differences in the magnitude of risk but both stenotic and regurgitant lesions were associated with elevated risk of cardiovascular complications.

Conclusions: Pregnant women with valvular heart disease have a greater burden of cardiovascular comorbidities and increased odds of obstetric and cardiovascular events at delivery. These women may benefit from specialized care from a Cardio-Obstetrics team.

Metformin improves hepatic and peripheral insulin sensitivity

Frederick Anokye-Danso, Kyewon Park, Xiaoyan Yin, Charles Addo-Yobo, Sangwon Kim and Rexford Ahima

Insulin resistance, defined as a failure of insulin to exert a normal biological action at the level of its target tissues, is a pathological defect associated with type 2 diabetes. Metformin, the most widely prescribed insulin-sensitizing drug, is thought to improve blood glucose control mainly through insulin-mediated suppression of hepatic glucose production. Putative molecular targets of metformin include AMPK, IPMK and glucagon. Although some studies have suggested that metformin may also influence glucose disposal in peripheral tissues, these findings are often based on acute treatment, intraperitoneal injection or very high concentrations of metformin causing weight loss and confounding glucose homeostasis. In the present study, we devised a clinically relevant model of chronic metformin treatment to evaluate effects of metformin on glucose regulation. Male C57BL/6J mice were fed a high fat diet (HFD) from age 10-18 weeks and received metformin (100 mg/kg) in drinking water (14-18 weeks). Control and metformin-treated mice were pair-fed to limit the impact of weight loss on glucose. After 4 weeks treatment, body weight was not statistically different between metformin and control groups, though metformin decreased fat content and increased thermogenesis. Hepatic glucose production measured by pyruvate tolerance testing was significantly blunted by metformin. Importantly, hyperinsulinemic-euglycemic clamp and radioisotopic tracer kinetic studies revealed that metformin enhanced insulin sensitivity in liver as well as skeletal muscle and adipose tissue.
**ABSTRACT 100**

**Left Atrium Right Atrium Network (LARANet): A Deep Neural Network For Biatrial Segmentation From MRI And CT Images**

Rebecca Yu, Rhaeda L. Ali, PhD, Pallavi Pandey, MD, Ryan P. Bradley, David D. Spragg, MD, FHRS, Hugh G. Calkins, MD, FHRS and Natalia A. Trayanova, PhD, FHRS.

**Background:** Structural remodeling in the left and right atrium (LA, RA) significantly impacts the success rates of catheter ablation in patients with Persistent Atrial Fibrillation (PsAF). Reconstruction of atrial anatomy and quantification of fibrotic substrate is clinically important for guiding catheter ablation. However, the first step to substrate quantification is atrial segmentation, which is time-consuming when done manually. Existing machine learning models segment the LA, but not the RA. We propose a novel two-step neural network to automatically segment the LA and RA from clinical cardiac images.

**Objective:** Assess feasibility of an automatic method for biatrial segmentation of cardiac images in a clinical workflow.

**Methods:** We developed a 2-stage 3D neural network (LARANet) that first identifies the atrial region-of-interest then refines the atrial epicardial segmentation (Fig. 1A). The network was trained on 169 patient late-gadolinium enhanced (LGE)-MRI and CT images, with corresponding ground truth segmentations. 135 image stacks were randomly selected to train and validate the network; remaining image stacks were used in hold-out testing.

**Results:** LARANet successfully segments both the LA and RA of never-before-seen clinical cardiac images with an average biatrial Dice Coefficient (DC) of 0.926 ± 0.024 (Fig. 1B, C). Existing benchmarks only evaluate LA segmentations, and LARANet performs equivalent to top models with a DC of 0.932 ± 0.026 (ns, p=0.85).

**Conclusion:** LARANet provides biatrial segmentations that accurately delineates the atria and exceeds existing methods in under 5 seconds. The success of LARANet will be significant for rapid substrate identification before catheter ablation.

**ABSTRACT 101**

**Molecular mechanism of HIV drug mediated weight gain**

IkRak Jung, Sunghee Jin, Becky Tu-Sekine, Fredrick Anokye-Danso, Todd T. Brown, Sangwon F. Kim

Antiretroviral therapy (ART) containing integrase strand transfer inhibitors (INSTI) has been associated with weight gain in both ART-initiation and switch studies, especially in women, but the underlying mechanisms are unclear. Estrogen promotes brown adipo-cyte differentiation while suppressing white adipose differentiation. Hence, we hypothesized that INSTIs may interrupt adipose function via inhibition of estrogen action.

Primary preadipocytes were isolated from 4 weeks female mouse (C57BL/6). Cells were treated with INSTI (dolutegravir (DTG) or bictegravir (BIC)) or doravirine (DOR) for 8 days during differentiation into mature white or brown adipocytes. Mature adipocytes were analyzed for lipid accumulation by Oil Red O Staining, adipogenic markers by qRT-PCR and immunoblotting. Estrogen receptor mediated transcriptional activity was measured by luciferase reporter containing estrogen response element. Finally, we examined the effects of DTG (10mg/kg for 5 days) on food intake, energy expenditure, oxygen consumption in female mice using Comprehensive Laboratory Animal Monitoring System.

We found that DTG and BIC mildly induced white adipocyte differentiation measured by white adipogenic markers (SREBP, CEBPa, and PPARg) and lipid accumulation. In contrast, brown adipogenic markers (CEBPb, PGC1a and FABP4) were significantly reduced by DTG or BIC exposure (50-80%). Uncoupling protein1 (UCP1), which is an essential for a thermogenic process in brown/beige adipocytes, was downregulated by more than 90% compared to no treatment group. In addition, a decrease in UCP1 in brown adipocytes was accompanied by a decrease in cytochrome oxidase complex IV (COX IV) in mitochondria as well as GAPDH, a key glycolytic enzyme. Moreover, estrogen receptor-reporter assay revealed that estrogen-mediated pathway was blocked by DTG by 70%. DOR had no effect on fat differentiation, UCP1 expression, or mitochondrial enzyme activity. In vivo, DTG administration to female mice inhibited oxygen consumption and energy expenditure by 15% without affecting food consumption.

In in vitro models, INSTI exposure had opposite effects on the differentiation of white and brown fat. In brown adipocytes, the inhibition of brown thermogenic function by DTG was associated with interruption of mitochondrial proteins (e.g. COX IV and UCP1), which may be mediated through estrogen receptor. These findings suggest a novel mechanism by which INSTIs may lead to weight gain, especially in women.
**ABSTRACT 102**

**DREADD Activation of Leptin Receptor Positive Neurons in The Nucleus of the Solitary Tract During Obstructive Sleep Apnea in Obese Mice**

Mateus R. Amorim, Thomaz Fleury-Curado, Huy Pho, Carla Freire, David Mendelowitz, Luiz G. S. Branco, Vsevolod Y Polotsky

**Introduction:** Obstructive sleep apnea (OSA) is periodic obstruction of the upper airway during sleep leading to intermittent hypoxemia, hypercapnia, and sleep fragmentation. OSA is highly prevalent, particularly in obesity. It leads to high neurocognitive and cardiovascular morbidity and mortality. The mechanisms alleviating OSA remain unknown. Previous studies have documented the role of leptin, an adipose tissue produced hormone, as a potent respiratory stimulant. A long functional isoform of leptin receptor, LepRb, was detected widely in the brain, especially in cell bodies within the nucleus of the solitary tract (NTS), an important region of the brainstem that processes afferent visceral information and controls breathing. We hypothesized that leptin acts on LepRb+ neurons in the NTS to increase ventilation and maintain upper airway patency during sleep in obese mice. Methods: We selectively expressed AAV8-hSyn-hM3(Gq)-mCherry into the NTS in LepRb-Cre-GFP diet-induced obese (DIO) mice (fed with a high-fat diet for 12 weeks) and examined the effect of DREADD ligand, J60, on genioglossus activity and breathing during sleep. Results: J60 did not stimulate breathing or upper airway in comparison with saline in obese mice during NREM and REM sleep. Conclusion: We conclude that the mechanisms alleviating OSA in DIO mice are independent of LepRb+ neurons in the NTS.

**ABSTRACT 103**

**Hydroxyurea is a potential risk factor for diminished ovarian reserve in young adults with sickle cell anemia**

Lydia H. Pecker, Sarah Hussain, Jaanvi Mahesh, Ravi Varadhan, Mindy S. Christianson, Sophie Lanzkron

Young women with sickle cell anemia (SCA) are at risk for diminished ovarian reserve (DOR), a risk factor for shortened reproductive lifespan and infertility. This study tested the hypothesis that (1) young women with SCA have lower ovarian reserve than age- and race-matched controls and (2) hydroxyurea is a risk for DOR. We measured ovarian reserve using antimullerian hormone (AMH) and antral follicle count (AFC) and obtained demographic, and SCA treatment history. We compared AMH levels in SCA subjects to controls and compared SCA subject characteristics by the presence of DOR (AMH <1.1ng/mL ± FSH > 10 - 40IU). Chi-squared and Mann Whitney U test were used as appropriate. Pilot data is from sixteen women with SCA and median age 25.5 years (IQR 21,29). All had prior hydroxyurea exposure. In subjects aged 19 – 25 years, AMH was lower in subjects than controls [2.48ng/mL vs 7.45ng/mL, p<.001]. 4/16 subjects with DOR currently took hydroxyurea and were >25yo. DOR was associated with lower AFC (5.5(IQR 4, 7) vs 12 (IQR 9,24) oocytes p=0.04) and higher mean corpuscular volume (MCV) (112 vs 92fL, p=0.02), a marker of hydroxyurea adherence. In young adult women with SCA, AMH is lower than in age- and race-matched controls supporting a concern for accelerated decline in reproductive lifespan. DOR is associated with taking hydroxyurea and higher MCV, suggesting that hydroxyurea is a risk for DOR. This data forms the basis for ongoing research on the pathobiological mechanisms and clinical significance of DOR in women with SCA.
ABSTRACT 104

Evaluating Home Healthcare Agency Responsiveness to the Needs of Older Adults in the Era of COVID-19

Maningbe B. Keita Fakeye; Sylvan Greyson; Alicia I. Arbaje; Dawn Hohl; Sarah McGann

Background: Older adults requiring skilled home health (SHHC) services after hospitalization are among those at highest risk of re-hospitalization and adverse events. Home health agencies need strategies to obtain and disseminate safety data to ensure safe transitions, especially during the pandemic.

Methods: Researchers utilized focus groups and semi-structured interviews to (1) understand the impact of the COVID-19 pandemic on hospital-to-SHHC transitions, and (2) identify strategies for ensuring safe transitions. We used purposive sampling to identify key stakeholders: SHHC front-line clinicians, SHHC leadership, older adults, and family caregivers.

Results: Contributors identified four key issues related to the pandemic: (1) fear and risk of viral transmission; (2) personal protective equipment (PPE) availability, training, and policy; (3) lack of caregiver training; and (4) missing equipment and care plan information. More patients and caregivers declined home health services, communicating a fear of potential transmission via home entry. Clinicians expressed confusion around the safety of reusing PPE. Due to social distancing policies, caregivers have not received hospital training to support older adults. Hastened hospital workflows have produced communication breakdowns leading to missing equipment and care plan information.

Conclusion: These findings will inform strategies for developing interventions to ensure safe care transitions.

ABSTRACT 106

HMGA1 Regulates Colon Epithelial Stem Cell Regenerative Function and Induces Distal Colon Tumorigenesis following Exposure to Enterotoxigenic Microbiota

Lingling Xian, Gongbo Guo, Shaoguang Wu, Li Luo, Jawara Allen, Cynthia Sears, Linda M. S. Resar

The High Mobility Group A1 (HMGA1) gene encodes a chromatin regulator and oncogenic protein that is highly expressed in stem cells and diverse tumors, including colorectal cancer. However, targetable mechanisms that induce HMGA1 or mediate its effects have remained elusive. To investigate HMGA1 in colon epithelium, we generated mouse models with varied levels. Modest overexpression of Hmga1 in colon stem cells expands the stem cell pool, increasing goblet cells and colonic mucous. In a DSS injury and inflammation model, Hmga1 promotes repair and tissue regeneration, suggesting that Hmga1 mediates signals from the microenvironment. To examine this further, we tested whether HMGA1 is modulated by the microbiome in mice harboring a heterozygous Apc inactivating mutation (Min+/-) following colonization with human symbiotic bacteria, enterotoxigenic Bacteroides fragilis (ETBF). Importantly, ETBF colonization is linked to human colon cancer and ETBF causes distal colon tumorigenesis in Min+/- mice by releasing E-cadherin to activate Wnt signals. We found that ETBF colonization induces Hmga1 expression. Surprisingly, loss of just a single Hmga1 allele decreases ETBF-induced distal colon tumorigenesis. Studies are underway to identify transcriptional networks and epigenetic alterations governed by Hmga1 in this setting. Together, this work not only provides new insights into the role of HMGA1 in colon epithelial homeostasis by maintaining the stem cell pool and promoting regeneration following injury, but also suggests that HMGA1 can be aberrantly induced by signals from the microbiome to promote tumorigenesis. Our results also highlight interactions between the microbiome and HMGA1 as a promising therapeutic target in colon carcinogenesis.
**ABSTRACT 107**

INCIDENT STENOSIS WITH NO BASELINE CORONARY ARTERY CALCIUM IN THE MULTICENTER AIDS COHORT STUDY

Sudipa Sarkar1, Sabina Haberlen1, Thomas S. Metkus1, Matthew J. Budoff2, Mallory D. Witt1, Lawrence A. Kingsley3, Frank J. Palella4, Wendy S. Post1, Todd T. Brown1

1Johns Hopkins University; Baltimore, MD; 2Lundquist Institute at Harbor-UCLA Medical Center, Torrance, CA; 3University of Pittsburgh; Pittsburgh, PA; 4Northwestern University, Chicago, IL

**Background:** Coronary artery calcium (CAC) is thought to underestimate subclinical coronary atherosclerosis in people with HIV (PWH). PWH are at more likely to have non-calcified plaque, which is not detected by non-contrast CT, which measures CAC. The objective of this exploratory analysis was to determine the association of HIV serostatus with incident moderate to severe coronary artery stenosis in men with HIV (MWH) and men without HIV and zero baseline CAC in the Multicenter AIDS Cohort Study (MACS).

**Methods:** MWH and men without HIV from the MACS with a baseline CAC of zero who underwent a follow-up CT angiogram (CTA) were considered for inclusion. Incident moderate to severe coronary artery stenosis was defined as coronary artery stenosis ≥ 50% at follow-up CTA. A chi-squared test was used to compare categorical variables, and a Student t-test was used to compare continuous variables. The following covariates were compared by HIV serostatus: age, race, study center, systolic blood pressure, antihypertensive medication use, total cholesterol, lipid lowering medication use, and smoking pack years.

**Results:** Of the 272 men included in the study, 160 were MWH and 112 were men without HIV. Of these, 7 participants had incident coronary artery stenosis at follow-up, and all of these participants were MWH. MWH were younger (mean (standard deviation (SD)) 50 (6) years) than men without HIV (mean (SD) 52 (7) years) (P<0.01). Among men without HIV, 57% were Non-Hispanic White and 32% were Non-Hispanic Black, compared to MWH, among whom 33% were Non-Hispanic White and 45% were Non-Hispanic Black (P<0.01). No differences in smoking pack years, hypertension, antihypertensive medication use, hyperlipidemia, lipid lowering medication use were noted by HIV serostatus.

**Conclusions:** Although a CAC of zero is a strong negative predictor for cardiovascular disease in the general population, some men with HIV and a baseline CAC of zero had incident coronary artery stenosis over a 5 year follow-up period.

**ABSTRACT 108**

Natural History of Intraductal Papillary Mucinous Neoplasms: A Prospective Study

Nancy R. Porter*, Margaret G Keane*, Vaishnavi Sawant+, Aadithyaa Raman, Elham Afgani, Queenster Narvey, Sarah Devlin, Atif Zaheer, Elliot Fishman, Ralph H. Hruban, Lindsey L. Manos, Christopher L. Wolfgang, Christopher Shubert, Kelly Lafaro, Richard Burkhart, William Burns, Jin He, Michael Goggins, Marcia I. Canto, Alison Klein*, Anne Marie Lennon*

*Joint first authors * Joint senior authors

**Background:** Pancreatic cancer is the 4th most common cause of cancer mortality with a 5-year survival of only 10%. Intraductal papillary mucinous neoplasms (IPMNs) are a common clinical finding and one of three recognized precursors of pancreatic cancer. Surveillance is recommended for patients with an IPMN, however there is little prospective data on the risk of malignant transformation in IPMNs, and the impact of surveillance on early cancer detection.

**Methods:** Patients with a pancreatic cyst attending our institution between 01/2010 - 12/2019 were prospectively enrolled and followed. Patients with a clinical diagnosis of an IPMN were included in this study.

**Results:** Of the 2422 participants with a pancreatic cyst, 2110 had a suspected IPMN. The median age of patients was 65 (range 18-92) years, 65% were female. There were 140 (7%) individuals who developed high-grade dysplasia or pancreatic cancer during the study period. The median follow-up of the 2110 participants was 12 (range 0 to 117) months and 37 (range 0 to 309) months after their cyst was first detected. Of these, 85% were diagnosed with HGD, stage I or II pancreatic cancer. The risk of progression to high-grade dysplasia or pancreatic cancer was 8% at 5 years and 12% at 10 years after diagnosis.

**Conclusion:** Within a dedicated surveillance program, 85% of patients with an IPMN that underwent malignant transformation were identified with high-grade dysplasia or localized cancer. This contrasts with the SEER data, where only 11% of pancreatic cancer patients had localized disease.
**ABSTRACT 109**

**Electrostatic transducer with tuned mechanical properties for improved body sound sensing**

Valerie Rennoll, Ian McLane, Adebayo Eisa, Mouinya Elhilali, James West

Stethoscopes are widely accessible tools to diagnose disease, but their use is limited outside of traditional hospital settings where overwhelming ambient noise masks body sounds. Typical stethoscope designs contain airborne sound transmission paths that exacerbate ambient noise corruption and decrease the energy transmitted from the body to the device. Noise suppression techniques on digital stethoscopes can decrease noise corruption, but the processing requires additional computational power. Here, we introduce a self-powered, electrostatic transducer with an acoustic impedance specifically tuned to match skin. The impedance matching provides improved pickup of body sounds and greater rejection of ambient noise without additional processing. Microstructures on the surface of an impedance-matched elastomer create small gaps between a deposited electrode and a charged polymer electret film. Mechanical vibrations from the skin compress the gaps and generate an electrical response. The transducer is experimentally evaluated using a body phantom in simulated noise environments and demonstrated to have a sensitivity greater than 2V/N and an SNR improvement of 35 dB. In comparison to both a traditional acoustic stethoscope and a digital stethoscope with noise suppression, the transducer is shown to have a greater coherence with lung sounds and less coherence with ambient noise in simulated noise levels from 65 to 90 dB.

**ABSTRACT 110**

**The Role of Hepatic Inositol Phosphate Multikinase (Ipmk) in Time Restricted Feeding in Mice**

Ik-Rak Jung, Becky Tu-Sekine, Frederick Anokye-Danso, Rexford S. Ahima, Sangwon F. Kim

Obesity is a major public health problem of the U.S. and is associated with diabetes, cardiovascular diseases and other diseases. Most research studies focus on excessive food consumption as the main cause of obesity. However, emerging data indicate that the timing of feeding can have significant effects on body weight and metabolism. Numerous studies in animals and small clinical studies in humans have shown that eating erratically over the 24 hour period or out of phase with the circadian rhythm predisposes toward weight gain, steatosis, dyslipidemia, insulin resistance and diabetes. Furthermore, studies indicate that restricting food intake to the active period synchronizes the circadian rhythm and metabolism, enhances weight loss and improves metabolic outcomes. Time restricted feeding (TRF) increases the amplitudes of clock gene expression and pathways mediating nutrient sensing and hepatic metabolism. However, the mechanisms mediating the effects of TRF are not fully understood. Here we characterized mice (10 week-old) fed a high-fat diet ad libitum (ALF) or from 7 pm to 7 am (TRF) for 2 weeks. The basal glucose production rate was similar between the two groups. Under hyperinsulinemic-euglycemic clamp, the glucose infusion rate (GIR) was significantly greater in TRF group compared to ALF group indicating an increase in insulin sensitivity. Using radioisotopic tracers, we demonstrated that the hepatic glucose production (HGP) was significantly reduced and the glucose disappearance rate was increased in TRF group compared to ALF group. Moreover, a biochemical analyses of liver tissues revealed that Inositol phosphate multikinase (IPMK) act as a key enzyme for inositol polyphosphate biosynthesis and play a role in insulin-, nutrient-, and energy-mediated metabolic signaling, was increased during TRF. Moreover, deletion of IPMK in hepatocytes decreased insulin stimulated AKT phosphorylation while increased lipid accumulation and gluconeogenesis. Importantly, hepatic deletion of IPMK attenuated the beneficial effects of TRF suggesting that IPMK in the liver may contributes to beneficial effects of TRF. Our findings provide the potential mechanism by which TRF confers the beneficial effects and may provide a novel therapeutic strategy for treating diabetes.
THErapeutic agent screening Microfluidic Electroporator (THEME) for precision medicine

Vignesh Chidambaram, Akshay Gupte, Jann-Yuan Wang, and Petros C. Karakousis

As clinicians have gained experience treating patients with

ABSTRACT 111

THErapeutic agent screening Microfluidic Electroporator (THEME) for precision medicine

Hyun Woo Sung, Sung-Eun Choi, Chris H. Choi, Mengxing Ouyang, Srivathsan Kalyan, Nathan Scott, Soojung Claire Hur

Functional assays performed on circulating tumor cells (CTCs) enable longitudinal monitoring for tumor drug resistance and identification of patient-specific targets for precision medicine. However, current approaches involving CTCs for personalized therapy are not compatible with assays requiring viable cells. To address this shortcoming, we describe a vortex-assisted microfluidic electroporation system designed to perform routine, minimally-invasive, personalized drug-efﬁcacy assessments using CTCs from the patient's blood. Gefitinib-resistant non-small cell lung cancer (HCC827 GR6) cells are used as validation for drug assay workflow before clinical translation. Cancer cells are trapped into microscale vortices and subsequently electroporated to deliver biomolecules to the cytosol in a sequential manner for multiplex drug combination assays. Planar microelectrodes are engineered to operate at substantially lower voltage ranges (15-20V) than conventional counterparts (&gt;100V) while maintaining high transfection efficiency, rendering compatibility for sensitive rare cell collection. On-chip design facilitates real-time monitoring of ﬂuorescent biomarker delivery for rapid optimization of cell electroporation parameters. Target delivery efficiency can be quantitatively assessed at single-cell resolution, demonstrating congruity in assays with rare cell counts. Cancer cells spiked into the blood can be puriﬁed without surface marker tags with trace blood contaminants (95 RBC:Cancer Cell and 0.14 WBC:Cancer Cell ratios). HCC827 GR6 cells were electroporated with the highest efﬁciency and viability (77% and 70%, respectively) at 20V. Individual electroporation delivery of Gefitinib and PHA-665752 resulted in viability of 63%, whereas combinatorial sequential delivery resulted in viability of 43% in resistant GR6 cells. These results suggest a potential translational application of the on-chip electroporator tool to bridge the divide between research and clinical pharmacology settings.

ABSTRACT 112

Coronary artery disease worsens while statins improve survival in patients with tuberculosis

Vignesh Chidambaram, Akshay Gupte, Jann-Yuan Wang, and Petros C. Karakousis

Background: Tuberculosis increases the risk of coronary artery disease and myocardial infarction. But the impact of cardiovascular diseases on the levels of inflammation and mortality in tuberculosis is not described. Though pre-clinical models have shown the usefulness of statins in improving tuberculosis outcomes, no clinical data is available currently.

Methods: Through a retrospective cohort study of patients with drug-susceptible tuberculosis, we analyzed the association of coronary artery disease with inflammatory markers and all-cause and infection-related mortality following tuberculosis treatment. The effect of statins on all-cause and infection-related mortality among patients with coronary artery disease were also assessed through regression analysis.

Findings: Among 2902 participants in our study, there were a total of 291 patients (10.1%) with coronary artery disease. Patients with coronary artery disease are associated with higher CRP levels (Adjusted OR 1.45,95%CI 1.05-1.98) and neutrophil-lymphocyte ratio (Adjusted OR 1.29,95%CI 1.10-1.38). All-cause mortality and infection-related mortality were higher in patients with coronary artery disease with adjusted hazard ratios of 1.36[95%CI 1.04-1.81] and 1.45[1.02-2.07], respectively. Among 291 patients with coronary artery disease, 62 patients (21.3%) received statin therapy. At 9 months of TB treatment, statin use was associated with a lower hazard for all-cause mortality (Adjusted HR 0.48,95%CI 0.25-0.93) and infection-related mortality (Adjusted HR 0.49,95%CI 0.21-0.98) in tuberculosis patients with cardiovascular diseases.

Interpretation: Coronary artery disease was associated with higher levels of inflammation and higher hazards of all-cause and infection-related mortality at 9 months. Statin therapy reduced the risk of mortality in patients with cardiovascular diseases.

ABSTRACT 113

Determining COVID-19 Patient Sub-groups and Outcomes via Triaged Predictions and Unsupervised Clustering

Hannah Paris Cowley, Michael Robinette, Jordan K. Matelsky, Daniel Xenex, Matthew Robinson, Scott Zeger, Brian T. Garibaldi, William Gray-Roncal

As clinicians have gained experience treating patients with COVID-19, they report recognizing different groups of patients early in their disease course with distinct risks for adverse outcomes, an observation that may enable more effective patient triage. We apply supervised and unsupervised machine learning, guided by clinical insights, to define COVID-19 patient sub-groups likely to achieve positive, poor, or unpredictable outcomes. We focus on readily-available clinical features during the first 48 hours of hospital admission: self-reported symptoms, comorbidities, vital signs, patient demographics, and blood laboratory test results. We identify sub-groups of patients for whom our triaged prediction approach is particularly well-suited and patients for whom additional information may be required to make high-quality predictions. Our results have the potential to aid clinicians in predicting disease outcome and choosing the appropriate treatment early in COVID-19 disease presentation, as well as to motivate the future study of patients with unexpectedly poor outcomes. Our tools are generalizable, and as additional features become available or patient populations shift, these methods may be easily adapted.
ABSTRACT 114


Shantanu Bailoor, Jung Hee Seo, Stefano Schena & Rajat Mittal

Transcatheter heart valves (THV) suffer from asymptomatic complications like subclinical leaflet thrombosis (SLT) which may result in fatal outcomes for the patient. SLT is detected incidentally during post-implant follow-up and common imaging techniques are either invasive or expose the patient to radiation, prohibiting their regular use. Moreover, the progression mechanism of SLT is not clearly understood and it may develop early or late post implantation. This makes designing a universal anticoagulant therapy difficult. These issues inform a critical need for a non-invasive and non-toxic, continuous monitoring modality of THVs which can alert to the onset of potential adverse outcomes at an early stage. We present an in-silico proof-of-concept assessment for a novel, microsensor-based technology for THVs which can provide persistent and longitudinal monitoring of prosthesis function. We generate a dataset of high-fidelity simulations of blood flow in a canonical aorta with healthy and mildly stenotic aortic valves. We describe changes in hemodynamics in the valve vicinity due to reduced leaflet motion (RLM) associated with asymmetric leaflet thickening. These changes are used to construct hemodynamic signatures of healthy and stenotic valves which can be analyzed to predict individual leaflet mobility. We use supervised learning methods to relate pressure measurements from strategic locations on the stent to the presence and severity of RLM. Our results show RLM can be accurately detected using pressure measurements at as few as two discrete locations per valve leaflet. Future extension of this work will assess the feasibility of such technology in patient-specific aorta models.

ABSTRACT 115

The Mineralocorticoid Receptor-Dependent Transcriptome Reveals Novel Aldosterone Signaling Pathways in the Distal Nephron

Hyun Jun Jung, Xiao-Tong Su, Lama Al-Qusairi, Boyoung Kim, David H. Ellison, Paul A. Welling

The mineralocorticoid receptor (MR, Nr3c2) is responsible for aldosterone-regulation of Na+ and K+ balance and blood pressure. A small group of aldosterone/MR-dependent genes have been identified, but their regulation cannot fully explain how aldosterone activates electrogenic Na+-K+ exchange in the aldosterone sensitive distal nephron (ASDN).

Here, we applied high-throughput transcriptome profiling using RNA-Seq in microdissected ASDN tubules of doxycycline-inducible Nr3c2 gene knockout (MRfl/fl/Pax8-rtTA/LC1) mice to define a comprehensive inventory of MR-dependent genes. After DOX treatment to induce MR KO, four groups were prepared to distinguish between K+ and MR effects: 1) control mice on normal K+ diet (CT-NK) or 2) high K+ diet (CT-HK) and 3) MR knockout mice on normal K+ diet (KO-NK) or 4) low K+ diet (KO-LK).

927 and 2010 differentially expressed genes were identified from comparisons of MR KO-NK vs. CT-NK and MR KO-LK vs. CT-HK, respectively. Diet effects were not detected. All known aldosterone-response genes including Sgk1 were significantly decreased in MR KO-LK compared to CT-HK. Computational identification of genome-wide GR and MR binding sites revealed that 526 of the significantly down-regulated genes in MR KO mice are potential MR-regulated genes. These MR-regulated genes suggest a new mechanism to understand how the Epithelial Sodium Channel (ENaC) is activated by proteolytic cleavage and vesicle trafficking and how aldosterone-dependent sodium reabsorption is tightly coupled to oxidative metabolism.

ABSTRACT 116


Apurva Sharma, Yumin Gao, Alaa Diab, Shireen Khouy, Pauline Huynh, Erin Spaulding, Seth S Martin, Francoise A Marvel

Background: Cardiovascular disease (CVD) remains the leading cause of death in the United States. Digital health interventions (DHI), such as consumer wearables and smartphone applications, have shown promise in promoting cardiovascular health and recovery. However, there are scarce data regarding clinician perspectives on the value and utility of DHI, and on factors promoting adoption of DHI in clinical practice.

Method: In this cross-sectional study, a web-based survey was administered to 88 clinicians directly involved in care of cardiac inpatients across multiple academic health care systems, between January 2020 and February 2021. Participants rated the importance of factors related to DHI use, reported their perceptions of DHI impact on their ability to care for patients with CVD, and shared perspectives on integrating the DHI into patient care. Descriptive statistics were analyzed and summarized as frequencies with percentages using Stata.

Results: 92% (81/88) of respondents believed that DHI would offer advantages in cardiovascular patient care. Increasing patient adherence was reported as the most important benefit of DHI adoption by 37% of clinicians, followed by improvement in the patient-clinician relationship (30%), and enabling remote care (18%). Clinicians under age 40, cardiologists, and internists were the groups more likely to consider DHI important in remote patient care, disease monitoring, and tele-visits, as compared to their counterparts over age 40 or in other clinical specialties.

Conclusion: Our results highlight clinician perspectives on the advantages of DHI and the potential for its adoption for secondary prevention of CVD.
Epigenetic Regulation of E-cadherin in Human Bronchial Epithelial Cells

Bonnie Ho-yee Yeung, Baishakhi Ghosh, Shreeti Thapa, Shyam Biswal, Venkataramana K. Sidhaye

The epithelial lining of the airway is the first barrier against environmental stimuli such as inhaled cigarette smoke (CS), a primary risk factor causing chronic obstructive pulmonary diseases (COPD). The integrity of the epithelial barrier is maintained by a complex of proteins composing different intercellular junctions, including the adherens junction protein, E-cadherin. Previously, we demonstrated that primary bronchial epithelial cells from COPD patients (CHBE) and CS-exposed normal human bronchial epithelial cells (NHBE) display a cellular plasticity with the downregulation of E-cadherin protein (encoded by the CDH1 gene) as compared to non-smoked NHBE. In silico analysis revealed human CDH1 promoter contains 4 CpG islands, thus we hypothesize that DNA methylation is one of the regulatory mechanisms to regulate CDH1 expression in CHBE and CS-exposed NHBE cells. Using bisulfite-cloning sequencing, we found that CDH1 promoter was highly methylated in CHBE and CS-exposed NHBE in CpG islands 3 and 4 in exon 2 as compared to NHBE cells alone. Exposing NHBE cells to CS could induce DNMT1/3A/3B, protein-coding genes that are responsible for DNA methylation. Treating with a DNA demethylating agent 5-aza-2’-deoxycytidine could reverse the decrease in CDH1 gene expression and abrogate the barrier dysfunction in CS-exposed NHBE. Our finding implicates CDH1 methylation is one of the causes for the barrier dysfunction in CS-exposed NHBE. Targeting locus-specific methylation at specific CpG sites is a potential therapy for CHBE and CS-induced dysfunctional changes.

Role of the fluid shear stress sensor TRPM7 in regulating tumor cell intravasation

Kaustav Bera, Christopher L. Yankaskas, Konstantin Stoletov, Soontorn Tuntithavornwat, Panagiotis Mistriotis, John D. Lewi, Miguel A. Valverde, Konstantinos Konstantopoulos

Tumor cell intravasation preferentially occurs in regions of low fluid shear because high shear is detrimental to tumor cell function. Here, we describe a molecular mechanism by which cells avoid high shear during intravasation. The transition from migration to intravasation was modeled using a microfluidic device where cells migrating inside longitudinal tissue-like microchannels encounter an orthogonal channel in which fluid flow induces physiological shear stresses. This approach was complemented with intravital microscopy, patch-clamp and signal transduction imaging techniques. Fluid shear-induced activation of the transient receptor potential melastatin7 (TRPM7) channel promotes extracellular calcium influx that in turn activates RhoA/myosin-II and calmodulin/IQGAP1/Cdc42 pathways to coordinate reversal of migration direction, thereby avoiding shear stress. Cells displaying higher shear sensitivity due to higher TRPM7 expression/activity levels, intravasate less efficiently in vitro and in vivo. This study provides a novel interpretation for the role of shear stress and its sensor, TRPM7, in tumor cell intravasation.
Despite the promise of electronic prescribing, medication errors persist under the current system and can cause serious harm to patients. Patient communication technologies such as After-Visit Summaries (AVS) and Patient Portals (PP) have the potential to mitigate such errors. Improving the accuracy and patient-centeredness of the AVS has been shown to reduce errors and improve patient comprehension. However, current evidence indicates that existing tools often fail to adequately support patients. This ongoing study analyzes qualitative data from a larger project on electronic cancelation messaging following a regimen change. We aim to elucidate current AVS/PP utilization practices and generate recommendations to improve their utility in this context. This pilot analysis examined four interviews each with prescribers and pharmacy staff from the first of three study sites, specifically a General Internal Medicine outpatient clinic and affiliated pharmacy. We also incorporated early observations from patient interviews, which are still under analysis. These initial findings support the existing evidence that both tools (and especially the AVS) are not being utilized to their full potential. We synthesized clinician feedback in order to suggest design changes to the interface that prescribers use to generate and customize the AVS/PP. Based on feedback from pharmacy staff, we also proposed that the information in the AVS/PP be used to communicate with other members of the care team in addition to the patient. Lastly, we explored how the use of these technologies have changed during the COVID-19 pandemic and identified changes that have positively impacted medication management.

**Background:** Approximately 1 in 4 patients with inflammatory bowel disease (IBD) are readmitted within 90 days. To reduce hospitalizations, regular follow-up appointments with gastroenterologists are essential for timely evaluation and optimization of therapies. However, the mean wait time for GI clinic appointments in North America significantly exceeds the target goal of 14 days.

**Methods:** We developed an evidence-based clinic appointment scheduling protocol based on exhaustive literature review. The protocol involved utilizing urgent scheduling slots and streamlined communication between the inpatient gastroenterology (GI) fellows, referring providers, and the dedicated IBD clinic scheduler. The inclusion criteria were adult IBD patients hospitalized within the past 90 days or newly referred seen at the GI clinics of the Johns Hopkins Bayview Medical Center and Greens Spring station. The wait times to clinic appointments with the IBD specialist (primary outcome) were extrapolated from a retrospective chart review. The patient satisfaction data using a 5-Likert scale (secondary outcome) was collected via in-person or Qualtrics surveys. The heterogeneous data at baseline and 12 weeks were analyzed using Mann-Whitney U test to evaluate the efficacy of the scheduling protocol.

**Results:** From February-November 2020, 16 IBD patients were included in the analysis. The mean age was 50.1 ± 17.1 years, and 75% were female. Hospital discharge follow-up appointments accounted for 62.5%. Compared to the pre-test group (median 25.0, IQR 42), the median wait time in the post-test group (median 27.0, IQR 22) improved by 2 days with no statistical difference (p=0.408). The patient satisfaction survey data had a poor response rate (47%) with the minimal change in the post-test group (p=0.533).

**Conclusion:** Although limited by the small sample size and implementation on the single gastroenterologist, implementation of our pilot project suggests potential roles of the appointment scheduling protocol in achieving timely and accessible GI care especially for patients with IBD with high readmission risks. Further studies with a clinic-wide implementation and larger sample sizes are warranted to investigate the effectiveness of the appointment scheduling intervention on timeliness of GI care and possibly clinical outcomes.
### ABSTRACT 121

**Predictors of Response and Effectiveness Of The Addition Of A GLP-1 Agonist To Insulin Therapy In Patients With Type 2 Diabetes**

*Colleen Gavigan, Tom Donner*

**Objective:** Assess the efficacy of a GLP-1 agonist in patients with type 2 diabetes already on insulin therapy and identify factors that may predict a beneficial clinical response.

**Methods:** This is a retrospective analysis of patients with type 2 diabetes who have been treated with insulin and had a GLP-1 agonist (liraglutide, semaglutide or dulaglutide) added. Baseline, 3-, 6- and 12-month data were collected.

**Results:** Eighty-one patients were included with a mean age of 61 years, BMI 34.4 ± 6.6 kg/m², duration of diabetes 16.9 ± 9.6 years, C-peptide 2.0 ± 1.1 ng/ml, HbA1C 8.2 ± 1.5% and daily insulin dose of 82.4 ± 72.3 units at baseline.

Among patients on prandial insulin at baseline, 46% had discontinued it by 1 year. In these patients mean baseline prandial dose (34.4 ± 15.7 units) was lower than that of patients who remained on prandial insulin (58 ± 41.2 units) at 1 year, but not significantly lower. There was no significant difference in change in HbA1C or percentage of patients who came off prandial insulin based on BMI (< or ≥30), insulin dose (< or ≥0.8 units per day) or duration of diabetes (< or ≥10 years).

**Conclusion:** In a cohort of patients with type 2 diabetes on insulin therapy the addition of a GLP-1 agonist reduced HbA1C, weight and insulin requirement. No significant predictor of response to GLP-1 agonists was identified when BMI, baseline insulin dose or diabetes duration were analyzed. Future analyses will include C-peptide as this database is expanded.

### ABSTRACT 122

**Reversing epithelial plasticity to abrogate Chronic Obstructive Pulmonary Disease (COPD) progression**


COPD is the 4th leading cause of death (affecting ~15% of the population) in the USA, with decline in lung function, evidence of poor mucociliary clearance and changes in airway epithelium as well as alveolar destruction. COPD is usually caused by chronic exposure to noxious inhaled pollutants (often cigarette-smoke, CS). The lung epithelium is the site of initial contact with inhaled agents and establishes a protective barrier to limit its access to subepithelial tissues, and this barrier is compromised in COPD. We hypothesize that epithelial plasticity due to repetitive injury is fundamental to COPD pathogenesis and tackling this disruption is required to abrogate and even reverse disease. We have found that both CS injured, and COPD epithelia have disrupted epithelial barrier, reduced ciliary beat function and monolayer height, and a transcriptional shift towards mesenchymal markers. However, our data suggests that plasticity that occurs is distinct from epithelial-to-mesenchymal transition as the monolayer has preserved apical-basal polarity and although motile, they do so with a distinct kinetic energy spectrum suggesting collective and not mesenchymal motion. Our preliminary data suggests that decreasing the polymerized fraction of actin, rescuing an adherens junctional protein, E-cadherin, and modulating the increase in aerobic glycolysis that is induced by CS, improves monolayer integrity. We find a decrease in the actin-binding protein, cofilin-1 as contributing to the plasticity occurring from CS. We anticipate that better defining the metabolic landscape that occurs from lung epithelial plasticity in response to chronic injury and COPD will give rise to novel therapeutic options.
Molecular Underpinning of Sexual Dimorphism in Potassium Regulation
Lama Al-Qusairi, P. Richard Grimm, Ava Zapf, Hyun Jun Jung, and Paul A. Welling

Although the ultimate aim of sexual dimorphism is species survival, differences between genders extend to physiologic processes other than reproduction. Recent studies have described surprising sexual dimorphisms in kidney physiology and disease including electrolyte handling, hypertension, acute and chronic kidney injury.

Renal potassium balance is one major sex-related differences in electrolyte handling. Women exhibit reduced basal plasma potassium (PK) compared to men. The mechanisms and implications of this sex-specific difference have not been yet investigated.

In this present study, we investigate the molecular underpinning of this sexual dimorphism. Similar to humans, we found C57BL6J WT female mice have a lower PK compared to males. C57BL6J males and females were fed control (1% K+) or K+-free diet (KFD) for 8 days. K+ balance and the key proteins involved in renal K+ handling in the distal nephron, site of regulated K secretion, were analyzed. We found males conserve K+ more efficiently than females, and exaggerated urinary K+ loss in response to KFD caused more profound hypokalemia to develop in females. Molecular analysis revealed the major K+ secreting channel in the distal nephron (ROMK) was more abundant in females versus males under control diet, which correlates with lower basal PK in females. We found males were able to efficiently reduce ROMK after 2 days of K+ deficiency while this takes more than 4 days in females. The activation of the epithelial Na+ channel (ENaC), which provides the driving force for K+ secretion, was decreased by KFD intake to the same extent in both sexes. This suggests that the sex-specific K+ regulation determined by differences in ROMK and not ENaC.

In-Silico Analysis of ROMK-positive cell-specific open chromatin regions at the ROMK promoter revealed several binding sites of sterol regulatory element–binding factor2 (SREBF2), a transcription factor expressed in ROMK-positive cells and known to be directly regulated by estradiol.

Together, our data highlight the physiological and molecular mechanisms underlying sexual dimorphism in K+ handling and suggest the ROMK channel as a key player in this process, under a potential regulation of Estrogen.

Tuberculosis Preventive Therapy for Tibetan Refugee Children and Adolescents in India
Kunchok Dorjee, Sonam Topgyal, Tenzin Tsewang, Elizabeth Bonomo, Dekyi Lhadon, Zorba Paster, Dawa Phunkyi, Tsetan D Sadutshang, Richard E Chaisson

Background: Tuberculosis (TB) rates among Tibetan refugee children and adolescents attending boarding schools in India are extremely high. We undertook a comprehensive case finding and TB preventive treatment (TPT) program in Tibetan boarding schools in Northern India.

Methods and findings: A mobile team annually screened children and staff for TB at 7 boarding schools in Himachal Pradesh, India, using symptom criteria, radiography, molecular diagnostics, and tuberculin skin tests. TB infection (TBI) was treated with short-course regimens of isoniazid and rifampin or rifampin. TB disease was treated according to national guidelines. Between April 2017 and December 2019, 6,582 schoolchildren (median age 14 years) were enrolled. Over 13,161 person-years of follow-up, 69 TB episodes occurred in schoolchildren, yielding annual incidence rates of 524/100,000 (95% CI 414–663/100,000) child-years. Of 1,412 schoolchildren diagnosed with TBI, 1,192 received TPT. Schoolchildren who received TPT had 79% lower risk of TB disease (adjusted hazard ratio [aHR] 0.21; 95% CI 0.07–0.69; p=0.010) compared to non-recipients. Protection was greater in recent contacts (aHR 0.07; 95% CI 0.01–0.42; p=0.004). Overall, between 2017 and 2019, TB disease incidence decreased by 87%, from 837/100,000 (95% CI 604–1,129/100,000) person-years to 110/100,000 (95% CI 36–255/100,000) person-years (p<0.001).

Conclusions: Following implementation of a school-wide TB screening and preventive treatment program, we observed a significant reduction in the burden of TB disease. The benefit of TPT was particularly marked for recent TB contacts. This initiative may serve as a model for other communities affected by TB.
**ABSTRACT 125**

**Self-latching drug delivery devices**

Arijit Ghosh, Ling Li, Liyi Xu, Ranjeet P. Dash, Neha Gupta, Jenny Lam, Qianru Jin, Venkata Akshintala, Gayatri Pahapale, Wangqu Liu, Anjishnu Sarkar, Rana Rais, David H. Gracias, Florin M. Selaru

Extended-release drug delivery systems delivered to the gastrointestinal (GI) tract can significantly increase the ease of administration of drugs and consequently adherence to therapeutics. This has led to significant progress in the design and development of gels and porous substrates to slow down the drug release. However, a significant challenge for extended release oral/rectal delivery is the normal GI motility, which eliminates the drug delivery device from the GI tract and therefore limits the duration of drug release. To counter this precise limitation, a variety of gastric resident devices such as mucoadhesive patches or particles have been developed with some success. We have developed active, self-latching, Theragrippers, which are inspired by hookworms and are capable of residing within the GI tract. Our sub-millimeter scale theragrippers have multiple digits with sharp tips and are powered by residual stress actuators, which are triggered by body temperature. They can function as self-latching drug delivery tools in any part of the GI tract. Using a combination of in vitro, ex vivo and in vivo models, we could demonstrate that the theragrippers fold with enough force to allow significant penetration into the mucosa which results in GI retention in vivo for 24 hours. To evaluate our extended release platform, we utilized the analgesic ketorolac as a model drug. Upon rectal delivery, the theragripper-mediated ketorolac delivery results in a six-fold increase in the elimination half-life of the drug. These results suggest that shape changing drug delivery devices, that latch onto the mucosa, could significantly enhance the efficacy of extended drug delivery.

**ABSTRACT 126**

**Associations of demographic and socioeconomic characteristics with fitness tracker ownership: Results from the Multi-Ethnic Study of Atherosclerosis (MESA)**

Resham J. S. Patel, Jie Ding, Francoise A. Marvel, Rongzi Shan, Timothy B. Plante, Michael J. Blaha, Wendy S. Post, Seth S. Martin

**Background:** Mobile health technologies can be applied for the prevention of cardiovascular disease (CVD), but a digital divide along demographic and socioeconomic boundaries has been identified for internet access and computing device ownership. This study aimed to evaluate if a similar divide exists for fitness tracker ownership. This study aimed to evaluate if a similar divide exists for fitness tracker ownership. This study aimed to evaluate if a similar divide exists for fitness tracker ownership.

**Methods:** The Multi-Ethnic Study of Atherosclerosis (MESA) is a population-based observational cohort study that includes participants free of CVD at baseline in 2000-2002. A follow-up phone survey was administered in 2019-2020. Multivariable logistic regression modeling was used to assess associations of age, sex, race/ethnicity, education level, annual family income, and health insurance status with fitness tracker ownership in 2019-2020.

**Results:** Among 2452 individuals (mean [SD] age 74.2 [8.7], 54% women, 25% Black, 21% Hispanic, 14% Chinese), fitness tracker ownership was reported by 9% (n=230) of participants. Lower odds of fitness tracker ownership were seen with older age (odds ratio 0.94 per 1 year increase; 95% CI 0.92-0.96), male sex (0.71; 0.53-0.95), Hispanic ethnicity (vs. White 0.55; 0.33-0.89), Chinese race (vs. White 0.46; 0.26-0.76), and annual family income less than $35,000 (vs. $75,000 0.47; 0.31-0.72). No significant associations were observed for Black participants (vs. White 1.00; 0.58-1.68) or with education level or health insurance status.

**Conclusion:** Important demographic and socioeconomic characteristics were associated with fitness tracker ownership, indicating the presence of a digital divide. Mobile device-based health interventions need to address these inequities.
Dermal fibroblast morphology and Collagen alignment as aging biomarker.

Kyu Sang Han, Pei-Hsun Wu, Denis Wirtz

Aging is a complex biological process that involves phenotypic changes in resident stromal cells and structural changes in extracellular matrix composition. Recent works identified dermal fibroblast cell morphology and inherent in vitro heterogeneity as biomarkers of aging. Here, we further evaluate the effect of aging in cellular/nuclear morphology in situ. We investigated the morphology of dermal reticular fibroblasts in a cohort of 89 skin tissue samples collected from individuals between 13 and 92 years old. We developed supervised deep learning models to locate dermal fibroblasts in H&E stained skin tissues and semantically segment the cell. We show that the aspect ratio of dermal reticular fibroblast nuclei increases proportionally with the age of donors. Our results further show a positive correlation between skin fibroblast nuclear aspect ratio of nuclei and local collagen fiber alignment. Our study also reveals that collagen structure is associated with aging. Together, our findings suggest that dermal reticular fibroblast nuclei morphology along with its microenvironment may be used as biomarkers of aging.

SARS-CoV-2 ORF3a Activates the NLRP3 Inflammasome

Kimberly E. Rousseau, Alexis Figueroa, Guido Massaccesi, Michael A. Chattergoon

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the agent of coronavirus disease 2019 (COVID-19), began circulating in 2019 and has since spread to pandemic levels. Clinical studies into the inflammatory syndrome in critically ill COVID-19 patients have highlighted aberrant immune responses and levels of pro-inflammatory cytokines. Among the dysregulated cytokines are interleukin (IL)-18 and IL-1β. These cytokines are produced after the activation and assembly of inflammasomes, multi-protein structures of the innate immune system that recognize and respond to viral infection, tissue damage, and other stimuli. The purpose of this study is to define the viral trigger for inflammasome activation by SARS-CoV-2 and to understand its mechanism for activation.

In this study, we employed a transfection reconstitution system to study SARS-CoV-2 for inflammasome activation. Plasmids encoding the inflammasome components and each SARS-CoV-2 protein of interest were co-transfected, allowing us to probe the relationship between these viral proteins and the NLRP3 inflammasome.

We report that the accessory protein open reading frame (ORF) 3a is sufficient to trigger assembly of the NLRP3 inflammasome and production of mature IL-18. MCC950, a selective small molecule inhibitor of the ATPase function of NLRP3, reduces IL-18 production in response to these viral proteins to mock levels.

The identification of SARS-CoV-2 ORF3a as the viral protein responsible for NLRP3 inflammasome activation is an important step in understanding the inflammatory response seen in severe COVID-19. Further study into the mechanism of this event has the potential to highlight clinical targets for drug interventions in severe cases of the disease.

Micro- and Nanosciences-Enabled Diagnostic Tools for COVID-19 and Beyond

Author: Jeff Wang; Principal investigator: Jeff Wang; Trainees submitting abstracts as an example of the depth of research in the lab: Fan-En Chen, Joon Soo Park, Alexander Trick, Pengfei Zhang

The COVID-19 pandemic underscores urgent but unmet needs for rapid and accurate point-of-care diagnostics. In response, my research team has been leveraging micro- and nanosciences and collaborating with researchers at the School of Medicine to develop point-of-care diagnostics for both SARS-CoV-2 testing and antigen testing through 4 projects. First, we have developed a portable and fully-integrated device that automates magnetic-based viral RNA purification and ultrafast and multiplexed qRT-PCR for the detection of SARS-CoV-2, Influenza A, and Influenza B in 8lt; 30 min with minimal manual operation. Second, we have developed a streamlined immune-PCR assay and have implemented the assay in another portable device to achieve rapid, sensitive, and simple-to-use antibody testing for COVID-19. Third, we have transformed a novel CRISPR-based assay from the traditional tube-based format to a digital detection format, akin to digital PCR. This digital CRISPR-based assay demonstrates more sensitive, more quantitative, and faster detection of SARS-CoV-2 than traditional tube-based assay.

Finally, we have integrated the CRISPR-based SARS-CoV-2 detection assay with our portable device to bring this novel detection method to the point of care. Importantly, these technologies can be readily applied to other diseases such as cancer and sexually transmitted infections. Together, these 4 collaborative projects highlight our research efforts in developing potentially transformative diagnostic technologies for COVID-19 and beyond.
The First Increase in Live Kidney Donation in The United States in 15 Years
Fawaz Al Ammary MD PhD, Yifan Yu MHS, Alexander Ferzola BA, Jennifer D. Motter MHS, Allan B. Massie PhD MHS, Sile Yu MD MPH, Alvin G. Thomas MSPH, Deidra C. Crews MD ScM, Dorry L. Segev MD PhD, Macey L. Henderson JD PhD, and Abimereki D. Muzaale MD MPH

Background: After more than a decade of decline, the first sustained increase in live kidney donation was observed in the US from 2017 to 2019. Understanding these trends in donation may provide opportunities to effectively sustain or even enhance this recent increase in donors.

Methods: We conducted a national registry study of 35,900 donors (70.3% white, 14.5% Hispanic, 9.3% black, 4.4% Asian) to understand the increase in 2017-2019 vs. 2014-2016 using Poisson regression stratified by donor-recipient relationship (biologically related, unrelated, and kidney paired donors).

Results: Among biologically related donors aged <35, 35-49, and ≥50 years, the number of donors did not change across race/ethnicity but increased by 38% and 29% for Hispanic and black ≥50. Among unrelated donors &lt;35, 35-49, and ≥50, white donors increased by 18%, 14%, and 27%; Hispanic donors &lt;35 did not change but increased by 22% and 35% for 35-49 and ≥50; black donors &lt;35 declined by 23% and did not change for 35-49 and ≥50; Asian donors did not change. Among kidney paired donors &lt;35, 35-49, and ≥50, white donors increased by 42%, 50%, and 68%; Hispanic donors &lt;35 and 35-49 increased by 36% and 55% and did not change for ≥50; black donors did not change; Asian donors &lt;35 did not change but increased by 107% and 82% for 35-49 and ≥50.

Conclusion: The increase in live kidney donation was driven predominantly by unrelated and paired white donors. Donation among unrelated black individuals should be promoted.

DISTRIBUTION OF LONG-ACTING (LA) CABOTEGRAVIR (CAB) IN PLASMA, MUCOSAL TISSUES, AND ASSOCIATED FLUIDS AFTER A SINGLE ULTRASOUND-GUIDED INTRAMUSCULAR (IM) INJECTION IN HEALTHY ADULT PARTICIPANTS
Ethel D. Weld, Jafar Sadik Shaik, Stacey Edick, Edward Fuchs, Sharon Riddler, Mark Marzinke, Ronald D’Amico, Kalpana Bakshi, Yu Lou, Craig Hendrix, Kelong Han, Susan L. Ford, David Margolis, William Spreen, Parul Patel, Craig Hendrix

Aim: Cabotegravir is an integrase strand transfer inhibitor in clinical development as long-acting (LA) injectable for HIV preexposure prophylaxis.

Methods: This phase I study assessed pharmacokinetics of cabotegravir LA in plasma and anatomical sites associated with sexual HIV-1 transmission after repeat oral and single intramuscular (IM) dosing in healthy adults. Following a 28-day oral lead-in period of cabotegravir 30 mg and a washout period of 14 to 42 days, participants were administered a single, ultrasonographic-guided gluteal IM cabotegravir LA 600-mg injection. Primary endpoint was cabotegravir concentrations through Week 12 after IM injection in plasma, cervical, vaginal, and rectal tissues and cervicovaginal and rectal fluids. Secondary endpoints included cabotegravir tissue-/fluid-to-plasma concentration ratios.

Results: Nineteen participants enrolled and 16 completed the study. Cabotegravir was detected in plasma and all tissues and fluids. Median plasma cabotegravir concentrations exceeded 1x protein-adjusted 90% maximal inhibitory concentration through Week 12. Median tissue-/fluid-to-plasma cabotegravir concentration ratios across all visits were 0.32 for rectal fluid and 0.08 to 0.16 for other tissues/ fluids. Pearson correlations between cabotegravir concentrations in plasma and cervical, vaginal, and rectal tissues were 0.94, 0.92, and 0.96, respectively. Injection-site reactions were common (88% of participants) and were mostly grade 1 in intensity (82%). Two participants reported 11 serious adverse events, which were unrelated to study treatment.

Conclusion: Concentrations of cabotegravir in tissues and fluids were proportional to plasma over time, with strong correlations between tissue and plasma concentrations. Cabotegravir LA tissue-to-plasma ratios from this study may be important for evaluating its efficacy for pre-exposure prophylaxis.
**ABSTRACT 132**

**QTc Interval Prolongation and In-hospital Mortality Among Patients Hospitalized with Covid-19**

Nino Isakadze, Marc Engels, MD, PhD, Dominik A Beer, DO, M. Scott Binder, MD, Anjali Wagle, MD, Rebecca McClellan, MS, CGC, Lisa Yanek, MPH, Bahareh Modanloo, Hugh Calkins, MD, FHRS and Andreas S. Barth, MD, PhD. Johns Hopkins University, Baltimore, MD

**Background:** QT interval prolongation is linked to an increased risk of life-threatening ventricular arrhythmias. The risk factors contributing to QT prolongation in patients with COVID-19 remain poorly defined.

**Objective:** To characterize risk factors associated with prolonged QTc interval among patients hospitalized with COVID-19.

**Methods:** We performed a retrospective study examining the QTc on the admission ECG of 2,456 hospitalized adult patients admitted for treatment of acute COVID-19 infection. Patients with atrial fibrillation or QRS widening >120ms were excluded.

**Results:** A prolonged and severely prolonged QTc was found in 34.28% and 4.23% of patients, respectively. In-hospital mortality in patients with a normal, prolonged, and severely prolonged QTc interval was 7.48%, 11.28%, and 17.31%, respectively (p<0.001 for trend). Factors associated with QTc prolongation were older age (57 vs. 61 vs. 63 yrs, p<0.001), CRP levels (9.2 vs. 12.7 vs. 12.3; p=0.002), ferritin levels (562 vs. 665 vs. 844, p = 0.006), pro-BNP levels (108 vs. 314 vs. 1064; p<0.001), female sex, presence of chronic lung or kidney disease, diabetes mellitus, heart failure, and hypertension. No statistically significant associations of QTc prolongation were found for race, body mass index, coronary artery disease, troponin I levels, TSH, and liver function.

**Conclusion:** QTc prolongation on admission ECG is common and associated with an increase in-hospital mortality, older age, higher inflammatory cytokine levels, heart failure, and presence of renal and pulmonary disease. Future studies are required to determine the utility of QTc interval on the admission ECG for risk stratification of patients hospitalized with COVID-19.

**ABSTRACT 133**

**Elevated Inflammatory Markers are Associated with Prolongation of the QTc Interval in Hospitalized Patients with Covid-19**

Nino Isakadze, Marc Engels, MD, PhD, Dominik A Beer, DO, M Scott Binder, MD, Anjali Wagle, MD, Rebecca McClellan, MS, CGC, Lisa Yanek, MPH, Bahareh Modanloo, Hugh Calkins, MD, FHRS and Andreas S. Barth, MD, PhD. Johns Hopkins University, Baltimore, MD

**Background:** QT interval prolongation increases the risk of life-threatening arrhythmias. Whether inflammation contributes to significant QTc prolongation in patients hospitalized with COVID-19 is currently unknown.

**Objective:** To evaluate QTc interval changes in the setting of acute elevation in inflammatory cytokine levels.

**Methods:** In this retrospective cohort study of hospitalized adults with COVID-19 infection, we evaluated patients who had serial and paired measurements of ECG and inflammatory markers. We included patients with elevation of CRP or ferritin levels at least five times above the upper limit of normal ("high") and the other value less than fifty percent of the peak value ("low"). We identified 93 and 42 patients in CRP and ferritin sub-cohorts, respectively.

**Results:** Mean ages were 59±16 and 56±14 years in the CRP and ferritin sub-cohorts, respectively. QTc interval was on average 23ms higher (462±51.2 vs. 439.1±36.0ms, p<0.001) when CRP level was high vs. low and 20ms higher (385.2±77.3 vs. 365.2±36.5ms, p=0.014) with ferritin high vs. low. WHO COVID-19 severity scale was elevated with higher inflammatory markers (CRP: 5.3±1.5 vs 4.7±1.6, p<0.001; ferritin: 5.6±1.4 and 4.5±1.5, p<0.001). No statistically significant differences were found for the number of QTc prolonging medications, creatinine level, potassium level and PR or QRS duration.

**Conclusion:** A transient QT interval prolongation is observed with an acute rise in inflammatory cytokine levels. Systemic inflammation should be considered as an important QTc prolonging condition which could contribute to the multifactorial QTc prolongation observed in patients with COVID-19.
**ABSTRACT 134**

**Prediction of Physiological Deterioration and Mortality in Mechanically Ventilated Patients Admitted to the ICU**

*Madi Kusmanov, Yunru Chen, Andy S. Ding, Morgan Sanchez, Shreyash Sonthalia, Timothy Bedard, Pedro Mendez-Tellez*

**Background:** Recent studies have shown that the degree of lung damage in ventilator-induced lung injury patients depends on the mechanical power (MP) of the ventilator, which describes the energy delivered by the ventilator per unit time. This study aims to build machine learning models that leverage knowledge of ventilator settings to predict morbidity and mortality in ventilated patients.

**Methods:** Patient data from the Phillips eICU Database were used to build predictive models. Inclusion criteria include age ≥18 years, ICU stay duration ≥48 hours, volume- or pressure-controlled mechanical ventilation, and ventilation duration ≥48 hours. Physiological deterioration was defined as any increase between daily Sequential Organ Failure Assessment (SOFA) scores within the first 7 days of ventilation. Classification models including logistic regression, random forest, and support-vector machines were evaluated for predicting mortality and physiological deterioration using area under the receiver operating characteristic curve (AUC).

**Results:** A total of 846 patients were included in this study. Preliminary models showed predictive power for deterioration defined using total SOFA scores (AUC: 0.65). For organ-specific deterioration, these models exhibited higher performance in predicting renal deterioration (AUC: 0.76) and pulmonary deterioration (AUC: 0.73). Prediction of mortality resulted in an AUC of 0.71.

**Conclusions:** Based on our preliminary data, these predictive models have the potential to be used in real-time to enable early clinician intervention for patients likely to deteriorate or expire. Implementation of additional physiological data as well as patient history is expected to further improve the performance of these models.

**ABSTRACT 135**

**Voice Analysis for COVID-19 Detection**

*Drew Grant, Ian McLane, Jim West*

The rapid spread and high mortality rate of the COVID-19 pandemic has created strong demand for rapid testing alternatives that are low cost, have a very high throughput, and are accessible to patients in their own homes. In the past, several studies have used signal processing and machine learning techniques to detect respiratory illnesses through speech and cough analysis, with varying degrees of success. We hypothesized that these signal processing and machine learning techniques could be expanded to accurately analyze cough and speech sounds for rapid detection of COVID-19 directly on patients’ smart phones. Using an online crowdsourced recordings database launched in India, called Project Coswara, we present preliminary results that show the system obtains an 89% balanced accuracy in detecting COVID-19 through cough analysis and an 80% balanced accuracy detecting COVID-19 through speech analysis. These two analysis processes are combined into a single classification system using a novel multiclass sound event detector, which discriminates between coughs, speech, and other ambient sounds in noisy environments with 92% balanced accuracy. We validate this model through the combination of other crowdsourced and web-scraped databases and to test the robustness of a real-time continuous COVID-19 detector deployable on smart phones.
**Validation of Automated CT Temporal Bone Segmentation for Applications in Neurotologic Surgery**

*Andy S. Ding, Alexander Lu, Zhaoshuo Li, Jeffrey H. Siewerdsen, Russell H. Taylor, Francis X. Creighton*

**Background:** Autonomous and semi-autonomous surgical robots have potential to improve surgical safety in neurotology. However, such methods require efficient and accurate segmentations of pre-operative imaging for registration to patient anatomy. This study investigates the accuracy of an automated method to segment relevant temporal bone anatomy in cone-beam CTs.

**Methods:** We developed an automated pipeline around the Symmetric Normalization registration method, which predicts segmentations of a new image based on a labeled atlas. To evaluate accuracy, we manually segmented 17 high resolution cone-beam CT images of the temporal bone, labeling each voxel corresponding to an anatomical region (e.g., ossicles, labyrinth, facial nerve, dura, etc.) with a different tag. Predicted segmentations were compared against manual segmentations using average Hausdorff distance and Dice coefficient. Runtime was documented to determine computational requirements of this method.

**Results:** Average Hausdorff distances and Dice coefficients between predicted and groundtruth segmentations were as follows: malleus [0.106±0.007 mm, Dice: 0.836±0.014], incus [0.129±0.029 mm, Dice: 0.846±0.029], stapes [0.257±0.081 mm, Dice: 0.877±0.072], labyrinth [0.175±0.081 mm, Dice: 0.847±0.063], and facial nerve [0.761±0.413 mm, Dice: 0.551±0.101]. A 24-core machine with 8 GB RAM completed one registration in 5 minutes.

**Conclusions:** We demonstrate submillimeter accuracy for automated segmentation of temporal bone anatomy compared to hand-segmented groundtruth labels using our template registration pipeline. This method is not limited by training data volume that plagues more complex deep learning models. Rapid runtime and low computational requirements further underscore this method's translational potential.

---

**Rapid Point-of-Care Immuno-PCR for COVID-19 Serological Testing**

*Pengfei Zhang, Liben Chen, Jiumei Hu, Alex Trick, Fan-En Chen, Kuangwen Hsieh, Yang Zhao, Branch Coleman, Kate Kruczynski, Christopher D. Heaney, Bill Clarke, and Tza-Huei Wang*

Serological tests play important roles in the fight against the Coronavirus Disease 2019 (COVID-19). Standard serological testing assays such as enzyme-linked immunosorbent assays (ELISA) and chemiluminescent immunoassays (CLIA) provide reliable and sensitive antibody detection but require significant laboratory infrastructure and lengthy assay time. Conversely, lateral flow immunoassays (LFIA) are suitable for rapid point-of-care tests but lack sensitivity. In response, we have developed a rapid and sensitive point-of-care immuno-PCR that can address the current gap in serological testing. To do so, we developed a novel magnetic-based, single-binding, and one-wash immuno-PCR assay and implemented this assay in a palm-sized magnetofluidic device that automates and accelerates the assay to 30 min. Within the automated magnetofluidic device, a programmable magnetic arm captures and transports magnetically-captured antibodies to assay reagents pre-loaded in a companion plastic cartridge, and a micro-scale thermocycler and a fluorescence detector perform ultrafast real-time PCR to detect the antibodies. We evaluated our point-of-care immuno-PCR with 107 clinical serum samples and achieved 98.3% specificity (n = 59) and 93.8% (30-min assay time) or 100% (45-min assay time) concordance with benchtop CLIA assays (n = 48). Our point-of-care immune-PCR thus offers a potentially powerful tool for serological testing for COVID-19 and beyond.
Overexpression of the orphan G-protein-coupled receptor GPRC5B leads to cell toxicity: implications for the pathophysiology of heart failure

Miguel Pinilla-Vera, Oscar Reyes Gaido, Olurotimi Mesubi, Mark Anderson

Rationale: heart failure affects 6.2 million Americans and this number is expected to increase to over 8 million by 2030. Despite a growing therapeutic armamentarium and major progress in understanding its pathophysiology, heart failure has high mortality and a profound negative impact in the quality of life of patients. Thus, it is imperative to study new molecular pathways implicated in the development of heart failure to prevent and treat heart failure more effectively. O-GlcNAcylation is a key posttranslational modification in which the enzyme OGT (O-GlcNAc transferase) adds γ-D-N-acetylglucosamine to serine/threonine residues. A complementary enzyme, OGA (O-GlcNAcase) removes O-GlcNAcylation. Importantly, increased O-GlcNAcylation is seen in the myocardium of patients with heart failure. Our group recently reported heart failure in mice with increased myocardial O-GlcNAcylation due to over expression of OGT in cardiomyocytes, indicating that excessive O-GlcNAcylation can be a cause of heart failure. Remarkably, cardiac dysfunction was repaired by crossing the OGT mice with mice over-expressing OGA. We performed an unbiased RNA-Seq analysis of OGT, OGA, OGTx-OGA and WT mouse hearts, and found that the expression of the orphan G-protein coupled receptor GPRC5B was significantly elevated in the failing myocardium of OGT transgenic mice in comparison to WT mice, and its expression level was reduced to that of the WT mice in the hearts of the rescued OGT mice after crossing with OGA mice.

Hypothesis: we hypothesize that GPRC5B mediated signaling plays a central role in the regulation of cardiomyocyte function and that increases in GPRC5B expression contribute to the development of heart failure.

Methods: GPRC5B-isoform specific expression in mouse whole heart and neonatal mouse ventricular myocytes (NMVMs) was performed by real-time quantitative PCR using isoform-specific primers. Human embryonic kidney cells (HEK293) and AC16 human cells, a cardiomyocyte-like cell line were transfected with a mGPRC5B-myc plasmid under control of CMV promoter or an IMHC-hGH empty vector. Cell viability was determined using MTT assay during baseline cell culture conditions. Stable AC16 cells overexpressing mGPRC5B under inducible control by doxycycline were generated by lentiviral transduction containing a pLVX-TRE3G-mcherry-IRES-mGPRC5B construct bearing a puromycin resistance gene. Cell proliferation assays were generated using CellTiter-Glo (Promega) following the manufacturer protocol under basal cell culture conditions. NMVMs from wild-type C57Bl6/J mice were obtained (Milteyi Biotec) according to the manufacturer protocol. shRNA specific for mGPRC5B was generated using a pLV-mcherry-shRNA lentiviral construct. A transgenic mouse with cardiomyocyte-specific overexpression of mGPRC5B was generated by pronuclear injections of linearized mouse GPRC5B (transcript NM_022420.2) cDNA fused with an N-terminal myc epitope tag. The resulting construct was cloned into the αMHC-hGH vector for myocardial expression. The embryos were implanted into pseudo-pregnant females to generate C57Bl6/J mice.

Results: isoform 1 and 2 of GPRC5B are expressed in WT-adult mice with baseline expression of isoform 2 around 10-fold over isoform 1. NMVMs demonstrate relatively high expression of isoform 2 of GPRC5B, around 100 fold higher than isoform 1. HEK293 cells and AC16 human cells exhibit decreased viability after transfection of mGPRC5B plasmid in comparison with non-transfected cells and cells transfected with an empty IMHC-hGH vector. AC16 cells stably expressing mGPRC5B under doxycycline induction demonstrated decreased proliferation in comparison to untransduced cells in absence of doxycycline. Transduction of NMVMs of WT mice with mGPCR5-mcherry lentivirus resulted in 53% reduction in the expression of mGPCR5B. Mice with cardiomyocyte-specific overexpression of mGPCR5B appear to develop normally in utero and reaching adulthood in similar proportions to their WT littermate counterpart.

Conclusions: overexpression of mGPCR5B in two human cell lines results in decreased viability and cell proliferation. The molecular and signaling mechanisms behind this phenotype are unknown. We have generated several molecular tools, including a transgenic mouse with cardiomyocyte-specific overexpression of GPRC5B to further dissect these molecular pathways.