69th Annual Meeting
of the
MGH Scientific Advisory Committee

SAC 2016

April 6 and 7, 2016
Simches Auditorium
185 Cambridge Street, 3rd Floor

Poster Session Abstracts

MASSACHUSETTS GENERAL HOSPITAL
RESEARCH INSTITUTE

Executive Committee on RESEARCH | *Fostering Innovation at MGH*

Contents

SAC 2016

Wednesday, April 6, 2016

Annual Celebration of Science at MGH

10:15 am–1:45 pm / Simches, Floors 2 & 3

SAC 2016 Poster Session (lunch available)

Session 1: 10:15–11:45 am

Session 2: 12:15–1:45 pm

2:00–5:00 pm / Simches 3.110

Scientific Presentations

Welcome

Peter L. Slavin, MD, President, MGH

Opening Comments, Introductions, and 2016 MGH Research Scholars

David N. Louis, MD, Chair,
Executive Committee on Research (ECOR)

2:15–3:00 pm

ECOR Report

David N. Louis, MD, Chair, ECOR

3:00–3:30 pm

2016 Martin Prize for Fundamental Research

Filip K. Swirski, PhD

Interleukin-3 amplifies acute inflammation and is a potential therapeutic target in sepsis

3:30–3:50 pm

Break

3:50–4:20 pm

2016 Martin Prize for Clinical Research

Andrew T. Chan, MD

Association of aspirin and NSAID use with risk of colorectal cancer according to genetic variants

4:20–4:50 pm / Simches 3.110

2016 Goodman Award

Robert Anthony, PhD

Glycan regulation of immunoglobulins

5:00–6:00 pm / Simches Cafe, Floor 3

Reception

SAC 2016

Thursday, April 7, 2016

Annual Celebration of Science at MGH

8:00–9:00 am / Simches 3.120

Executive Breakfast

9:00–9:30 am / Simches 3.110

Keynote Address—Thematic Centers: Experiments in Science

Daniel Podolsky, MD

President, University of Texas Southwestern Medical Center

9:30–10:15 am

Thematic Center Survey Results

David N. Louis, MD

Chair, ECOR

10:15–10:45 am

Wellman Center for Photomedicine

R. Rox Anderson, MD

10:45–11:00 am

Break

11:00–11:30 am

Center for Systems Biology

Ralph Weissleder, MD, PhD

11:30 am– 2:00 pm

Center for Computational and Integrative Biology

Brian Seed, PhD

12:00–1:30 pm / Simches 3.120 & 3.130

Lunch—SAC Members with invited Faculty

1:30–2:00 pm

Center for Regenerative Medicine

David Scadden, MD

2:00–2:30 pm

Center for Human Genetic Research

Sekar Kathiresan, MD

2:30–3:15 pm

MGH Thematic Centers: Open Discussion

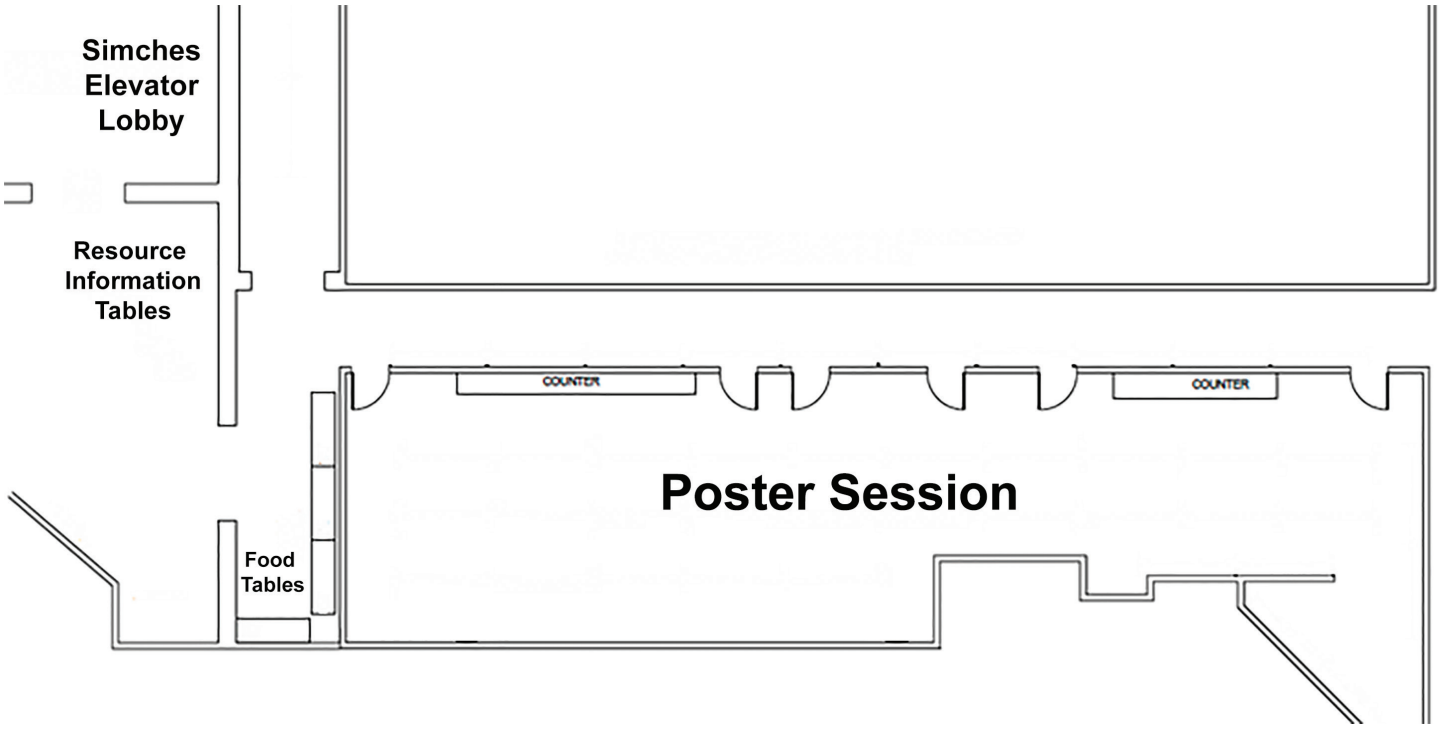
3:15–3:45 pm / Simches 3.120

Executive Discussion (SAC Members only)

3:45–4:15 pm

Debriefing (SAC Members and ECOR Leadership)

Poster Session Floor Plan: Simches 2nd Floor–SERI



SAC 2016 Poster Categories and Session Breakdown

Session 1

Simches 2nd Floor—SERI

Bioengineering and Devices	1–5
Bioinformatics, Technology & Innovation	6–10
Biomedical Imaging	11–19
Cancer	20–46
Cardiovascular	47–51
Cellular Biology	52–55

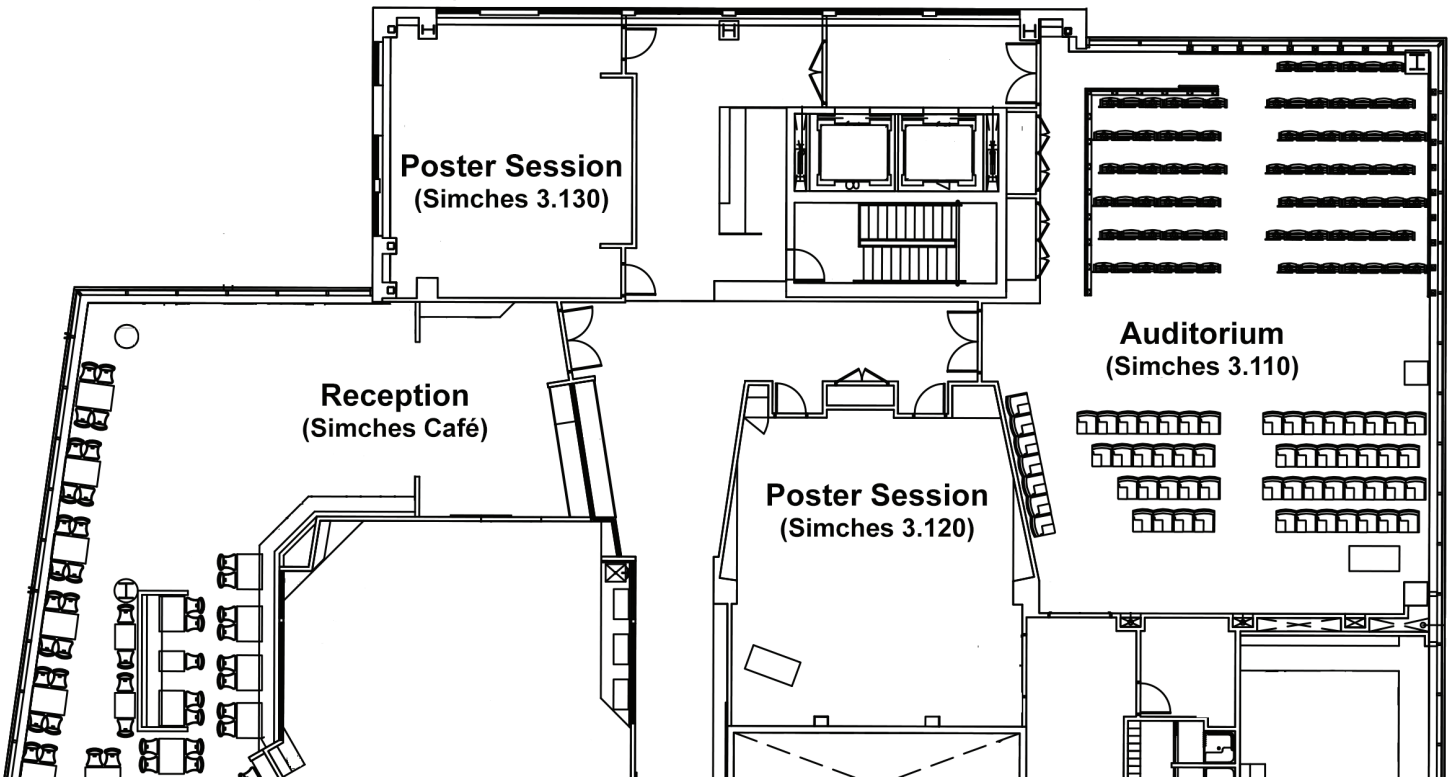
Simches 3rd Floor—Room 3.120

Cellular Biology	56–61
Community Health Population Research	62–64
Endocrinology	65–74
Health Disparities/Equities Research	75–78

Simches 3rd Floor—Room 3.130

Health Services and Policy Research	79–82
Immunology/Inflammation	83–88

Poster Session Floor Plan: Simches 3rd Floor



SAC 2016 Poster Categories and Session Breakdown

Session 2

Simches 2nd Floor—SERI

Simches 3rd Floor—Room 3.120

Simches 3rd Floor—Room 3.130

The James P. Timilty Middle School Students

We would like to acknowledge the hard work of the students whose work is on display today. The school's participation is coordinated by the MGH Youth Programs Team in the MGH Center for Community Health Improvement (CCHI). Students are matched with an MGH Volunteer mentor and meet at MGH every other Friday morning, over the course of 4 months, to complete projects. Below is a list of the participating students; their MGH mentors are shown in parenthesis.

Jaydam Aponte, 7th Grade
What Is the Effect of the Dam On the Salinity and Dissolved Oxygen Levels in the Charles River?
(Nicholas Carrellas & Kristina Conroy, Psychiatry)

Mariana Crisanto, 8th Grade
Does Gender Affect What Side of the Brain Controls You the Most?
(Nicole Rivilis, Neurology)

Lakeisha Harper, 8th Grade
Do Colored Lights Affect Your Heart Rate?
(Maida Broudo, Termeer Center for Targeted Cancer Therapy)

Anne Hector, 8th Grade
The Process of Quenching
(Michelle DeLelys, Rachel Nicholson, & Steph Tsui, Pathology)

Cynthia Hydes, 8th Grade
Which Type of Bivalves is Most Effective in Filtering Pollutants from Natural Water?
(Danielle Le Hals, Radiation Oncology)

Marangela James, 8th Grade
Making a Hero Engine
(Irma Vlasac, Center for Human Genetics Research)

Ismael Lazala, 8th Grade
Human Reaction to Colorful Confusion
(Deepak Bhere, Radiology)

Melissa Mendez, 8th Grade
Does Gender Affect Blood Pressure While Exercising?
(Erin McGivney, Executive Committee on Research)

Gabin Mendoza, 8th Grade
How Does Playing Video Games Affect Your Body?
(Aoife Kilcoyne, MD & Alexi Otrakhi, MD, Radiology)

Poster Index

Poster Index

Poster Index

Poster Index

Poster Index

Poster Index

Poster Index

BIOENGINEERING & DEVICES

Poster Number 1

Ishita Basu, PhD
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Effects of electrical stimulation on cortical and deep brain local field potentials: potential use in closed loop brain stimulation paradigms

INVESTIGATORS: I. Basu, M. A. Kramer, B. Crocker, A. S. Widge, E. N. Eskandar

Brain stimulation has gained popularity as a means of ameliorating drug resistant pathologies such as movement disorders, epilepsy, pain and some psychiatric disorders. Most existing brain stimulators modulate a single brain area in an open-loop fashion. This works fairly well for pathologies that arise from single well-localized brain regions. Movement disorders may even have simple, single-site biomarkers that can support closed-loop control. However, pathologies such as complex neuro-psychiatric disorders reflect network-wide alterations in activity and connectivity. Closed-loop neuromodulation has remained impractical in this case, because attempts to isolate biological or electrical signatures for neuro-psychiatric disorders have largely failed. Developing an efficient closed loop brain stimulation paradigm for neuro-psychiatric disorders requires a network level model of the neuronal signals and the effect of stimulation. This in turn requires a thorough descriptive analysis of the effects of stimulation on the temporal and spectral features of local as well as networked brain structures. We explore the influence of external electrical stimulation at different cortical and subcortical regions while a subject is resting and during the performance of psychophysical tasks such as fear extinction and emotional conflict resolution. We show that the stimulation evoked potential at the neighboring electrode contacts and at other distant regions depend on the amplitude, frequency and duration of stimulation and these features can be used to model the dynamics of the brain that is affected by stimulation. This in turn can be used to predict the effects of stimulation in a therapeutic closed loop stimulation paradigm.

Poster Number 2

Chen-Han Huang, PhD
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A portable no-filter imaging system for biomolecular detection

INVESTIGATORS: C. H. Huang, Y. I. Park, K. Park, C. M. Castro, H. Y. Lin, R. Weissleder, H. Lee

Upconversion nanoparticles (UCNPs) have unique optical properties which render the particles a prominent luminescent label in multiplex bioassays and bioimaging. These superiorities include multiple sharp emission bands, long lifetime, tunable emission, photostability, and low cytotoxicity. UCNPs are excited at near-infrared (NIR) wavelengths where biomolecules are optically transparent, and emit visible light suitable for detection by semiconductor imagers. We herein introduce a new, portable diagnostic technology based on UCNP imaging. After UCNPs' excitation, the imaging system integrates the long-lived luminescence. This approach significantly simplifies the detection system; there is no need for any optical filter sets, and an imager system can be directly used. Importantly, the method eliminates the interference from excitation light and short-lived background autofluorescence, improving the overall signal-to-noise ratio. We optimized the assay to detect molecular targets: (i) we engineered UCNP structure and composition to lengthen the luminescence decay time; (ii) we established an optimal surface chemistry for bioconjugation; (iii) we built a compact, portable detection system. We applied the developed platform to detect protein targets as well as bacterial DNA. Target markers were captured on microbeads, and subsequently labeled with UCNPs. The optical assay achieved the sensitivity comparable to that of ELISA. The signal acquisition was fast (~1 min) and amenable to a high-throughput imaging scheme. The system could be a powerful tool for molecular assays, particularly in point-of-care settings.

BIOENGINEERING & DEVICES

Poster Number 3

Hyungsoon Im, PhD
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Smartphone-based low-cost molecular diagnostics for global oncology field applications

INVESTIGATORS: H. Im, D. Pathania, L. Fexon, C. Min, A. Kilcoyne, M. Avila-Wallace, A. R. Sohani, M. Pivovarov, C. M. Castro, R. Weissleder, H. Lee

Developing a low-cost, rapid and accurate diagnostic technology is a key requisite to cope with growing global cancer challenges. This is of particular importance in low- or middle-income countries where treatment opportunities are often missed due to limited resources and the absence of timely diagnoses. Here, we report a digital diffraction diagnostic (D3) technology that transforms ubiquitous smartphones into a molecular detection tool. This approach labels malignant cells with marker-specific microbeads, which produces unique diffraction patterns detectable by the smartphone camera in bright-field settings. Recorded patterns are sent to a remote server, and digitally reconstructed into microscopic cellular images. The system detected individual tumor cells and enabled quantitative molecular profiling per cell, akin to a flow cytometer with imaging capability, but in a miniaturized system. We showed that the system can be used to screen for pre-cancerous or cancerous cells in clinical cervical specimens. The system generated readouts in less than 1 hour and showed excellent agreement with gold-standard pathology. With its capacity for imaging and wireless communication, the smartphone-based D3 system would be a powerful point-of-care tool for cancer screening in resource limited settings.

Poster Number 4

Benjamin Kleinstiver, PhD
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High-fidelity CRISPR-Cas9 nucleases with no detectable off-target effects

INVESTIGATORS: B. P. Kleinstiver (co-first author), V. Pattanayak (co-first author), M. S. Prew, S. Q. Tsai, N. T. Nguyen, Z. Zheng, J. K. Joung

CRISPR-Cas9 nucleases are widely used for genome editing but can induce unwanted off-target mutations at genomic locations that resemble the intended target. These so-called off-target effects can confound research applications and are important considerations for potential therapeutic use. Existing strategies for reducing genome-wide off-targets of the broadly used Streptococcus pyogenes Cas9 (SpCas9) have proven to be imperfect, possessing only partial efficacy and/or other limitations that constrain their use. Here we describe a high-fidelity variant of SpCas9, called SpCas9-HF1, that contains alterations in the amino acid sequence designed to reduce non-specific contacts to the target strand DNA. SpCas9-HF1 retains on-target activities comparable to wild-type SpCas9 with >85% of single-guide RNAs (sgRNAs) tested in human cells. Strikingly, with eight different sgRNAs targeted to standard non-repetitive sequences in human cells, SpCas9-HF1 rendered all or nearly all off-target events imperceptible by genome-wide break capture and targeted sequencing methods. Even for atypical, repetitive target sites, the vast majority of off-targets induced by SpCas9-HF1 were not detected. With its exceptional precision, SpCas9-HF1 provides an important and easily employed alternative to wild-type SpCas9 for research and therapeutic applications. Our findings also suggest a general strategy for improving or optimizing the genome-wide specificities of other Cas9 orthologues and engineered variants.

Poster Number 5

Hsing-Ying Lin, PhD
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Electrochemical-magneto Assay Platform for On-site Food Testing

INVESTIGATORS: H. Lin, C. Huang, S. Jeong, J. Park, D. Pathania, R. Weissleder, H. Lee

A rapid rise of food allergies in the past two decades, together with the fact that the major therapy is to avoid allergen-containing food, urges the need for fast, on-site detection of food allergens. Here, we present a portable assay platform for quantitative check on food-borne allergens. The sensing is based on a novel electrochemical and magneto assay: we use target-specific magnetic beads to capture and enrich allergens in food extracts, and the analytical signal is generated through electrochemical reactions. By combining magnetic enrichment and enzymatic amplification, this approach improves the detection sensitivity, and also allows for sensor miniaturization. As a proof-of-concept, we implemented a portable, eight-channel device that can connect to a smartphone through bluetooth. We applied the system to detect common allergens in food: nut proteins, gluten, casein, and ovalbumin. Our assay achieved higher detection sensitivity (down to 0.003 ppm level) than conventional ELISA. The sensing was also much faster (<30 min), and could be performed on site. The developed system could be a promising tool for streamlined food allergen detection for the food industry and daily life.

Poster Number 6

Sara Golas, MA
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The Effect of a Personal Emergency Response Service at Partners Healthcare at Home

INVESTIGATORS: S. B. Golas, S. O. Agboola, J. C. Kvedar, K. Jethwani

Background: Increasing longevity coupled with the rising rates of chronic diseases has led to the increase design of interventions for decreasing management costs and promoting independent functioning in the elderly and patients with debilitating chronic diseases. One of such interventions is the Philips Lifeline's Personal Emergency Response Service (PERS). The PERS consists of a call button worn on the wrist or as a pendant that activates an intercom system in the home when pressed and directly connects the user with a response agent at a PERS call-center. The system tracks the types and outcomes of all incidents.

Objectives: This retrospective study evaluates the effect of PERS on clinical outcomes and healthcare costs in Partners Healthcare at Home (PHH) patients and also evaluates the accuracy of the PERS algorithm to predict emergency transports among PHH/PERS subscribers.

Methods: EMR data of PHH/PERS subscribers' health status, clinical outcomes, healthcare utilization and costs were extracted from Partners data repositories and medical alerts from the Philips Lifeline algorithm database.

Results: 3335 PHH patients used the interventions. Of these, 52% had inpatient admissions during 2011–15 (5258 total admissions). Of total admissions, 15.2% had 30-day readmissions, 88.3% were non-elective, 79.6% had emergency charges, and 90.7% had government payers. The most frequent principle diagnosis was obstructive chronic bronchitis with acute exacerbation. Admissions with this diagnosis accounted for 3.7% of admissions and 2.2% of total costs.

Conclusion: An optimized PERS algorithm can help identify patients at high risk of readmissions and develop appropriate targeted interventions.

Poster Number 7

Derek Klarin, MD
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Genetic Determinants of Venous Thromboembolism: A Genetic Association Study in the UK Biobank

INVESTIGATORS: D. M. Klarin, P. Natarajan, R. P. Cambria, S. Kathiresan

Background: Biobanks with phenotypes ascertained from electronic health records are being assembled all over the world but the fidelity of such phenotypes for genetic analysis is uncertain. With >500,000 participants enrolled, UK Biobank is the world's largest repository for phenotypic and genotypic information in individuals of European ancestry.

Methods: In UK Biobank, we studied venous thromboembolism (VTE), a leading cause of cardiovascular mortality. VTE case status was defined as ICD-10 Codes I26.9, I80.1, I80.2. We studied association of each of 820,000 variants in 4060 VTE cases and 146,000 controls.

Results: We identified 29 variants across 11 loci that associated with VTE below a genome-wide significance threshold. Top results recapitulated previously reported associations with known hemostatic loci: Factor V, Factor XI, Fibrinogen Gamma Chain and ABO blood group (all $P < 5x10\text{-}8$). For example, carriers of an Arg534Gln change at the Factor V gene (Factor V Leiden, 2.2% frequency) were at 3.4-fold increased risk for VTE (CI 3.13 – 3.65, $P = 2.5x10\text{-}57$), an effect size consistent with prior reports. In an evaluation restricted to null mutations (nonsense, splice site, frameshift), we identified a stop variant in the Phosphodiesterase-3B (PDE3B) gene (Arg783Ter, 0.01% frequency) with a suggestive association (OR=3.85, CI 2.7-5.4 , $P = 0.00013$). Experimental studies have previously implicated the phosphodiesterase-3 pathway in the biology of thrombosis.

Conclusion: This proof-of-concept analysis in UK Biobank suggests that, for common diseases like VTE, electronic health record phenotyping is able to recover previously reported association signals as well as suggest novel ones.

Poster Number 8

Ramya Palacholla, MD
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FeatForward: Design of a Randomized Controlled Trial of a Multi-modal mHealth Intervention to Improve Physical Activity Behavior in Patients with High Cardio-metabolic Risk Factors

INVESTIGATORS: R. S. Palacholla, S. O. Agboola, K. Jethwani, J. C. Kvedar, A. J. Centi

Background: Physical activity is an important modifier of cardio-metabolic risk factors and mobile applications are increasingly being used to engage patients in self-managing these risk factors. We aim to investigate the effects of a mobile application, FeatForward, on physical activity levels and other cardio-metabolic risk factors in patients with chronic conditions.

Intervention: FeatForward is a hyper-personalized Smartphone app with the ability to respond to individual behavior patterns simulating an intelligent health coach, to achieve better health outcomes. The app includes machine learning components for tailoring message frequency based on users' activity levels, social networking and educational features to keep the participants engaged. Additionally, the app has a care provider portal integrated with the medical records that allows physicians to monitor the patients' progress and communicate when appropriate.

Methods: 300 patients with chronic conditions (hypertension, diabetes) will be randomized into two groups: an intervention group that receives a Smartphone with the FeatForward app and a Smartwatch to track physical activity and other biometric parameters; and a control group that receives a Smartphone with the S-Health app and a Smartwatch. The difference in physical activity levels between the control and intervention group will be measured as the primary outcome at baseline, 3 and 6 months. Secondarily, we will assess changes in cardio-metabolic risk factors, quality of life, usability, satisfaction, participant engagement and physician engagement.

Conclusions: Given the high prevalence of physical inactivity in the society, findings from this study, may help participants engage in healthier lifestyles and lead to improved health outcomes.

Poster Number 9

Surya Reis, PhD
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Chemical Optoepigenetics—Light-controlled Small Molecule Modulation of Gene Transcription

INVESTIGATORS: S. A. Reis, B. Ghosh, J. A. Hendricks, D. M. Szantai-Kis, L. Törk, K. N. Ross, J. Lamb, W. Read-Button, B. Zheng, H. Wang, C. Salthouse, S. J. Haggarty, R. Mazitschek

Epigenetic gene regulation is a dynamic process orchestrated by chromatin- modifying enzymes. Many of these master regulators exert their function through covalent modification of DNA and histone proteins. Aberrant epigenetic processes have been implicated in the pathophysiology of multiple human diseases. Small-molecule inhibitors have been essential to advancing our understanding of the underlying molecular mechanisms of epigenetic processes. However, the resolution offered by small molecules is often insufficient to manipulate epigenetic processes with high spatio-temporal control. Here, we present a novel and generalizable approach, referred to as 'Chemo-Optical Modulation of Epigenetically-regulated Transcription' (COMET), enabling high resolution, optical control of epigenetic mechanisms based on photochromic inhibitors of human histone deacetylases using visible light. We have designed class and isoform selective inhibitors of human histone deacetylases (HDACs) and demonstrated that these COMET probes exhibit up to three orders of magnitude increased potency when exposed to blue light and allow for optically controlled HDAC-dependent modulation of gene expression in living human cells. To allow for high-throughput profiling of biochemical and cellular activity with COMET, we designed and optimized microprocessor-controlled (using the open-source Arduino platform) 12×8 light-emitting diode (LED)-arrays that are compatible with 96- well microtiter plates and may be used in standard tissue culture incubators due to their self-contained nature and small footprint. COMET probes may translate into novel therapeutic strategies for diseases where conditional and selective epigenome modulation is required.

Poster Number 10

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Genome Editing of Human iPS Cells to Model Recurrent Genomic Disorders such as 16p11.2 and 15q13.3 Microdeletion / Microduplication syndromes

INVESTIGATORS: J. Tai, A. Ragavendran, P. Manavalan, A. Stortchevoi, C. M. Seabra, S. Erdin, R. L. Collins, I. Blumenthal, X. Chen, Y. Shen, M. Sahin, C. Zhang, C. Lee, J. F. Gusella, M. E. Talkowski

Recurrent microdeletion and microduplication syndromes (rMDS) are among the most common causes of human neurodevelopmental and psychiatric disorders. These recurrent rearrangements are mediated by non-allelic homologous recombination (NAHR), which occurs between two highly homologous flanking segmental duplication (SD) sequences and can result in either copy loss (microdeletion) or the reciprocal copy gain (microduplication) of an identical genomic segment. Unfortunately, each individual recurrent genomic disorder is relatively rare, and accurate modeling of their impact in model systems represents a major challenge. The capacity to mimic these rMDSs in an otherwise isogenic pluripotent stem cell could provide an invaluable tool for modeling rMDS in humans. As a proof-of-principle, we describe a CRISPR/Cas9 genome engineering method, Single-guide CRISPR/Cas targeting Of Repetitive Elements (SCORE), that targets homologous sites in each flanking SD could efficiently model NAHR-mediated rMDS. Our methods successfully generated reciprocal copy number variation (CNV) of 16p11.2 and 15q13.3 rMDS regions, 740 kb and 1,989 Mb respectively, including alteration of one copy-equivalent of the segmental duplications that mediate NAHR *in vivo*. Genome-wide analyses suggest that the method efficiently generates rMDS without off-target CNVs and that RNAseq can reliably cluster transcriptional signatures from subjects with *in vivo* rMDS and their corresponding *in vitro* models. Our data suggest that this SCORE approach will provide broad applicability to *in vitro* rMDS modeling and, with further development, may also permit efficient correction of these defects.

BIOMEDICAL IMAGING

Poster Number 11

Iman Aganj, PhD
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Mid-Space-Independent Symmetric Deformable Image Registration

INVESTIGATORS: I. Aganj, J. E. Iglesias, M. Reuter, M. R. Sabuncu, B. Fischl

Image registration—i.e., computation of a set of dense spatial correspondences among images—is a central step in most population and longitudinal imaging studies. Since linear transformation is usually not sufficient to account for cross-subject variation and temporal changes in the anatomy, deformable image registration often becomes necessary.

Aligning a pair of images in a mid-space is a common approach to ensuring that deformable image registration is symmetric—that it does not depend on the arbitrary ordering of the input images. The results are, however, generally dependent on the choice of the mid-space. In particular, the set of possible solutions is typically affected and biased by the constraints that are enforced on the two transformations (that deform the two images), which are to prevent the mid-space from drifting too far from the native image spaces. The use of an implicit atlas has been proposed to define the mid-space for registration. In this work, we show that by aligning the atlas to each image in the native image space, we can make implicit-atlas-based pairwise registration independent of the mid-space, thereby eliminating the need for anti-drift constraints and the disadvantages that these constraints bring about. We present a novel symmetric data term that only depends on a single transformation morphing one image to the other, and show improvement achieved by the proposed method using diffeomorphic registration experiments on brain MR images.

Poster Number 12

Pauline Desogere, PhD
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Type I collagen-targeted positron emission tomography probe for pulmonary fibrosis detection and staging in preclinical models

INVESTIGATORS: P. Desogere, L. F. Tapias, L. P. Hariri, N. Rotile, T. A. Rietz, C. K. Probst, F. Blasi, H. Day, J. Elliot, M. Mino-Kenudson, A. M. Tager, M. Lanuti, P. Caravan

Pulmonary fibrosis is a scarring of the lungs that can arise from radiation injury, drug toxicity, and environmental causes. The most devastating form of pulmonary fibrosis is idiopathic pulmonary fibrosis (IPF), a chronic, progressive fibrosing interstitial pneumonia of unknown cause that primarily affects older adults. IPF imaging uses high resolution computed tomography scanning, which may diagnose IPF non-invasively in some but not all patients, but cannot accurately predict prognosis or therapy response to any of the currently available treatments. New methods of diagnosis and monitoring in IPF are needed.

Fibrosis is characterized by excess deposition of type I collagen. Here, we showed that positron emission tomography imaging with a new type I collagen-targeted probe, 68Ga-CBP8, is sensitive for the detection of pulmonary fibrosis in the commonly used bleomycin mouse model. 68Ga-CBP8 showed high specificity for pulmonary fibrosis and high target to background ratios in diseased animals. Lung PET signal and *ex vivo* quantification of lung 68Ga-CBP8 uptake correlated linearly (r2=0.84) with increased lung collagen levels in fibrotic mice.

We further demonstrated that this probe could be used to monitor treatment response in a second mouse model of pulmonary fibrosis associated with vascular leak. This model captures an important aspect of IPF pathogenesis, in which fibrosis is thought to result from exaggerated host responses to relatively mild environmental injuries.

We then showed that 68Ga-CBP8 is sensitive to collagen changes in human IPF lung tissues.

These studies suggest that 68Ga-CBP8 represents a promising candidate for noninvasive imaging of human pulmonary fibrosis.

Poster Number 13

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HIgh b-value and High Resolution Integrated Diffusion (HIBRID) MR Imaging

INVESTIGATORS: Q. Fan, A. Nummenmaa, J. R. Polimeni, T. Witzel, S. Y. Huang, V. J. Wedeen, B. R. Rosen, L. L. Wald

Purpose: The cerebral cortex is rich in gyral folding and finer cortical axonal fiber structures. Thus, high spatial resolution diffusion MRI is desired for resolving detailed cortical fiber paths, whereas high b-value is beneficial in improving the angular resolution of crossing structures in white matter (WM). While spatial resolution and b-value are necessarily traded-off due to finite SNR, diffusion MRI experiments cannot be optimized for cortex and WM simultaneously. We developed the HIgh b-value and High Resolution Integrated Diffusion (HIBRID) imaging to hybrid both of the complementary imaging capabilities.

Methods: MRI data were acquired from a healthy adult subject on the Siemens 3T Connectom Scanner. A spin echo-Echo Planar Imaging sequence was used to acquire diffusion data with 1.0mm, 1.5mm and 2.0mm isotropic resolution at b=1500 s/mm2 and 2.0mm isotropic resolution at b=8000 s/mm2. The detailed cortical structures revealed with various spatial resolution were compared. To combine the 1.0mm-b1.5k data with 2.0mm-b8k data, the weighting on the 1.0mm-b1.5k data was set to 1 in cortical gray matter. For the rest of the brain, the distance from the white-gray boundary surface was used to determine the weighting. To examine the reliability of the combined data, Jackknife resampling was performed to estimate the variance of orientation distribution function by leaving 20% diffusion weighting directions out each iteration for 100 iterations.

Results: Finer cortical structures were better revealed with higher imaging resolution. The combined HIBRID data gained improved spatial resolution in cortex while preserving high angular resolution in WM with reasonable reliability.

Poster Number 14

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White Matter Atrophy in Cerebral Amyloid Angiopathy

INVESTIGATORS: P. Fotiadis, A. Schultz, T. Hedden, S. Martinez-Ramirez, Y. Reijmer, A. Ayres, K. Schwab, A. Viswanathan, R. Sperling, K. Johnson, S. Greenberg, E. Gurol, Alzheimer's Disease Neuroimaging Initiative (ADNI)

Cerebral Amyloid Angiopathy (CAA) is a small vessel disease that leads to leukoaraiosis, lacunar infarcts, and cortical tissue loss. We hypothesized that CAA is also associated with white matter atrophy (WMA). We compared volumetric multimodal MRIs from 72 prospectively enrolled non-demented patients with probable CAA (per Boston criteria), to 3 other well-studied cohorts: 289 Healthy Controls (HC) from the Harvard Aging Brain (HAB) study, 231 HC and 198 patients with Alzheimer's Disease (AD) from the Alzheimer's Disease Neuroimaging Initiative (ADNI). Using advanced neuroimaging softwares (FreeSurfer, FSL, MATLAB) and in-house algorithms, we calculated white matter volume (WMV), white matter hyperintensity volume (WMHv), cortical thickness, and number of lobar microbleeds (MB). Measures were obtained from the contralateral hemisphere when intracerebral hemorrhage was present, and all volumes were corrected for total intracranial volume. CAA patients were significantly younger (mean age: 70.1) compared to both HC cohorts (ADNI-HC: 76.0, $p<0.001$, HAB-HC: 73.8, $p<0.001$), and to patients with AD (75.5, $p<0.001$). Here we show that despite being younger, patients with CAA presented significantly lower global WMV (28% ± 2.6) than both ADNI-HC (29.2% ± 2.2, $p < 0.001$), HAB-HC (29.0% ± 2.5, $p = 0.001$), and patients with AD (28.7% ± 2.2, $p = 0.02$). Also, WMA strongly and independently correlated with lobar MB counts (rho = -0.26, $p = 0.03$), a marker of CAA severity. Consistent spatial patterns of white matter atrophy especially in posterior regions when compared to both HC and AD might represent the "WMA signature of CAA."

Poster Number 15

Tonya Gilbert, PhD
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Insights into neuroepigenetics through human histone deacetylase imaging

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Epigenetic dysfunction is implicated in many neurological and psychiatric diseases, including Alzheimer's disease and schizophrenia. Consequently, histone deacetylases (HDACs) are aggressively pursued as therapeutic targets. However, a fundamental knowledge gap exists regarding the expression and distribution of HDACs in healthy individuals for comparison to disease states. Current neuroepigenetic models cannot measure dynamic human-environmental interactions occurring throughout the course of life and disease progression. Here, we report the first-in-human evaluation of neuroepigenetic regulation. Using positron emission tomography (PET) with [11C]Martinostat, an imaging probe selective for a subset of HDACs, we found *in vivo* HDAC expression is higher in cortical gray matter than white matter. We also showed HDACs are expressed with strikingly conserved regional distribution patterns within and between healthy individuals, suggesting that HDAC expression is a state function. Among gray matter regions, HDAC expression is lowest in the hippocampus and amygdala. Since HDACs inhibit gene expression, lower levels of [11C]Martinostat uptake may serve as a surrogate marker for transcriptionally active brain regions undergoing dynamic neuronal plasticity. Through biochemical profiling of postmortem human brain tissue, we confirmed [11C]Martinostat selectively binds HDAC2 and HDAC3, the HDAC subtypes most associated with cognitive function, in the cortical gray matter (superior frontal gyrus). While [11C]Martinostat showed binding to HDAC1 and HDAC2 in the white matter (corpus callosum), the interaction was more than 100 times weaker, demonstrating for the first time, tissue-specific target engagement of an HDAC inhibitor. This foundational study allows unprecedented access to *in vivo* epigenetic information in the healthy and diseased human brain.

Poster Number 16

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Non-invasive Positron Emission Tomography with 18F-Labeled Nanoparticles Detects Macrophages in Atherosclerotic Plaques and Acute Myocardial Infarction

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Background: Macrophages play a critical role both in normal physiology (tissue resident macrophages) as well as disease states (plaque macrophages triggering myocardial infarction (MI) and stroke). Understanding macrophage relative numbers, distribution profiles, mobilization and flux rates across diseases and normal physiologic conditions could enhance the effects of emerging macrophage targeted therapies.

Methods: We have developed macrophage specific dextran nanoparticles (DNP) with ultrafast targeting and excretion from the blood pool enabling the use of rapidly decaying fluorine-18 for positron emission tomography (PET) detection of macrophages. These materials were employed for PET in murine models atherosclerosis and MI, diseases where inflammation associates with adverse prognosis. *In vivo* PET/MR imaging of mice with atherosclerosis and acute MI was correlated with *ex vivo* counting, autoradiography and flow cytometry data. Pharmacokinetic profiling experiments in a non-human primate were pursued for extrapolation to performance in humans.

Results: *In vivo* PET-CT imaging using 18F-DNP showed 0.39 ± 0.04 SUV in murine atheroma (*ex vivo* %IDGT 1.0 ± 0.3) and *ex vivo* autoradiography colocalized with Oil Red O staining of atherosclerotic plaques. Furthermore, 18F-DNP PET imaging in MI after coronary ligation showed high *in vivo* PET signal in inflamed ischemic myocardium and good correlation with *ex vivo* measurement. Cellular uptake profiling revealed a high avidity for macrophages.

Conclusion: 18F-DNP provides non-invasive *in vivo* detection of surging macrophage numbers in atherosclerotic plaques and acute MI. Preliminary pharmacokinetic data from non-human primates indicate promising extrapolation for translation to human clinical trials.

Poster Number 17

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Preliminary evaluation of a novel class of carbon-11 labeled sulfonamide carbamates and ureas for imaging monoacylglycerol lipase

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Monoacylglycerol lipase (MAGL) is a 33 kDa member of the serine hydrolase superfamily that preferentially degrades 2-arachidonoylglycerol (2-AG) to arachidonic acid in the endocannabinoid system. Inhibition of MAGL is not only of interest for probing the cannabinoid pathway but also as therapeutic and diagnostic target for neuroinflammation. Since only limited attempts have been made to image MAGL *in vivo*, the search for a suitable PET ligand for this target is urgently sought. Herein we developed two 11C-labeled MAGL inhibitors based on a novel sulfonamide core and performed preliminary PET studies in rodents.

A library of twenty sulfonamido carbamates / ureas with a series of alterations in terminal aryl moieties, linkers and leaving groups was synthesized and evaluated in a MAGL / fatty acid amide hydrolase (FAAH)/ cannabinoid receptors (CB1/CB2) competitive binding assay, an artificial membrane permeability assay and a hydrolysis stability assay. The most promising compounds, namely N-((1-(1H-1,2,4-triazole-1-carbonyl)piperidin-4-yl)methyl)-4-chlorobenzenesulfonamide (TZPU), and 1,1,1,3,3,3-hexafluoropropan-2-yl 4-(((4-chlorophenyl)sulfonamido)methyl)piperidine-1-carboxylate (SAR127303), were radiolabeled at the 11C-carbonyl position via [11C]COCl2. PET imaging of [11C] TZPU in rats showed a limited brain uptake (0.4 SUV) and no significant washout during the scan. [11C]SAR127303 demonstrated high whole brain uptake (2 SUV peaked at 3 min) and the distribution was heterogeneous with the highest radioactivity in the cerebellar cortex and striatum, followed by hippocampus, cerebellum and the lowest in pons, which is consistent with MAGL distribution in the rat brain. [11C]SAR127303 shows promise as a proof-of-concept radiotracer for neuroimaging of MAGL. PET studies in nonhuman primates are underway and will be presented.

Poster Number 18

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Tau and amyloid PET imaging in a Colombian kindred with autosomal-dominant Alzheimer's disease: A preliminary report

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Background: Examining rare families with autosomal dominant Alzheimer's disease (ADAD) provides a unique model for studying the trajectory of Alzheimer's-related pathology. We used PET imaging to characterize the relation between amyloid burden and tau accumulation in the brains of young, asymptomatic PSEN1 E280A mutation carriers from a Colombian kindred with ADAD. We hypothesized that amyloid-beta deposition precedes tau tangle formation both within and beyond the medial temporal lobe in ADAD.

Methods: Six cognitively-unimpaired carriers (mean age 32-years) and six noncarriers (mean age 36-years) traveled to Boston for amyloid (PiB) and tau (T807) PET imaging. PiB-PET cerebral-to-cerebellar DVRs and T807-PET SUVRs were compared based on mutation status. PiB and T807 utilized structural regions-of-interest as defined by Freesurfer.

Results: Compared with non-carriers, asymptomatic carriers had significantly higher mean cortical PiB DVRs (carriers: 1.16 +/-0.06, non-carriers: 1.05 +/-0.02, p<0.001). The group differences for T807 SUVRs were in the expected direction but were non-significant (e.g. entorhinal-cortex; carriers: 1.21 +/-0.40, noncarriers: 1.02 +/-0.06, p= 0.28). There was also a trending association between amyloid burden and T807 binding in entorhinal cortex (r= 0.57, p=0.07), which was in part driven by the oldest mutation carrier in this sample (age 38), who was still several years away from clinical onset at age 44.

Conclusion: Initial findings from this ongoing study are consistent with the hypothesis that mutation carriers express amyloid deposition before tau deposition. Studying amyloid and tau concomitantly in ADAD is a promising method for clarifying the temporal relationship between the emergence of tau and amyloid pathology.

Poster Number 19

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Role of high-speed pediatric body CT at ultra-low radiation dose and no anesthesia

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The purpose of our study is to perform high-speed motion free CT at ultra-low dose with no anesthesia and compare image quality, diagnostic confidence with standard CT.

Study cohort comprised of all consecutive pediatric CT performed with high helical pitch of 3 or more and table speed of 450mm/sec {HPCT}. Comparison group included age matched CT with standard pitch of 0.9{SPCT}. Image quality evaluation included subjective assessment of lesion margins, motion artifacts and noise as well as objective noise measured as standard deviation of HU values. Statistical analysis was performed with Student t-test.

35 patients {8.8±6.2 years M:F17:18} underwent HPCT {n=20 chest and n=15 abdomen} on a dual source MDCT (SOMATOM Definition Flash, Siemens Healthcare}. HPCT were performed in children >10 (n=19), followed by 5-9 (n=6), 1-4 (n=6) and <1 year old (n=4). None of the HPCT cases required sedation or anesthesia, including all 10 patients <5 years and 1 case of multi-phase abdominal CT performed to evaluate a hepatic lesion. HPCT was associated with a 49.3% dose reduction {CTDIvol: 3.3±2.2 vs SPCT 6.5±4.0 mGy; p=0.0002). Subjective image noise was graded as similar to SPCT and no motion artifacts were reported. Overall image quality was rated as diagnostically acceptable, including adequate resolution of small sub-centimeter lymph nodes and branch vessels/bronchi. No significant difference in objective noise was found with HPCT {11.9 ±6.5} compared to SPCT {13.0±5.1}(p=0.22).

High-speed motion free pediatric CT can be performed without sedation and up to 50% lower radiation dose as an potential alternative to MRI.

Poster Number 20

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Dual inhibition of Ang-2 and VEGF receptors normalizes tumor vasculature and prolongs survival in glioblastoma by altering macrophages

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Glioblastoma (GBM) is the most common, incurable primary brain tumor in adults. Current standard of care including chemo-radiation confers modest survival benefits of less than 1.5 years. GBMs are highly dependent on angiogenesis and anti-angiogenic (anti-VEGF) therapy holds the promise to prolong survival. Unfortunately, GBM patients rapidly become refractory to VEGF pathway inhibition.

We previously demonstrated that Angiopoietin-2 (Ang-2) may confer resistance to anti-VEGF receptor (VEGFR) treatment, since ectopic overexpression of Ang-2 compromises the benefits of VEGF pathway inhibition in murine GBM. Additionally, circulating Ang-2 levels in GBM patients rebound after an initial decrease following cediranib (a pan-VEGFR tyrosine kinase inhibitor) administration. Here we tested whether dual inhibition of Ang-2 and VEGFR could improve survival in two GBM models, GI261 and U87. Dual therapy using cediranib and MEDI3617 (an anti-Ang-2 neutralizing antibody) improved survival over each therapy alone by delaying GI261 growth and increasing U87 necrosis, effectively reducing viable tumor burden. Consistent with their vascular modulating function, dual therapy enhanced morphological normalization of vessels. Dual therapy also led to changes in tumor-associated macrophages (TAMs). Inhibition of TAM recruitment using an anti-colony stimulating factor-1 antibody (anti-CSF-1) compromised the survival benefit of dual therapy. Thus, dual inhibition of Ang-2 and VEGFR prolongs survival in preclinical GBM models by reducing tumor burden, improving normalization, and alerting TAMs. This approach may represent a potential therapeutic strategy to overcome the limitations of anti-VEGFR monotherapy in GBM patients by integrating the complementary effects of anti-Ang2 treatment on vessels and immune cells.

Poster Number 21

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SCD1 is a new therapeutic target in Glioblastoma

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Glioblastoma is the most malignant and common form of primary central nervous system tumors with high mortality and resistance to therapy. Cancer cells often require de novo fatty acid synthesis. Stearoyl CoA Desaturase 1 (SCD1) is important for the biosynthesis of monounsaturated fatty acids and is crucial for lipid homeostasis.

We identified an increased SCD1 activity in a panel of highly aggressive, treatment resistant primary patient-derived gliomas. Activation of different transcription factors, responsible for promoting tumorigenesis and treatment resistance, was accompanied by an increased SCD1 expression further confirming the role of SCD1 in tumorigenesis. In culture, highly proliferative glioma stem cells showed increased vulnerability to SCD1 inhibition using genetic knockdown of SCD1 or targeted inhibitors. Cell death induced by SCD1 inhibition could be reversed by addition of oleic acid, the primary product of this enzyme thereby confirming the high dependence of gliomas on this lipid synthesis pathway. Knockdown of SCD1 completely abrogated tumor formation in orthotopic glioma mouse models. We identified CAY10566 as a highly potent small-molecule inhibitor of SCD1. This compound was administered using intranasal delivery to mice bearing orthotopic brain tumors. This alternative drug delivery method increases targeting to the brain, bypasses the blood-brain barrier and reduces systemic toxicity. Mice treated with CAY10566 showed reduced tumor volume along with a significant increase in overall survival.

In summary, we have identified a new metabolic vulnerability in highly aggressive gliomas and showed that *in vivo* delivery of SCD1 inhibitors presents a new avenue to treat brain tumors.

Poster Number 22

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CPI-613 a promising candidate for combination with FDA approved olaparib treatment in OvCa

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In 2014, the FDA approved olaparib (a PARP-inhibitor targeting DNA repair) as monotherapy for the treatment of advanced serous ovarian cancer (OvCa) patients with a BRCA mutation. Clinical trials with olaparib in OvCa have demonstrated an increase in progression free survival but no significant difference in overall survival. This prompted us to hypothesize that treatment with olaparib failed to impact ovarian cancer stem cell (CSC) populations, contributing to recurrent disease.

We identified OvCa CSC as cells that co-express CD133 and CD117 in the established serous OvCa cell lines OVCAR4, OVCAR8 and CaOV4 and in primary cell lines derived from serous carcinomas obtained with IRB approval at the time of initial surgery. We showed that olaparib treatment increased sphere forming capacity and the expression of stemness related genes suggesting that olaparib has the unintended effect of stimulating CSC in maintaining tumor's growth. Previous work has shown that the metabolic inhibitor CPI-613 (inhibitor of tricarboxylic acid (TCA) cycle) preferentially targets ovarian CSC due to their altered metabolism. Treatment with CPI-613 reduces sphere forming capacity of OvCa cells. Pre-treatment with CPI-613 negated the olaparib induced increase in CD133 and CD117 OvCa positive cells and sphere forming capacity. These results suggest pretreatment with CPI-613 negatively impacts CSC and overcomes the unintended effect of olaparib on CSC function. Sequential treatment may therefore prevent or delay the anticipated tumor recurrence.

In conclusion, targeting CSC with non-traditional strategies could augment clinical outcomes and CPI-613 represents a promising candidate for combination with FDA approved olaparib treatment in OvCa.

Poster Number 23

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Ado-trastuzumab emtansine (T-DM1) controls tumor progression of established HER2-positive breast cancer brain metastases in mice

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Brain metastases represent a major problem in the treatment of HER2-positive breast cancer due to the poor efficacy of HER2-targeted therapies in the brain microenvironment. The antibody drug conjugate ado-trastuzumab emtansine (T-DM1) has shown efficacy in trastuzumab-resistant systemic breast cancer. We tested the hypothesis that T-DM1 could overcome trastuzumab resistance in murine models of brain metastases. Using intravital imaging, molecular techniques and histological analysis we determined tumor growth, mouse survival, cancer cell viability, tumor drug distribution, and HER2 signaling.

Here we show that T-DM1 can overcome resistance to trastuzumab therapy in HER2-positive breast cancer brain lesions due to the cytotoxicity of the DM1 component. The results of our studies indicate that T-DM1 is effective in the brain microenvironment and will directly inform clinical trials in patients with HER2-positive breast cancer brain metastases.

Poster Number 24

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ePAL: Pain Management in Cancer Patients Using a Smartphone Application—Preliminary Results from a 2-Arm Randomized Control Trial

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Background: ePAL is multidimensional mobile application based on clinical guidelines to help cancer patients better manage their pain by encouraging improved self-management of pain and more regular pain assessments.

Objective: To evaluate the effect of ePAL in controlling cancer pain and improving quality of life in patients with cancer pain being treated at an academic palliative care clinic.

Methods: The ongoing 2-arm randomized control trial included 65 patients randomized into control and intervention groups. Pain severity and quality of life metrics were assessed at the enrollment, midpoint (week 4), and closing (week 8) of the study. Pain severity was assessed using the Brief Pain Inventory (BPI) and Quality of Life was assessed with the Functional Assessment of Cancer Therapy (FACT-G).

Results: At the time of analysis, 41 subjects have completed the study. In the control group, overall pain severity increased from 3.51 (SD, 1.57) at enrollment to 3.81 (SD, 2.26) and to 3.79 (SD, 1.8) at closeout. Whereas in the intervention group, average pain severity scores decreased from 3.32 (SD, 2.08) to 3.20 (SD, 1.71), and to 3.13 (SD, 2.23). Quality of life increased from baseline in the intervention group [[67.24 (SD, 15.67), 69.51 (SD, 14.61), and 69.97 (SD, 14.57)] but declined in the control group [68.37 (SD, 14.62), 66.55 (SD, 14.20), and 65.25 (SD, 15.55)].

Conclusion: While the study is still ongoing, preliminary results show that ePAL may reduce overall pain severity and could improve overall quality of life.

Poster Number 25

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Mitochondrial phenotype is a biomarker of cancer cell metabolism and chemotherapy response

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Mitochondria show phenotypic variation in number, mass and shape in normal and malignant cells. Past research has primarily focused on short-term molecular mechanisms underlying fission/fusions that control these phenotypic changes. Less is known about longer-term mitochondrial behavior such as the overall makeup of cell populations' mitochondrial phenotypic patterns and whether these patterns can be used as biomarkers of drug response in human cells. Here, we have developed an image-based analytical technique to phenotype mitochondrial morphology in different cancers, including patient-derived cancer cells and cell lines. We demonstrate that i) cancer cells of different origins, express highly diverse mitochondrial phenotypes; ii) a given phenotype is characteristic of a cell population and fairly constant over time; iii) mitochondrial patterns correlate with cell metabolic measurements and iv) therapeutic interventions alter mitochondrial phenotypes following treatment. These observations shed light on the role of mitochondrial dynamics in the biology and drug response of cancer cells. On the basis of these findings, we propose that image-based mitochondrial phenotyping may be a new biomarker to broadly asses cancer phenotypes and drug response in fine needle aspirates.

Poster Number 26

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A geometric knowledge-based algorithm to predict patient-specific benefits of proton therapy

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Introduction: There would be many advantages to an algorithm which can quickly identify whether a patient is a good candidate for proton therapy. For example, a physician at a hospital without proton capabilities could make a more informed decision to refer a patient to a proton therapy center. It could also aid arm selection in model-based clinical trials. In this study, a geometric knowledge-based algorithm has been developed in order to predict patient-specific benefits of proton therapy with respect to an X-ray treatment modality (IMRT).

Methods: The algorithm exploits correlations between dose and distance-to-target in order to predict the dose-volume histogram (DVH) of each organ-at-risk (OAR). DVH variations between patients are assumed to originate from geometric differences. Clival chordoma was chosen to test the algorithm, owing to its geometric complexity and the multitude of nearby OARs. The algorithm was trained on 20 patients and validated with a further 20 patients. Validation was achieved by comparing properties of the predicted DVHs to those of actual proton plans.

Results: DVHs were predicted for the brainstem, cochleas, optic nerves, optic chiasm, pituitary gland, parotid glands and spinal cord. The predicted equivalent uniform dose (EUD) was found to be in good agreement with that of the actual proton plan. Each prediction was also compared to an IMRT plan, to evaluate how accurately the benefits of proton therapy were predicted.

Conclusions: We have developed a geometric knowledge-based algorithm and demonstrated that it can predict patient-specific benefits of proton therapy with high accuracy.

Poster Number 27

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Increasing the Efficacy of Radiotherapy on Metastatic Osteosarcoma using the Angiotensin Receptor Blocker Losartan or the CXCR4 Inhibitor AMD3100

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Osteosarcoma (OS) is the most common primary malignant bone tumor. Most OS tends to rapidly develop distant metastases. Tumor hypoxia and CXCR4 expression are poor prognostic factors in metastatic OS. To study if reducing hypoxia and inhibiting CXCR4 expression would inhibit metastatic OS progression, we tested OS response to radiotherapy combined either with losartan (an angiotensin receptor blocker that reduces fibrosis and hypoxia in tumors) or AMD3100 (an inhibitor of the hypoxia-induced CXCR4 receptor) using an established Os-P0107 model. Os-P0107 tumors show higher spontaneous metastases (to lungs, or liver, lymph nodes, kidneys, and bones) in C3H mice within 3 months after removal of 15 x 15 mm primary tumors and higher CXCR4 expression in their primary and metastatic tumor tissues. When Os-P0107 isografts reached 6 mm in diameter after their subcutaneous implantation, losartan (L, 40 mg/kg BW, by gavage) or AMD3100 (AMD, 5 mg/kg BW, i.p.) was applied daily for 7 days followed by 20 Gy of single dose local radiation (LR) at day7 (L+LR or AMD+LR, n=80). Both treatments significantly reduced local tumor volume when compared to the control group ($p=0.01$ and $p=0.05$, respectively), but LR alone did not cause significant impact. In summary, a highly metastatic and CXCR4 expressing Os-P0107 model has been used to show that losartan or AMD3100 combined with radiotherapy could improve local tumor control, but not significantly affect distant dissemination. The safety and efficacy of a triple combination (L+AMD+LR) for both local and distant disease control in the model are currently being evaluated.

Poster Number 28

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Metformin reduces desmoplasia in pancreatic cancer by reprogramming stellate cells and tumor-associated macrophages

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Background: Pancreatic ductal adenocarcinoma (PDAC) is a highly desmoplastic tumor with a dismal prognosis for most patients. Fibrosis and inflammation are hallmarks of tumor desmoplasia. We have previously demonstrated that preventing the activation of pancreatic stellate cells (PSCs) and alleviating desmoplasia are beneficial strategies in treating PDAC. Metformin is a widely used glucose-lowering drug. It is also frequently prescribed to diabetic pancreatic cancer patients and has been shown to associate with a better outcome. However, the underlying mechanisms of this benefit remain unclear. Metformin has been found to modulate the activity of stellate cells in other disease settings. In this study, we examine the effect of metformin on PSC activity, fibrosis and inflammation in PDACs.

Methods/Results: In overweight, diabetic PDAC patients and pre-clinical mouse models, treatment with metformin reduced levels of tumor extracellular matrix (ECM) components, in particular hyaluronan (HA). *in vitro*, we found that metformin reduced TGF-ß signaling and the production of HA and collagen-I in cultured PSCs. Furthermore, we found that metformin alleviates tumor inflammation by reducing the expression of inflammatory cytokines including IL-1β as well as infiltration and M2 polarization of tumor-associated macrophages (TAMs) *in vitro* and *in vivo*. These effects on macrophages *in vitro* appear to be associated with a modulation of the AMPK/STAT3 pathway by metformin. Finally, we found in our preclinical models that the alleviation of desmoplasia by metformin was associated with a reduction in ECM remodeling, epithelial-to-mesenchymal transition (EMT) and ultimately systemic metastasis.

Conclusion: Metformin alleviates the fibro-inflammatory microenvironment in obese/diabetic individuals

Poster Number 29

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Formation of lymph node metastases is not angiogenesis dependent

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Background: To date, antiangiogenic therapy has failed to improve overall survival in cancer patients when used in the adjuvant setting (local-regional disease with no detectable systemic metastasis). The presence of lymph node metastases worsens prognosis, however their reliance on angiogenesis for growth has not been reported.

Methods: Here, we introduce a novel chronic lymph node window (CLNW) model to facilitate new discoveries in the growth and spread of lymph node metastases. We use the CLNW in multiple models of spontaneous lymphatic metastases in mice to study the vasculature of metastatic lymph nodes. We further test our results in patient samples. Finally, we test the ability of antiangiogenic therapy to inhibit metastatic growth in the CLNW.

Results: We reveal the surprising lack of sprouting angiogenesis during metastatic growth, despite the presence of hypoxia in some lesions. Treatment with two different antiangiogenic therapies showed no effect on the growth or vascular density of lymph node metastases. We confirmed these findings in clinical specimens, including the lack of reduction in blood vessel density in lymph node metastases in patients treated with bevacizumab.

Conclusion: We provide pre-clinical and clinical evidence that sprouting angiogenesis does not occur during the growth of lymph node metastases, and thus reveal a new mechanism of treatment resistance to antiangiogenic therapy in adjuvant settings. The targets of clinically approved angiogenesis inhibitors are not active during early cancer progression in the lymph node, suggesting that inhibitors of sprouting angiogenesis as a class will not be effective in treating lymph node metastases.

Poster Number 30

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Comprehensive assessment of cancer missense mutation clustering in 3D protein structures

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Large-scale tumor sequencing projects enabled the identification of many new cancer gene candidates through computational approaches. Here, we describe a general method to detect cancer genes based on significant 3D clustering of mutations relative to the structure of the encoded protein products. The approach can also be used to search for proteins with an enrichment of mutations at binding interfaces with a protein, nucleic acid, or small molecule partner. We applied this approach to systematically analyze the PanCancer compendium of somatic mutations from 4,742 tumors relative to all known 3D structures of human proteins in the Protein Data Bank. We detected significant 3D clustering of missense mutations in several previously known oncoproteins including HRAS, EGFR, and PIK3CA. Although clustering of missense mutations is often regarded as a hallmark of oncoproteins, we observed that a number of tumor suppressors, including FBXW7, VHL, and STK11, also showed such clustering. Beside these known cases, we also identified significant 3D clustering of missense mutations in NUF2, which encodes a component of the kinetochore, that could affect chromosome segregation and lead to aneuploidy. Analysis of interaction interfaces revealed enrichment of mutations in the interfaces between FBXW7-CCNE1, HRAS-RASA1, CUL4B-CAND1, OGT-HCFC1, PPP2R1A-PPP2R5C/PPP2R2A, DICER1-Mg(2+), MAX-DNA, SRSF2-RNA, and others. Together, our results indicate that systematic consideration of 3D structure can assist in the identification of cancer genes and in the understanding of the functional role of their mutations.

Poster Number 31

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Disparities in bilateral mastectomy use among women with early-stage breast cancer

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The use of bilateral mastectomy (BM) has increased over the past decades among women diagnosed with early-stage breast cancer even though the long term benefits of undergoing BM for those women with unilateral cancers are unclear. We surveyed women who were diagnosed with localized or regional stage invasive breast cancer at age 40-64 in Pennsylvania and Florida between January 1, 2007 and December 31, 2009. Patients were asked whether they received treatments such as lumpectomy, mastectomy, bilateral mastectomy, and adjuvant treatments. Family history, education, income, insurance status, and physician information were also ascertained by self-report, and age, stage at diagnosis, and receptor status were determined from cancer registry data. Of 2349 study participants (1481 whites and 868 blacks), black women were less likely to undergo BM than white women (10% vs 19%, p<0.001). After adjusting for clinical factors, family history, BRCA testing, and clustering within surgeons, white women were still more likely to undergo BM (OR 2.04, 95% CI 1.54-2.69, P<.001). However, racial differences in the use of BM appeared to be associated with differences in the level of worry about the recurrence of a second breast cancer with white women being more likely to believe that they are likely to develop a second breast cancer (p<0.001). Since this likelihood varied between women who had and had not had BM, more research is needed to understand why there is such difference in worry and whether this represents underuse among black women or overuse among white women.

Poster Number 32

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Ang-2/VEGF bispecific antibody reprograms macrophages and resident microglia to anti-tumor phenotype and prolongs glioblastoma survival

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Glioblastoma (GBM) is a uniformly lethal primary brain tumor affecting more than 12.000 patients every year in the US alone. Standard therapy of this highly angiogenic tumor entity comprises surgical maximal safe resection and radiochemotherapy. The addition of antiangiogenic—VEGF neutralizing—therapy to the standard of care regimen failed to improve overall survival of GBM patients. Clinical and preclinical data suggest that the rapidly developing resistance to anti-VEGF therapy may be mediated by Angiopoietin-2 (Ang-2). Here we investigated whether dual Ang-2/VEGF inhibition could overcome anti-VEGF treatment resistance. We treated mice bearing orthotopic syngeneic (GI261) GBMs or human (MGG8) GBM xenografts with antibodies inhibiting VEGF (B20), or Ang-2/VEGF (CrossMab, A2V). We examined the effects of treatment on the tumor vasculature, immune cell populations, tumor growth and survival in both the GI261 and MGG8 tumor models. We found that in the GI261 model, which displays a highly abnormal tumor vasculature, A2V decreased vessel density, delayed tumor growth and prolonged survival compared with B20. In the MGG8 model, which displays a low degree of vessel abnormality, A2V induced no significant changes in the tumor vasculature, yet still prolonged survival. In both the GI261 and MGG8 models, A2V reprogrammed pro-tumor M2 macrophages toward the anti-tumor M1 phenotype. Our findings indicate that A2V may prolong survival in mice with GBM by reprogramming the tumor immune microenvironment and delaying tumor growth.

Poster Number 33

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Regulation of Ribosome Biogenesis by snRNAs and RNA binding proteins

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Ribosome biogenesis is indispensable for all cells. Impaired ribosome biogenesis underlie several malignancies such as Myelodysplastic Syndromes (MDS) that can lead to leukemias. We have uncovered a novel regulation of ribosome biogenesis by the RNA-binding protein FXR1. FXR1 is frequently overexpressed in several cancers and is critical for post-transcriptional regulation of gene expression. Here we demonstrate that FXR1 depletion decreases general translation by 50% in THP1 human monocytic leukemic cells. The decreased translation correlates with significantly reduced levels of ribosome subunits. Consistent with previous reports indicating association of FXR1 with ribosomes, we find several 60S and 40S subunit components are decreased upon FXR1 depletion. Distinctly, although both ribosomal proteins and ribosomal RNAs are decreased, tRNAs are not affected by FXR1 reduction. This decrease in ribosomal subunits is more pronounced in serum starved cells , where rRNA and ribosomal protein mRNA transcription is also decreased as these cells conduct limited non-canonical translation. To understand underlying causes of this phenotype, we examined a key early regulator involved in rRNA processing, U3 RNA (snRNA/snoRNA) and U2, a major splicing factor. Genes encoding ribosomal proteins are heavily spliced, thus any defect in splicing is likely to affect ribosomal protein production. Both U2 and U3 are decreased upon FXR1 reduction, potentially explaining the reduction in ribosome biogenesis observed in FXR1 knockdown. Thus, we elucidate a novel regulation of ribosome biogenesis executed by FXR1 via its impact on U3 and U2 snRNAs.

Poster Number 34

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LKB1 loss links the serine metabolic network to DNA methylation and tumorigenesis

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Intermediates generated in cell metabolism also serve as substrates for covalent modification of chromatin, enabling the potential coupling of metabolic states and epigenetic control. Here, we identify such a network as a major mediator of cell transformation downstream of the LKB1 tumour suppressor. LKB1 encodes a serine-threonine kinase that integrates nutrient availability, metabolism and growth, although the mechanisms for LKB1-dependent tumour suppression remain elusive. By developing primary epithelial cell models and employing transcriptional, proteomics, and metabolic analyses, we find that oncogenic cooperation between LKB1 loss and KRAS activation, alterations commonly coinciding in human cancer, is fueled by pronounced induction of the serine-glycine-one carbon network coupled to S-adenosylmethionine (SAM) generation. In concert, DNA methyltransferases (DNMT1 and DNMT3A) are upregulated, leading to global elevation in genomic 5-methylcytosine levels. Correspondingly, LKB1 deficiency renders cells independent of exogenous serine for growth, but highly sensitive to inhibition of serine biosynthesis and DNA methylation *in vitro* and *in vivo*. Thus, we define a hypermetabolic state resulting from loss of LKB1 that links rewiring of glucose metabolism and chromatin regulation. This state both potentiates and is critically required for the tumourigenic program of LKB1-mutant cells, suggesting novel points of therapeutic intervention in defined patient subsets.

Poster Number 35

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YAP Subcellular Localization and Hippo Pathway Transcriptome Analysis in Pediatric Hepatocellular Carcinoma

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Introduction: Currently, no effective chemotherapy exists for pediatric hepatocellular carcinoma (HCC). There is evidence that YAP, the transcriptional co-activator of the Hippo pathway, plays an important role in hepatocyte proliferation, and nuclear YAP activity is increased in adult HCC. We sought to examine whether YAP nuclear localization is also increased in pediatric HCC.

Methods: Tumor and adjacent, non-neoplastic liver tissue from 7 pediatric patients with HCC were analyzed for YAP expression using immunofluorescence. Hippo-pathway specific array and real-time PCR were used to assess the expression of Hippo-pathway related genes.

Results: The percentage of nuclei staining positive for YAP was increased in tumor cells compared to non-neoplastic liver in 86% of the HCC tumors. Overall, YAP nuclear localization did not correlate with increased YAP mRNA levels. YAP target gene mRNA levels were increased in the samples that had the most significant increase in YAP nuclear localization. Co-localization studies with Ki67 demonstrated that most YAP-positive cells were in the G0 state.

Conclusions: There are significant increases in nuclear and cytoplasmic YAP in pediatric HCC. Only a small subset of YAP-positive cells were in active cell cycle, and not all actively proliferating tumor cells were YAP-positive, suggesting that these cells may also have non-cell autonomous action.

Poster Number 36

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Global translatome analysis in chemotherapy resistant quiescent cancer cells

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Tumors promote cells that adapt to unfavorable environments by entering quiescence, a reversible state to escape irreversible arrest or apoptosis. Quiescent (G0) cells are a clinically relevant fraction in cancers like leukemia that resist clinical therapy and can eventually lead to cancer recurrence. G0 involves gene expression reprogramming, upregulating factors required for survival and cell specific functions. G0 cells do not show much transcriptional upregulation; however, profound changes in gene expression were observed, indicating post-transcriptional regulation in G0. Consistently, we recently identified that the general translation mechanism is altered in G0 cancer cells. Post-transcriptional mechanistic features including an RNA binding protein, and microRNAs, small RNAs that target distinct mRNAs to alter gene expression, and are involved in maintaining G0 in myocytes and cancers, were found to have altered functions in G0 cells, where they are associated with alternate translation factors to regulate translation. We conducted global translational profiling in G0 leukemic monocytes and other cancers that exhibit resistance to chemotherapy and altered microRNA functions and translation to identify the novel therapeutic targets. We find expression of critical regulators that can lead to metastatic changes and an inflammatory response. These include tumorigenic cytokines in quiescent cancer cells that are important for the persistence of the G0 state and chemoresistance and could lead to tumor persistence. Significantly, inhibiting these pro-inflammatory cytokines induces apoptosis of G0 cancer cells. Importantly, we find that the translation expression profile in G0 leukemic cells induced by nutrient deprivation is similar to chemoresistant cells that are isolated.

Poster Number 37

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Improving radiation therapy outcomes by targeting mitochondria using gold nanoparticles

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Gold nanoparticles (GNPs) have shown great potential as radiosensitizers in radiation therapy. Since damage to the genome affects the viability of a cell, it is generally assumed that GNPs have to localize within the nucleus, which is difficult to achieve in practice. However, there is substantial evidence suggesting that indirect processes, induced when the nucleus is not directly targeted by radiation, also influence cellular radiation responses. The mitochondrion with its many essential functional roles, is starting to be recognized as an effective extra-nuclear radiation target. Furthermore, GNPs may naturally attach to the mitochondrial membrane. Using Monte Carlo (MC) simulations, we investigate the significance of macroscopic dose enhancement from GNPs in cells irradiated with x-rays or protons. We found that adding a 1% mass fraction of gold to the cytosol increases the energy deposited in mitochondria more than the nucleus as a result of the larger surface area of the mitochondrion. We also investigate the track structure of secondary electrons in the mitochondria when GNPs are attached to the organelle outer membrane surface. These simulations show an increased number of ionization events within the mitochondrion structure, especially for photons. Large numbers of ionization clusters will induce damage to the mitochondrial DNA leading to mitochondrial dysfunction. Thus our simulations show that the mitochondrion is a potentially viable indirect radiation target for GNPs. If GNPs can be successfully delivered to a sufficient number of mitochondria in a tumor prior to irradiation, mitochondrial induced cell death could be a prevalent cause of apoptosis.

Poster Number 38

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Laparoscopic Staging for Presumed Stage I Epithelial Ovarian Cancer: Analysis of the National Cancer Data Base

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Purpose: To evaluate the association of laparoscopic staging with survival among women with presumed stage I epithelial ovarian cancer.

Patients and Methods: We conducted a retrospective propensity score matched cohort study using the National Cancer Data Base. We included women who underwent surgical staging for apparent stage I epithelial ovarian cancer diagnosed in 2010 and 2012. Propensity to undergo laparoscopic surgery was calculated using a logistic regression model which included demographic, socioeconomic, and clinical variables. We employed an optimal 1:1 matching algorithm to select a propensity matched cohort, and performed survival analysis using the Kaplan-Meier method and Cox regression.

Results: Of the 4,798 patients who met study criteria, 1,112 (23.2%) underwent laparoscopic staging. Compared to patients staged by laparotomy, those undergoing laparoscopic staging were more frequently white, privately insured, from wealthier zip codes, and received care in community cancer centers. Patients undergoing laparoscopy had smaller tumors, with histology that was more often serous, and less frequently mucinous. In a well balanced propensity score matching cohort of 2,192 patients, there was no difference in overall survival between surgical approaches ($p = 0.13$). Compared to laparotomy, laparoscopic staging was associated with shorter hospital stay ($p<0.001$) and slightly higher lymph node count ($p=0.005$), but there were no differences in frequency of 30 day readmission ($p = 0.37$), or 90 day mortality ($p=0.10$).

Conclusion: Surgical approach was not associated with survival in women with presumed stage I epithelial ovarian cancer.

Poster Number 39

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Reactive Oxygen Species Induction in Oncolytic Virus Therapy of Glioblastoma

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Reactive oxygen species (ROS) are signaling molecules that play versatile roles in regulating various cellular pathways in both physiological and pathological conditions such as cancer and virus infection. The effects of ROS on viral replication are context-dependent and the role of ROS in oncolytic virus therapy of cancer is poorly understood. In this study, we investigated ROS induction and its potential impacts on oncolytic herpes simplex virus (oHSV) therapy of the malignant brain tumor glioblastoma. We employed a clinically relevant glioblastoma model that is based on patient-derived glioblastoma stem cells (GSCs) that recapitulate the genotype and phenotype of primary tumors. Two oHSVs were used; G47Δ that lacks γ34.5 and α47 and has a LacZ insertion inactivating ICP6, and MG18L that contains deletion of the Us3 gene and a LacZ insertion inactivating ICP6. Measurement of intracellular ROS by H2-DCFDA and Mito SOX assays showed that both oHSVs (G47Δ and MG18L) robustly induced intracellular ROS in GSCs at 24 and 48 hours post infection, which was specifically seen in infected cells. Antioxidants, reduced glutathione (GSH) and N-acetylcystein (NAC), potently suppressed oHSVs-induced intracellular ROS. Antioxidants-mediated suppression of intracellular ROS did not affect viral replication or viral spread. However, cell viability assays (MTS and Cell Titer Glo) and dead cell staining (trypan blue or propidium iodide) demonstrated that both antioxidants protected GSCs from early cell death caused by the viruses. Thus we show that oHSV is a potent inducer of ROS upon infection of GSCs.

Poster Number 40

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Sentinel lymph node metastases in breast cancer: A contributor to distant metastases?

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Cancer metastasis remains a major cause of mortality in patients. Although significant progress has been made in understanding the mechanisms of this complex process, the findings have yet to be translated into improved survival rates in patients with metastatic disease. The presence of lymph node metastasis in most breast cancer patients is associated with tumor aggressiveness, poorer prognosis and often results in the need for systemic therapy. However, whether tumor cells in the lymph node exit and contribute to distant metastases remains controversial. To track the fate of tumor cells that metastasized to the lymph node, we engineered breast tumor cell lines to express Dendra2, a photo-convertible protein that fluoresces green in the native state and on light activation converts to red fluorescence. Using a novel chronic lymph node window that allows time-lapse imaging over a period of 10 days, we were able to track the movement of cancer cells in the dynamic microenvironment using high- resolution multi-photon microscopy. Our studies show that tumor cells entering the lymph node through the afferent lymphatic vessel proliferate in the sub-capsular sinus and later begin to invade the lymph node parenchyma. Importantly, by photo-conversion of tumor cells in the lymph node, we are able to track the tumor cells that escaped the lymph node and entered the blood circulation. The circulating tumor cells that transited through the lymph node were viable and proliferated *in vitro*. Our data indicate that metastatic breast cancer cells can exit the node and potentially colonize distant organs.

Poster Number 41

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Dynamic redox regulation in Ras-driven tumorigenesis

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Oncogenic Ras mutations are frequently observed in human cancers and are associated with poor prognosis and chemoresistance. Thus, there is a substantial unmet need to elucidate the key metabolic vulnerabilities of Ras-driven cancers that in turn can be exploited for therapeutic benefits. We focus on an indispensible regulator of TOR signaling, REDD1, which is induced upon hypoxia and energy stress. Our previous work has defined REDD1 as a key metabolic regulator that controls ROS, autophagy and glycolytic metabolic reprogramming. While deregulation of REDD1 function has been linked to numerous human cancers including pancreatic ductal adenocarcinoma, the relevant mechanism remains unknown. To fully assess the cooperation between REDD1 loss and KRas activation, I developed two pre-clinical mouse models, employing both genetic ablation and somatic knockdown of REDD1 in Ras-induced lung and pancreatic cancer models, respectively. Here, I consistently observed dramatic enhancement of Ras-mediated tumor progression in both models. Strikingly, the KRasG12D;Redd1-/- lung tumors generated through intratracheal delivery of adenovirus-Cre recombinase to LSL-KRasG12D; Redd1+/+ and Redd1-/- mice display highly aggressive behavior as evidenced by hematogenous metastasis to distant organs. Functional analysis revealed that KRasG12D; Redd1-/- primary cells displayed mitochondrial respiratory defects and elevated antioxidant capacity, resulting in addiction to glutamine through reductive carboxylation for generation of cellular building blocks as detected by metabolic profiling analysis. In summary, we find that REDD1 deficiency co-operates with oncogenic KRas activation *in vivo*. Ongoing work seeks to define and validate the molecular mechanisms particularly the redox dysregulation underlying this dynamic cooperation.

Poster Number 42

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Feasibility of the LUM Imaging System for real-time, intraoperative detection of residual breast cancer in lumpectomy cavity margins

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Background: Tumor-free margins are critical for local control in breast conserving surgery. Currently, 20–40% of lumpectomy patients have positive margins that require surgical re-excision. We assessed the LUM 015/LUM 2.6 imaging device, LUM system, for real-time detection of residual tumor in breast cancer patients.

Methods: Lumpectomy cavity walls and shaved cavity margins (SCM) of patients undergoing lumpectomy surgery for invasive cancer or ductal carcinoma in situ (DCIS) were assessed using the LUM system. LUM015, a cathepsin-activatable fluorescent agent, was injected intravenously prior to surgery. Sites of fluorescence were correlated with histopathology findings.

Results: 10 patients undergoing lumpectomy surgery received LUM015 dye and were scanned intraoperatively. Median age was 61 years (range 48-78), 70% percent had invasive ductal carcinoma (IDC) with DCIS, 10% invasive carcinoma with ductal and lobular features and DCIS, and 20% pure DCIS. Median tumor size was 1.8cm (range 0.4 – 2.5). 102 margin surfaces were assessed intraoperatively. The LUM system showed 100% sensitivity, 85% specificity, 56% positive predictive value, and 100% negative predictive value for detection of tumor at or near the margin (<2mm). There were no false negative results, but false positive readings were observed in 13% of margins. Two patients underwent re-excision surgery; in both cases, the LUM system correctly identified residual tumor in lumpectomy cavity walls at the initial surgery.

Conclusions: The LUM system allows instantaneous identification of residual tumor in the lumpectomy cavity of breast cancer patients and may reduce rates of positive margins. Additional studies are underway to optimize this approach.

Poster Number 43

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Hybridization-based RNA expression assays for profiling putative biomarkers for immunotherapy response in cancer

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Recent successes of immune checkpoint blockade represent a turning point in cancer treatment. Significant and durable anti-tumor responses have been observed in a subset of patients by therapeutically inhibiting immunosuppressive signals in the tumor, enabling the immune system to mount an effective anti-tumor response. However, despite complete responses observed in a subset of patients, not all patients will similarly benefit. Thus, there is a need to identify novel biomarkers that can help stratify patients for single or combination immunotherapies.

We have developed an immune-related RNA expression assay using the Affymetrix/eBioscience QuantiGene Plex (QGP) platform to identify new correlates of immunotherapy response. This multiplexed hybridization-based approach simultaneously evaluates the expression of >120 immune-related genes that provide a signature related to tumor neoantigen burden, inflammation, immune infiltrate type, immune infiltrate orientation, immune cell trafficking, immunosuppression, and immune exhaustion. We are testing this QGP immunopanel assay in archival patient tissues (FFPEs) across different cancer types, including those treated with immune checkpoint inhibitors at the MGH. Targets of interest are further evaluated using RNA ISH to gain further context of the RNA marker in the larger tumor microenvironment.

We present preliminary observations of immune-related signatures across a variety of tumor types and their association with therapeutic response. In addition, new RNA ISH assays have been developed to identify the presence of T-cell exhaustion using markers such as Tim-3 and Lag-3. Using these platforms, we aim to discover novel biomarkers that could be used to more effectively stratify patients to existing and emerging immunotherapies.

Poster Number 44

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AKT Inhibition Promotes Non-autonomous Cancer Cell Survival

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Small molecule inhibitors of AKT (v-akt murine thymoma viral oncogene homolog) signaling are being evaluated in patients with various cancer types, but have so far proven therapeutically disappointing for reasons that remain unclear. Here, we treat cancer cells with subtherapeutic doses of Akti-1/2, an allosteric small molecule AKT inhibitor, in order to experimentally model pharmacologic inhibition of AKT signaling *in vitro*. We then apply a combined RNA, protein, and metabolite profiling approach to develop an integrated, multiscale, molecular snapshot of this "AKTlow" cancer cell state. We find that AKT-inhibited cancer cells suppress thousands of mRNA transcripts, and proteins related to the cell cycle, ribosome, and protein translation. Surprisingly, however, these AKT-inhibited cells simultaneously upregulate a host of other proteins and metabolites posttranscriptionally, reflecting activation of their endo-vesiculo-membrane system, secretion of inflammatory proteins, and elaboration of extracellular microvesicles. Importantly, these microvesicles enable rapidly proliferating cancer cells of various types to better withstand different stress conditions, including serum deprivation, hypoxia, or cytotoxic chemotherapy *in vitro* and xenografting *in vivo*. These findings suggest a model whereby cancer cells experiencing a partial inhibition of AKT signaling may actually promote the survival of neighbors through non-cell autonomous communication.

Poster Number 45

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Extreme vulnerability of IDH1 mutant cancers to NAD+ depletion

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Recently-discovered recurrent mutations in canonical metabolic enzymes highlight the importance of altered metabolism in cancer pathogenesis. Heterozygous mutation of IDH1 in cancers modifies IDH1 enzymatic activity, reprogramming metabolite flux and markedly elevating 2-hydroxyglutarate (2-HG), resulting in altered HIF activity, widespread chromatin alteration, stem-like cells differentiation, and CpG island methylator phenotype. Using several IDH1 mutant solid cancer cell lines including patient-derived glioma stem-like cells, we found that 2-HG depletion did not inhibit tumor growth or alter epigenetic state of IDH1 mutant cells. We therefore searched for other metabolic targets, with the hypothesis that mutant IDH1 generates an oncogenic metabolic state with specific metabolic dependencies. We systematically profiled metabolites in endogenous IDH1 mutant cancer cells after mutant IDH1 inhibition and discovered a profound vulnerability to depletion of the coenzyme NAD+. Mutant IDH1 lowered NAD+ levels by downregulating the NAD+ salvage pathway enzyme nicotinate phosphoribosyltransferase (Naprt1), sensitizing to NAD+ depletion via concomitant nicotinamide phosphoribosyltransferase (NAMPT) inhibition. NAD+ depletion activated the intracellular energy sensor AMPK, triggered autophagy and resulted in cytotoxicity. NAMPT inhibitor treatment significantly extended overall survival and depleted NAD+ within the tumor tissue in an IDH1 mutant orthotopic xenograft model. Thus, we identify NAD+ depletion as a metabolic susceptibility of IDH1 mutant cancers.

Poster Number 46

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SREBP1-driven Lipogenesis Pathway in Melanoma

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Cancer cells have peculiar metabolic pathways that allow them to survive, proliferate, and metastasize under adverse (e.g. anaerobic and nutrient-deficient) conditions. Three hallmark phenotypes of cancer cells are: elevated de novo fatty acid biosynthesis (lipogenesis), aerobic glycolysis, and DNA synthesis. Aggressive tumors, constitutively synthesize very high levels of lipid to support elevated proliferation and evade apoptosis. In contrast, most normal tissues rely on circulating lipids, and hence have limited lipogenesis. My research work is investigating whether the abnormal lipid metabolism in malignant cancers may represent a linchpin and unique therapeutic target. We found the genetic upregulation of sterol regulatory element binding factor 1 (SREBF1) and a subunit of mediator complex (Med15) was associated with the skin cutaneous melanoma patients with low overall survival and disease free survival rates. We found that both antisense oligonucleotides that depleted SREBF1 mRNA and small molecules that inhibited SREBF1 transcription activity led to cell death of melanoma cell lines. We showed that SREBF1 was the master regulatory factor of a principal step in neoplastic lipogenesis: SREBF1 controlled the mRNA expression of a range of genes that encode essential lipid producing enzymes such as fatty acid synthase (FASN), stearoyl CoA desaturase (SCD) and acetyl CoA carboxylase (ACACA) to synthesize fatty acids in melanoma cell lines. SREBF1's mechanism of action rested on RNA polymerase II (polII) to transcribe lipogenic genes via tethering Med15 at gene bodies. Our work indicates that SREBF1 is a potential therapeutic target for treatment of late-stage melanoma.

Poster Number 47

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Novel Oral Anticoagulant and Warfarin Use Over Time in a Primary Care Network

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There is limited data about the uptake of novel oral anticoagulants (NOACs) for stroke prevention in patients with atrial fibrillation (AF). We examined the use of OACs among patients with AF at high risk of stroke following the introduction of NOACs. The study cohort includes adult patients with AF cared for in a primary care network between 2010 and 2014. We considered patients to be using a medication if they had at least 1 prescription in that year. We examined anticoagulation usage over time among all patients with AF, and among patients at high risk of stroke (CHA2DS2-VASc ≥ 2). We assessed trends over time using the Cochran-Armitage test for trend. In 2014, there were 4959 patients with AF, representing 3.1% of the total population. Over the study period, NOAC usage increased from 0.31% of AF patients in 2010 (dabigatran) to 16.4% (apixaban 2.5%; dabigatran 4.1%; rivaroxaban 9.8%) in 2014 (p-value <0.001). The proportion of AF patients taking warfarin decreased from 60.3% in 2010 to 45.2% in 2014 (p<0.001). There was little change in the proportion of patients not prescribed any anticoagulation treatment from 2010 (39.4%) to 2014 (38.4%) (p=0.09). Among patients with a CHA2DS2-VASc score ≥ 2, the proportion of patients anticoagulated did not increase following introduction of NOACs (2010: 66.2% ; 2014: 66.4%, p=0.99). Increased use of NOACs appears to be replacing rather than adding to warfarin use. Anticoagulation rates and the proportion of patients at high risk of stroke that are anticoagulated have not increased over time.

Poster Number 48

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Cardiotoxic Brain Infarcts: A Voxel-Based Neuroanatomic Correlation Study

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Introduction: Acute brain infarcts can lead to injury to the heart in the absence of primary cardiac causes, with serious outcomes ranging from myocardial damage to sudden death.

Hypothesis: To investigate the neuroanatomic substrate of myocardial injury occurring after ischemic stroke using a method that is free from the bias of a-priori hypotheses as to any specific location.

Methods: We examined the relationship between infarct location and plasma Troponin-T elevation (cTnT-E) and QT-segment prolongation on ECG (QTc-P) in a prospective study of 1208 consecutive patients. Infarct outlines on diffusion-weighted images were co-registered to a standard template, spatially smoothed, and re-sampled at 1 mm isotropic resolution. We calculated t-statistics at each voxel, generated p-value maps using a permutation-based approach, and identified the clusters of contiguous voxels associated with cardiac abnormalities with a p-value <0.05.

Results: We found two clusters, one for cTnT-E and one for QTc-P. Both clusters were within the right insula. Patients with infarcts that encompassed these clusters were less likely to achieve functional independence and more likely to die at 90-days; OR (95%CI) for favorable outcome was 0.39 (0.27-0.56) for cTnT-E and 0.55 (0.39-0.81) for QTc-P after adjustment for infarct volume and other important confounders. For 90-day mortality, the adjusted OR was 0.50 (0.32-0.77) for cTnT-E and 0.63 (0.40-0.99) for QTc-P.

Conclusion: Infarcts encompassing the right insula are independently associated with myocardial injury and adverse clinical outcomes. Identification of a cerebral site for stroke-related myocardial injury could facilitate development of novel strategies to improve stroke outcome.

Poster Number 49

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Rare, Damaging Mutations in Lipoprotein Lipase, Elevated Triglycerides, and Risk for Coronary Artery Disease

INVESTIGATORS: A. V. Khera, H. H. Won, S. Kathiresan, Myocardial Infarction Genetics Consortium

Background: For complex traits such as coronary disease, identification of individuals who share a driving pathophysiology may lead to more effective therapies. Rare mutations in relevant genes provide one such opportunity for participant stratification. Here, we determine if damaging mutations in the lipoprotein lipase (LPL) gene, a key regulator of triglyceride hydrolysis, associate with higher levels of triglyceride levels and risk for coronary artery disease.

Methods: We sequenced the exons of LPL in 10 case control cohorts (Total N = 22,533, including 12,395 controls and 10,318 participants with coronary artery disease). Rare (allele frequency <1%) damaging mutations included loss of function variants, missense variants annotated as pathogenic in the ClinVar clinical genetics database, and missense variants predicted to be damaging by each of 9 computer prediction algorithms.

Findings: Damaging mutations in the LPL gene were identified in 14 of 12,395 control participants (0.11%, 1 in 885) and 37 of 10,138 (0.36%, 1 in 274) individuals with coronary disease. Multivariate median regression analysis noted a 46 mg/dl increase in triglyceride levels (95%CI 14 – 77 mg/dl; p = 0.005) in mutation carriers as compared to non-carriers; no such differences were noted in HDL or LDL cholesterol levels. Adjusted logistic regression analysis noted a 3.1 fold increased risk of coronary disease (95%CI 1.7 – 5.9; p = 0.0004) in LPL mutation carriers.

Interpretation: About 1 in 885 individuals carry a damaging mutation in the LPL gene that is associated with higher plasma triglycerides as well as substantially increased risk for coronary disease.

Poster Number 50

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Low-density Lipoprotein Cholesterol, Familial Hypercholesterolemia Mutation Status, and Risk of Coronary Artery Disease

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Background: Although many assume that individuals with severe hypercholesterolemia (LDL-C ≥190 mg/dl) have familial hypercholesterolemia (FH), the prevalence of a FH mutation in this population has not been adequately studied. Secondly, whether a FH mutation confers additional coronary artery disease (CAD) risk in those with severe hypercholesterolemia is largely unknown.

Methods: Three genes causative for FH (LDLR, APOB, and PCSK9) were sequenced in 5,540 cases with CAD, 8,577 controls, and 11,908 prospective cohort study participants. FH mutations included null variants in LDLR, missense mutations in LDLR predicted to be damaging, and variants linked to FH in ClinVar, a clinical genetics database.

Results: Amongst 8,577 control participants, 430 had a LDL-C ≥190 mg/dl and of these, only eight (1.9%) carried a FH mutation. Similarly, only 17 of 956 (1.8%) prospective cohort participants with a LDL-C ≥190 mg/dl carried a FH mutation. In an extrapolation to the US population based on NHANES data, we estimate that 14.5 million adult Americans have an untreated LDL-C ≥190 mg/dl and of these, 412,000 also carry a FH mutation. For participants with LDL-C ≥190 mg/dl and no FH mutation, odds for CAD were increased six-fold (CI 5.2–6.9) when compared to a reference group with LDL-C <130 mg/dl and no mutation. By contrast, for participants with both LDL cholesterol ≥190 mg/dl and a FH mutation, odds of CAD were increased 22.3 fold (CI 10.7–53.2).

Conclusion: Only a small fraction of severely hypercholesterolemic individuals carry a FH mutation. However, these individuals are at substantially higher risk for CAD.

Poster Number 51

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An HDAC9-BRG1-MALAT-1 Nuclear Complex Involved in Thoracic Aortic Aneurysm (TAA) Pathogenesis

INVESTIGATORS: C. L. Lino Cardenas, Y. Cheng, M. E. Lindsay

TAA is an important source of morbidity and mortality affecting both children and adults. Gene discovery in families with aortic disease has identified two major categories of gene alterations. The first involves mutations in genes encoding various components of the transforming growth factor beta signaling cascade. These conditions are known collectively as the TGF-B vasculopathies.The second group encodes components of the smooth muscle contractile apparatus, and these disorders are termed the smooth muscle contraction vasculopathies. Patients with TGFBVs and SMCVs display similar anatomic distributions of aortic disease, progressive aortic enlargement, and risk of AoD. We therefore reasoned that the shared phenotype of ascending aortic aneurysm and clinical outcomes displayed in TGFBVs and SMCVs imply the possibility of a shared pathogenic molecular mechanism. To investigate our hypothesis loss-of-function gene perturbations were modeled in human aortic smooth muscle cells (AoSMCs) using small interfering RNAs (siRNAs). Microarray gene expression assays identified upregulation of the epigenetic modulator HDAC9, a class II histone deacetylase, as a shared feature of TGFBVs and SMCVs. Interestingly, the HDAC9 locus has been recently implicated in separate genome wide association screens (GWAS) for large vessel ischemic stroke, cerebral aneurysm and coronary artery disease, however its implication in thoracic aortic aneurysm is a completely novel observation. Further work has implicated the long non-coding RNA MALAT-1 in HDAC9-dependant nuclear function and that MALAT-1 and the protein BRG1 form a ternary complex with HDAC9. This work implicates the formation of a HDAC9-MALAT1-BRG1 pathogenic RNA-protein complex involved in AoSMC epigenetic cellular reprogramming in TAA.

Poster Number 52

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Isolation and co-culture of rat parenchymal and non-parenchymal liver cells to evaluate cellular interactions and response

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Liver is the central organ in human body, and first line of defense between host and external environment. Liver response to any external perturbation is a collective reaction of resident liver cells. Most of the current *in vitro* liver models focus on hepatocytes, the primary functional unit, and metabolic component, undermining interactions and cues from surrounding environment and non-parenchymal cells (NPCs). Recent studies suggest that contributions of NPCs are vital, particularly in disease conditions, and outcomes of drugs and their metabolites. Along with hepatocytes, NPCs—namely Kupffer (KC), sinusoidal endothelial (LSEC) and stellate cells (SC) are major cellular components of the liver. Incorporation of these primary cells in *in vitro* liver platforms is essential to emulate the functions of the liver, as well as its overall response. Herein, we isolate NPC cell fractions from rat livers and co-culture the cells in a transwell format incorporating primary rat hepatocytes with LSECs, SCs, and KCs. Our results indicate that the presence and contributions of multiple cells within the co-culture capture the interactions and modulates the responses between hepatocytes and NPC. The isolation and co-culture methods could provide a stable platform for creating *in vitro* liver models that provide defined functionality beyond hepatocyte alone.

Poster Number 53

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A specialized mechanism of translation mediated by FXR1a-associated microRNP in cellular quiescence

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MicroRNAs predominantly decrease gene expression; however, specific mRNAs are translationally upregulated in quiescent (G0) mammalian cells and immature Xenopus laevis oocytes by an FXR1a-associated microRNP (microRNA-protein complex) that lacks the microRNP repressor, GW182. Their mechanism in these conditions of decreased mTOR activity and therefore, reduced canonical (cap-and-poly(A)-tail-mediated) translation, remains undiscovered. Our data reveal that mTOR inhibition in THP1 cells enables microRNA-mediated activation. Activation requires shortened/no poly(A)-tailed targets; polyadenylated mRNAs are partially activated upon PAIP2 overexpression, which interferes with poly(A)-bound PABP, precluding PABP-enhanced microRNA-mediated inhibition and canonical translation. Consistently, inhibition of PARN deadenylase prevents activation. P97/DAP5, a paralog of the canonical translation factor, eIF4G, which lacks PABP- and cap binding complex-interacting domains, is required for activation and thereby, for the oocyte immature state. P97 interacts with 3′-UTR-binding FXR1a-associated microRNPs and with PARN, which binds mRNA 5′ caps, forming a specialized complex to translate recruited mRNAs in these altered canonical translation conditions.

Poster Number 54

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Targeting Ca2+ homeostasis rescues lysosomal phenotypes in neuronal cell models of juvenile NCL

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Juvenile neuronal ceroid lipofuscinosis (NCL, also referred to as Batten disease) is a lysosomal storage disorder with mostly neurodegenerative symptoms, caused by autosomal recessive mutations in CLN3, which encodes a late endosomal/lysosomal transmembrane protein. Previous work established evidence of abnormal Ca2+ homeostasis in CLN3 deficiency models including juvenile NCL patient cells, identifying particularly elevated intralysosomal Ca2+ levels compared to control cells. We have therefore tested whether treatments expected to reduce the abnormally elevated intralysosomal Ca2+ could restore lysosomal system abnormalities and neuronal cell health using genetically accurate mouse and human patient-based cell models. Overexpression of MCOLN1, which encodes a cationic efflux channel (TRPML1) of the late endosome/lysosome, in CLN3 mutant mouse neuronal cells normalized lysosomal morphology and reduced the accumulation of lysosomal storage material. Similarly, in a human patient induced pluripotent stem cell (iPSC)-derived neuronal cell system, treatment with the TRPML1 agonist, MLSA1, significantly improved lysosomal morphology and neuronal cell health. CLN3 patient iPSC-derived neuronal cells were more metabolically healthy, and a defect in neuronal differentiation was significantly improved following MLSA1 treatment. These studies emphasize the likely role of CLN3 in lysosomal Ca2+ homeostasis and imply that further development of treatments targeting this defect could lead to novel therapies for this devastating and fatal childhood disorder.

Poster Number 55

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DIGIT is a conserved long noncoding RNA that regulates GSC expression to control endoderm differentiation of human embryonic stem cells

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Endoderm differentiation is one of the earliest steps in lineage commitment, leading to development of the gastrointestinal organs, lungs and thymus. Transforming growth factor beta (TGF-beta) signaling initiates endoderm differentiation of embryonic stem cells. Analyses of the functions of protein-coding genes induced by TGF-beta signaling have led to significant insights into the control of endodermal differentiation; however, the identity and function of long noncoding (lnc) RNAs regulated by TGF-beta signaling remain largely unknown.

Using high-throughput RNA sequencing, we identified the lncRNAs induced during endoderm differentiation. We then used chromatin immunoprecipitation and sequencing (ChIP-seq) to define a subset of these lncRNAs that are direct targets of TGF-beta signaling effector SMAD3. In this subset, we discovered a conserved lncRNA, DIGIT, that was divergently transcribed from the development regulator gene Goosecoid (GSC) and induced during endoderm differentiation. We show that CRISPR-mediated deletion of the SMAD3 binding site proximal to lncRNA DIGIT results in loss of DIGIT and GSC expression and inhibits endoderm differentiation.

Disruption of DIGIT transcription by the CRISPR system and depletion of the DIGIT transcript reveal that DIGIT is a key regulator of endoderm differentiation. We find that DIGIT promotes endoderm differentiation of hESCs by regulating GSC expression and demonstrate that induction of endogenous GSC expression using a dead (d) Cas9 protein fused to an activator domain (dCas9-VPR) is sufficient to rescue endoderm differentiation in DIGIT-deficient hESCs. Our study defines DIGIT as a direct target of TGF-beta signaling and a key noncoding developmental regulator that is conserved between humans and mice.

Poster Number 56

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The MAPK/ERK-signaling pathway regulates the expression and distribution of tight junction proteins in the mouse proximal epididymis

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The initial segment (IS) in rodents is functionally and structurally distinct from other epididymal segments and plays an important role in sperm maturation. The MAPK/ERK1/2 pathway is maintained active in the IS by testicular luminal factors, and plays crucial roles in the maintenance and differentiation of the IS epithelium. Tight junctions (TJs) are constituents of the blood-epididymis-barrier, which mediates the paracellular transport of ions, solutes and water, and controls epithelial cell differentiation, thereby contributing to the establishment of a unique luminal environment. We examined here the role of the MAPK/ERK1/2 pathway in the regulation of TJ proteins in the IS. Inhibition of mitogen activated protein kinase kinase (MAPKK or MEK1/2) with PD325901, followed by reduction of ERK1/2 phosphorylation (pERK), decreased zonula occludens (ZO)-2 expression and increased ZO-3 expression in TJs, but had no effect on ZO-1 expression. In control mice, in addition to being located in TJs, claudin (Cldn)-1, Cldn-3 and Cldn-4 were detected in the basolateral membrane of epithelial cells, with enriched expression of Cldn-1 and -4 in basal cells. PD325901 reduced the expression of Cldn-1 and Cldn-4 at all locations without affecting Cldn-3. Occludin was undetectable in the IS of control mice, but PD325901 triggered its expression in TJs. No effect was observed for any of the proteins examined in the other epididymal regions. Our results indicate the participation of the MAPK/ERK1/2 pathway in the regulation of cell-cell events that control the formation and maintenance of the blood-epididymis-barrier.

Poster Number 57

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ROCK Isoforms ROCK 1 and ROCK 2 are Critical for the Development of Pulmonary Fibrosis in Several Different Cell Specific Mechanisms

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Background and Rationale: The actin cytoskeleton directs many pro-fibrotic cellular responses in pulmonary fibrosis, including epithelial cell apoptosis, endothelial cell barrier disruption and fibroblast migration and differentiation. The Rho kinases ROCK1 and ROCK2 regulate the actin cytoskeleton, making them logical targets for anti-fibrotic therapy. We used a genetic approach to identify the specific contribution(s) of each ROCK isoform to pulmonary fibrosis. Mice haploinsufficient, i.e. heterozygous for a mutant allele, for ROCK1, ROCK2 or both were challenged with intratracheal (IT) bleomycin, and their phenotypes were assessed.

Main Conclusions: We found that haploinsufficiency of ROCK1 or ROCK2 protects mice from bleomycin-induced pulmonary fibrosis. Significantly fewer lung epithelial cells were apoptotic early after bleomycin challenge in ROCK1+/- mice compared with WTs (3.68 +/-0.82 versus 9.7 +/-0.73 cells/HPF, p=0.04). ROCK2+/- mice had 7.3 +/-0.91 cells/HPF and ROCK1+/-2+/- mice had 4.1 +/-0.4 cells/HPF, neither of which were significantly different from WTs. Vascular leak induced by bleomycin challenge was markedly reduced in the lungs of mice haploinsufficient for ROCK1 or ROCK2 versus WTs. Evans blue vascular permeability index of WT mice (0.036 +/- 0.004) was significantly decreased in both ROCK1+/- (0.008 +/- 0.006, p=0.02) and ROCK2+/- mice (0.009 +/- 0.006, p=0.02). Fibroblast migration induced by bronchoalveolar lavage from bleomycin-challenged mice was reduced by siRNA targeting ROCK1 or ROCK2 to 77.9% (p=0.046) or 85% (p=0.058) of control fibroblasts. Myofibroblast differentiation identified by aSMA expression was also reduced in both ROCK haploinsufficient mice. ROCK signaling by both isoforms therefore is critically involved in multiple pro-fibrotic cellular functions.

Poster Number 58

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YB-1 mediated translational control of the senescence secretome in epidermal progenitors

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The integrity of stratified epithelia depends on the ability of progenitor cells to keep a fine balance between proliferation, differentiation and senescence. While accumulating evidence links various transcriptional and epigenetic pathways to stem cell maintenance in the epidermis, posttranscriptional regulation of gene expression by mRNA-binding proteins, a rate-limiting step in sculpting the dynamic of the cellular proteome, remains poorly understood. We applied an unbiased systematic approach to capture the mRNA interactome of epidermal progenitors and identified the RNA-binding protein YB-1 (Y-box binding protein-1) as a novel regulator of stem cell function *in vitro* and *in vivo*. YB-1 expression was restricted to the epidermal progenitor population and its genetic ablation led to stem cell defects in hair follicles, interfollicular epidermis and sebaceous glands. Global polysomal profiling revealed that YB-1 negatively controls protein translation by binding to the 3'UTR regions of a subset of cytokines involved in the promotion of cellular senescence. Our study has uncovered a new level of posttranscriptional control of senescence-associated cytokine biosynthesis by YB-1, which is required for maintenance of epidermal tissue homeostasis.

Poster Number 59

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Vascular Leak-Induced Thrombin-PAR1 Signaling Drives Pulmonary Fibrosis Following Lung Injury

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Background and Rationale: Increased vascular permeability has been demonstrated in the lungs of patients with idiopathic pulmonary fibrosis (IPF), but whether this vascular leak is mechanistically linked to the development of fibrosis is unknown. We have previously described a mouse model of pulmonary fibrosis in in which disruption of endothelial barrier integrity caused by administration of FTY720, a functional antagonist of S1P1 signaling, shifts the outcome of a mild lung injury, induced by low-dose bleomycin challenge, from lung repair to lung fibrosis. We hypothesized that increased extravascular coagulation induced by increased vascular permeability in this model promotes the development of fibrosis in a manner dependent on thrombin signaling through it major receptor protease-activated receptor-1 (PAR1), but independent of fibrin deposition.

Main Conclusions: Here we found dramatic protection from fibrosis produced in our vascular leak-dependent model with treatment with the direct thrombin inhibitor dabigatran. Dabigatran treatment was associated with decreased PAR1 activation, αvβ6 intergrin induction, and TGF-β activation. Treatment with an anti-αvβ6 mAb also protected from the development of pulmonary fibrosis in this model. In contrast, despite achieving therapeutic anticoagulation, treatment with the vitamin K antagonist warfarin did not protect from lung fibrosis in this model. Warfarin treatment also failed to decrease PAR1 activation, αvβ6 induction, or TGF-β activation. We conclude that vascular leak promotes pulmonary fibrosis by promoting extravascular coagulation, but that the pro-fibrotic effects of extravascular coagulation are mediated by thrombin signaling through a PAR1-αvβ6-TGF-β axis, rather than by coagulation, i.e. fibrin generation and deposition, itself.

Poster Number 60

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Single cell analysis of the bone marrow niche reveals novel regulators of hematopoietic regeneration

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Sluggish hematopoietic recovery post-transplant or following chemotherapy is a major cause of morbidity and mortality. We undertook a study of the bone marrow niche to identify novel extrinsic factors which influence hematopoietic stem and progenitor cell (HSPC) behavior. We transplanted fluorescently labeled HSPC into neonatal recipients with GFP-labeled osteolineage cells (OLC), an established cellular component of the niche, extracted individual OLCs located either close (proximal OLC) or further away (distal OLC) from transplanted HSPC and compared their transcriptional profile by single cell RNA-Seq, thus defining a proximal OLC signature. Using this signature, we selected three secreted factors with previously unknown niche function—cytokine Interleukin 18, cell adhesion molecule Embigin and secreted RNAse Angiogenin—for testing their effect on HSPC *in vivo*.

We found that all three molecules acted as regulators of quiescence while affecting different HSPC subsets: IL18 predominantly regulated short-term progenitors, while Embigin and Angiogenin also controlled long-term HSC. Testing each molecule in a therapeutically relevant setting revealed their potential clinical applications. Absence of IL18 significantly accelerated short-term multi-lineage post-transplant reconstitution and recovery post-chemotherapy, such as 5-fluorouracil (5FU), and improved post-transplant animal survival. Neutralization of Embigin also resulted in increased post-transplant HSPC proliferation and faster post-5U recovery. Somewhat counter-intuitively, ex-vivo treatment of mouse or human HSPC with Angiogenin, while promoting quiescence, endowed these cells with a markedly enhanced repopulation capacity following transplantation.

Thus, our study of HSPC niche regulatory function revealed the complexity of non cell-autonomous quiescence-controlling pathways and identified novel molecular tools to enhance hematopoietic regeneration.

Poster Number 61

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Nascent RNA Profiling Reveals Differentially Expressed LncRNAs Across S-Phase

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Different cell types in multi-cellular organisms heritably maintain different gene expression patterns despite carrying the same genome; a phenomenon termed epigenetics. Importantly, the regulatory interactions that allow these cell type specific expression profiles are maintained through numerous rounds of cell division. Yet, little is known about the factors responsible for the reestablishment of parental chromatin structure when cells divide. We hypothesize lncRNAs have a role in inheritance of silenced states through Polycomb group (PcG) protein targeting and that lncRNA levels will be up regulated at the G1/S border or during S phase. With the goal of better understanding the mechanisms by which cell type specific epigenetic states are established and inherited, I set to develop a new technology that utilize EU Click chemistry to isolate and investigate the nascent transcripts through out S phase in human RPE1 cells, with a focus on newly synthesized lncRNAs. This novel genome scale approach provided candidates for the functional analysis of lncRNAs and their potential role in chromatin maturation during S phase.

Poster Number 62

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Application of the 2015 ACS Screening Mammography Guidelines: Risk Assessment is Critical for Women Ages 40-44

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Introduction: The 2015 American Cancer Society (ACS) screening mammography guidelines suggest that women at 'average risk' of breast cancer may not require screening before age 45. Based on these guidelines, we assessed women ages 40-44 in our breast practice who would be eligible for screening mammograms, MRIs, and genetic testing.

Methods: We reviewed a database of all new female patients at a single institution from 3/3/2011–10/26/2015. Those with a ≥20% lifetime risk of breast cancer were considered MRI-eligible. Those with a ≥5% risk of a BRCA mutation, or met NCCN guidelines, were considered eligible for genetic testing. According to ACS guidelines, those meeting either criterion were considered above average risk and qualified for screening mammography.

Results: 6964 women age ≥40 were reviewed. Of these, 909 (13%) were ages 40-44 and constitute our cohort. Of this group, our risk assessment identified 352 women (39%) deemed above average risk by ACS criteria and were eligible to start screening at age 40. Of the cohort, 127 (13.8%) qualified for screening MRI, 59 (6.5%) were at risk for a suspected genetic mutation, and 166 (18.3%) qualified for both MRI and genetic testing.

Conclusion: Following the new ACS guidelines, 39% of women in our cohort would have been eligible for screening mammography at age 40. Some were at risk for a genetic mutation and/or qualified for an MRI. It is essential that women 40-44 have formal risk assessment, in order to identify those who qualify for screening mammography, MRIs, and genetic testing.

Poster Number 63

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The Impact of Cigarette Smoking on the Life Expectancy of People with HIV in the United States

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Background: In the US, >40% of people living with HIV (PLWH) smoke cigarettes. We sought to estimate the impact of smoking on life expectancy (LE) of PLWH, and to project the potential LE gains from smoking cessation.

Methods: We used a simulation model of HIV disease and treatment to project LE, based on current, former, or never smoking status, of PLWH in the US. We populated the model with published age- and sex-specific data on mortality associated with different smoking groups. We derived the potential impact of smoking cessation on LE for PLWH initiating care at different ages. We also projected the total life-years that could be gained if a proportion of HIV-infected smokers quit smoking.

Results: Among PLWH entering care at age 40y, men and women who continued to smoke lost 6.7y and 6.3y of LE compared to never smokers; men and women who quit smoking upon entering care and remained abstinent regained 5.7y and 4.6y of LE. Younger patients with higher initial CD4 counts gained the most from smoking cessation, but even those who quit at age 60y gained >2.0y of LE. Smoking cessation and sustained abstinence by 25% of the approximately 250,000 HIV-infected smokers aged 30-64y and in HIV care could save >265,000 life-years.

Conclusions: Among PLWH in the US initiating HIV care at age 40y, the loss in LE from continued smoking is >6 years per person. Smoking cessation, regardless of age, can markedly improve survival and should be a major priority in HIV treatment programs.

Poster Number 64

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Choice architecture and WIC fruit and vegetable purchases in a Latino community: randomized, controlled corner store intervention

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Background: Changes to the Special Supplemental Nutrition program for Women, Infants, and Children (WIC) in 2009 increased access to fruits/vegetables by providing cash-value vouchers, but more integrated policy and community-based approaches are needed to reduce disparities in healthy food choices.

Methods: We conducted a randomized, controlled trial of 6 WIC-certified corner stores in a low-income, Latino community. Three stores were assigned to "choice architecture" intervention that increased visibility and quality of fresh fruits/vegetables. Primary outcome was WIC fruit/vegetable voucher (FVV) sales, comparing changes in sales trends from baseline (December 2012–October 2013) to follow-up (December 2013–April 2014) for intervention vs. control stores. Secondary outcomes, from customer exit surveys, were changes between baseline and follow-up in self-reported purchase of fresh fruits/vegetables by customers using WIC or Supplemental Nutrition Assistance Program (SNAP).

Results: During baseline, WIC FVV sales decreased in both intervention and control stores. During follow-up, FVV sales increased in intervention but decreased in control stores (p=0.036). Exit surveys were completed by 575 customers; 23% used WIC, and 37% used SNAP. Compared to baseline, intervention store customers using SNAP increased purchase of fruits/vegetables at follow-up more than control store customers (6% vs. -15%, p=0.007). For customers on WIC, there was a similar but not statistically significant difference between intervention and control (18% vs. -2%, p=0.11).

Conclusions: A simple choice architecture intervention increased purchases of fruits/vegetables by corner store customers using WIC. Policies that incentivize WIC-certified stores to stock and prominently display good quality fresh produce could improve healthy choices of low-income families.

Poster Number 65

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Estrogen replacement improves verbal memory and executive control in oligo-amenorrheic athletes in a randomized controlled trial

INVESTIGATORS: C. Baskaran, B. Cunningham, F. Plessow, V. Singhal, R. Woolley, K. E. Ackerman, E. A. Lawson, K. Eddy, M. Misra

Both estrogen and exercise may have cognition enhancing benefits, yet young oligo-amenorrheic athletes (OA) with estrogen deficiency have not been evaluated for cognitive deficits. Our objective was to determine whether 6 months of estrogen replacement will impact cognitive domains in OA. We hypothesized that estrogen replacement would improve verbal memory (VM) and executive control in OA.

Methods: We performed cognitive assessments at baseline and after 6 months in 48 OA (14-25 years) randomized to estrogen (EST+) (oral 30 mcg ethinyl estradiol (n=16) or transdermal 100 mcg 17-beta-estradiol patch (n=13)), or no estrogen (EST-) (n=19) in an ongoing clinical trial. Neurocognitive testing included California Verbal Learning Test II (CVLT-II) (for VM), and Delis-Kaplan Executive Function System Color-Word Interference Test (DKEFS-CWIT) (executive control).

Results: On an average subjects (age: 19.9±3.1 years BMI: 20.6±2.3 kg/m2) participated in 10.3±5.9 hours/week of weight-bearing activities of lower limbs. The EST+ group performed better for CVLT-II VM scores for immediate recall over 6 months of therapy compared to EST- ($p<0.05$) even after controlling for BL scores and age. Changes in D-KEFS-CWIT over 6 months did not differ between the groups. However, the EST+ group had greater improvements in inhibition switching completion time over 6 months compared with the EST- group after controlling for BL scores and age ($p=0.01$).

Conclusion: OA show improvements in VM and executive control following 6 months of estrogen replacement. These findings in athletes, who are in their prime of neurocognitive development, underscore the need for future studies exploring cognition in OA.

Poster Number 66

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Neuroactive steroids and affective symptoms in women across the weight spectrum

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Allopregnanolone (allo), a progesterone (prog) metabolite, is a neuroactive steroid and potent positive GABAA modulator. Small studies have suggested low allo levels in patients with major depressive disorder and in patients with PTSD. However, little is known about allo across the weight spectrum, including anorexia nervosa and obesity. We hypothesized that serum allo would be associated with affective symptoms independent of weight in 36 women 1:1 age-matched across 3 groups: DSM-5 AN (BMI<18.5 kg/m2), healthy controls (HC)(BMI=19-24 kg/m2), and obese/overweight (OB)(BMI≥25 kg/m2). AN were amenorrheic. HC and OB were eumenorrheic and in the follicular phase. Serum hormones were measured by mass spectrometry. Mean Hamilton depression (AN 11.9±5.4 vs. OB 5.0±4.4 vs. HC 1.3±1.6, $p<0.05$) and anxiety scores were highest in AN (AN 9.9±4.3 vs. OB 3.1±0.9 and HC 1.6±2.2, $p<0.0001$). Mean serum allo was lower in AN and OB than HC (AN 95±56 and OB 74±29 vs. HC 199±168 pg/mL, $p<0.02$), despite comparable mean serum prog (AN 53±59, HC 62±43, OB 45±37 pg/mL). Allo levels were negatively associated with severity of depression ($r= -0.45$,$p=0.03$) and anxiety ($r= -0.37$,$p=0.04$) independent of BMI, with 15% of the variability in depression and 11% of the variability in anxiety attributable to allo, despite no association with serum prog. Therefore, women at both extremes of the weight spectrum have low mean serum allo, which is negatively associated with depression and anxiety severity, independent of BMI. Neuroactive steroids such as allo may be potential therapeutic targets for depression in traditionally treatment-resistant groups such as AN.

Poster Number 67

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Oxytocin secretion in men

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Oxytocin (OXT) is increasingly recognized for its role in the regulation of energy metabolism. Data regarding resting state OXT secretory patterns in men are limited; we therefore examined the secretory dynamics of peripheral OXT in healthy men, hypothesizing a pulsatile pattern.

Serum OXT levels were obtained every 5 minutes from 2200h-0800h to obtain 120 samples/subject. OXT was measured following extraction (Assay Designs ELISA; intra-assay CV 17%, sensitivity 3.5 pg/ml). Secretory dynamics were analyzed using deconvolution analysis.

Five healthy normal-weight men (mean±SEM age 22.8±1.2 years, BMI 21.7±0.4 kg/m2) were included. The CV for OXT levels among all subjects was 24%. Within each individual, the CV for OXT levels ranged from 33-51%. OXT area under the curve was 5421±1331 pg/ml/10h and the mean OXT level was 9.1 pg/ml. Assuming a basal secretion of zero and a half life of 5 minutes we demonstrated a mean of 22±3 significant pulses over the ten hour period. The mean pulse height, pulse mass and inter pulse interval were 1.81±0.48 pg/ml, 30.34±10.29 pg/ml and 27±4 minutes, respectively. Deconvolution analysis resulted in high residuals indicating a residual difference between the actual data points and the fitted curve. The shape of secretory events could not be completely resolved, suggesting a need for more frequent sampling.

These data define biological variation and demonstrate a pulsatile pattern of peripheral OXT secretion. To optimize the resolution of deconvolution analyses, future studies using samples <5 minutes apart to evaluate OXT secretory patterns and their impact on energy metabolism are warranted.

Poster Number 68

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Genetically driven hyperglycemia increases risk of coronary artery disease m type 2 diabetes

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Observational evidence suggests that type 2 diabetes (T2D) is associated with coronary heart disease (CHD). However, whether hyperglycemia itself increases CHD risk remains unclear. To elucidate the putative role of glycemia on CHD, we tested the hypothesis that genetically raised fasting glucose (FG) was associated with increased CHD risk independently of the risk conferred by T2D. We conducted a Mendelian randomization analysis using summary-level statistics from large genome-wide association studies (GWAS) for FG (n=133,010) and CHD (n=63,746/130,681 cases/controls). Excluding FG-related genetic variants that increase T2D risk from DIAGRAM (n=34,840/114,981 cases/controls) at nominal significance (P value<0.05), we identified 12 independent FG risk-increasing genetic variants. Using 11 FG risk-increasing candidate variants as the polygenic instrument (one was excluded for heterogeneity), we found that genetically raised FG increased CHD risk (odds ratio [OR] = 1.34 [1.07–1.68] per 1 mmol/L increase in FG, P=0.01, heterogeneity P value=0.88). The 11 FG-raising genetic variants explained 5.03% of the genetic variance in FG the Framingham Heart Study (n=5,113). The association was preserved after omitting genetic variants that have pleiotropic associations with blood pressure, blood lipids and body mass index (OR 1.33 [1.02–1.73] per 1 mmol/L increase in FG, P=0.03, heterogeneity P value=0.89). In conclusion, our findings quantify the causal relationship between isolated genetically increased FG and CHD risk separately from the genetic effect of T2D and other major CHD risk factors. Our results provide additional evidence for the design and interpretation of clinical trials of glycemic interventions on CHD outcomes.

Poster Number 69

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Intranasal Oxytocin Increases Proactive Cognitive Control and Strengthens Resting-State Functional Connectivity Within the Cognitive Control Network in Overweight and Obese Men

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Background: Obesity is a major public health concern in need of novel treatments. Cognitive control, suppressing behavioral impulses to support goal achievement, represents a promising therapeutic target, as obese individuals show increased impulsiveness and hypofunctioning of the cognitive control network (CCN). Oxytocin (OXT) has been shown to reduce caloric intake and weight. However, the underlying mechanisms are unclear. We hypothesized that OXT would increase cognitive control and strengthen resting-state functional connectivity (rs-fc) within the CCN as a potential mechanism for explaining OXT effects on food intake.

Methods: We performed a randomized, double-blind, placebo-controlled crossover study with 24 IU intranasal OXT in 10 healthy overweight/obese men. After receiving OXT or placebo, participants completed a task assessing strategy and ability to suppress behavioral impulses (stop-signal task). Furthermore, we investigated differences in fMRI BOLD signal in CCN during rest and while participants viewed food versus non-food images.

Results: After receiving OXT, subjects displayed fewer incidences of impulsive behavior ($p=0.049$) and decreased response speed ($p=0.012$). Furthermore, OXT increased BOLD signal in the anterior cingular cortex (ACC; $p<0.02$) when subjects viewed high-calorie foods compared to objects. OXT also enhanced rs-fc between key CCN regions, i.e., ACC and dorsolateral prefrontal cortex ($p<0.005$) as well as posterior parietal cortex ($p<0.002$).

Conclusion: OXT improves cognitive control in overweight/obese men. Since subjects could not reliably differentiate between OXT and placebo, we assume that the observed changes occurred without deliberation, e.g., via interoceptive signaling. Additional research is required to establish the predictive value of altered cognitive control for food intake.

Poster Number 70

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Absence of Vitamin D Receptor (VDR)-Regulated PPARg Suppression Causes Alopecia in VDR Null Mice

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VDR null mice (VDR-/-) exhibit alopecia, lack of postmorphogenic hair cycling, reduced keratinocyte stem cell (KSC) number/function, and lipid-laden dermal cysts. Herein, we delineate the underlying molecular defect(s) and demonstrate a genetic rescue strategy. RNA-Seq and Ingenuity Pathway Analysis of VDR-/- versus Wild Type (WT) KSCs identified 181 differentially regulated genes (>2 fold) involved in lipid metabolism. Among these, 157 genes--such as, PPARg, PGC1b, and LPL, a classic PPARg target--were upregulated in VDR-/- KSCs, demonstrating that the VDR is a transcriptional repressor. ChIP-Seq of KSCs identified VDR binding sites in the regulatory regions of the PPARg(Site1), PGC1b(Site1), and LPL(Site2) genes. Both the VDR and PPARg proteins are transcriptional regulators; ChIP-qPCR analyses in WT keratinocytes demonstrated that while VDR is recruited to PPARg(Site1), PGC1b(Site1), and LPL(Site2), PPARg bound only to PGC1b(Site1) and LPL(Site2). In VDR-/- keratinocytes, PPARg bound to PPARg(Site1). Thus, the VDR precludes PPARg from binding to PPARg(Site1). Co-immunoprecipitation analyses in transiently transfected COS-7 cells and WT keratinocytes demonstrated a physical interaction between the VDR and PPARg proteins. To determine whether increased PPARg expression contributes to alopecia in VDR-/- mice, VDR-/- with PPARg haploinsufficiency in keratinocytes (VDR-/-Keratin14CrePPARgf/+) were depilated at postnatal day 18. Induction of hair cycling by depilation induced mRNA expression of genes characteristic of anagen in WT and VDR-/-Keratin14CrePPARgf/+, but not VDR-/-. This was accompanied by hair follicle proliferative regeneration and hair coat formation in VDR-/-Keratin14CrePPARgf/+. No dermal cysts were observed in VDR-/-Keratin14CrePPARgf/+. Therefore, the VDR suppresses PPARg to maintain healthy KSCs/keratinocytes, and prevent alopecia and dermal cysts.

Poster Number 71

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Bone mineral density, body composition, and psychopathology of anorexia nervosa spectrum eating disorders in DSM-IV versus DSM-5

INVESTIGATORS: M. Schorr, J. T. Thomas, K. T. Eddy, L. E. Dichtel, E. A. Lawson, E. Meenaghan, M. Lederfine Paskal, P. K. Fazeli, A. T. Faje, M. Misra, A. Klibanski, K. K. Miller

Context: The Diagnostic and Statistical Manual of Mental Disorders, DSM-5, revised diagnostic criteria for anorexia nervosa (AN) by eliminating the amenorrhea requirement, liberalizing weight and psychological criteria, and adding "atypical AN" for individuals with AN psychological symptoms but not low weight. Understanding the implications of these new diagnostic criteria for bone density (BMD) screening is important.

Objective: To determine whether BMD is impaired in women diagnosed with AN using the new, more liberal diagnostic criteria

Design: Cross-sectional

Setting: CRC

Participants: 168 women, 18-45y: 1) women with AN by DSM-IV(n=37), 2) women diagnosed with AN by DSM-5 who would not have fulfilled DSM-IV criteria (n=33), 3) women with ATYPICAL AN(n=77), 4) healthy controls (HC)(n=21)

Measures: DXA, Eating Disorder Examination-Questionnaire, Eating Disorder Inventory-2, Hamilton Depression and Anxiety Rating Scales.

Results: BMD Z-score <-1.0 was present in 78% of DSM-IV, 82% of DSM-5, and 69% of ATYPICAL($p=0.28$). Mean Z-scores were comparably low in DSM-IV and DSM-5, intermediate in ATYPICAL, and highest in HC($p<0.05$). ATYPICAL without prior low weight (24%) had lower, but ATYPICAL with no history of amenorrhea (17%) had similar, mean spine Z-scores to HC($p<0.05$). DSM-IV, DSM-5 and ATYPICAL had comparable psychopathology.

Conclusions: Despite the liberalization of weight, menstrual and psychological diagnostic criteria, a high percentage of women diagnosed with AN using DSM-5 criteria have low BMD and should be screened for bone loss. Normal weight (including in the past) does not fully protect against bone loss. The deleterious effect of eating disorders on BMD extends beyond those with low-weight and amenorrhea.

Poster Number 72

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Marrow Adipose Tissue in Relation to Bone Density in Adolescent Girls with Anorexia Nervosa and Normal Weight Controls

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Background: Anorexia nervosa (AN) increases fracture risk and risk is higher if AN develops earlier in life. Although AN is associated with a reduction in total fat mass, adults with AN have increased marrow adipose tissue (MAT), which is inversely associated with bone mineral density (BMD). This is consistent with the recognized concept of a common progenitor stem cell that can differentiate along the adipocyte or osteoblast lineage depending on specific transcription factors and the hormonal milieu. Hormone deficiencies including estrogen would increase adipocyte differentiation and may explain the higher MAT and lower BMD seen in adults with AN. However, MAT also increases in normal adolescence, a time when bone is being actively accrued. The impact of AN on the quantity of MAT at different sites, and its association with BMD have not been evaluated in this age group. Here, we evaluate MAT content in adolescents with AN and normal weight controls (C) in relation to BMD.

Here we show that MAT content was greater in AN than C: L4 (0.82±0.45 vs. 0.49±0.23; p=0.016), femoral diaphysis (7.05±2.69 vs. 4.55±2.38; p=0.014) and femoral metaphysis (5.73±2.14 vs. 2.76±1.44; p=0.0001). There were inverse correlations between BMD and MAT at various sites. L4 MAT was an independent inverse predictor of lumbar BMD after controlling for BMI and the number of menses in the previous year.

We conclude that MAT content may be an independent predictor of BMD.

Poster Number 73

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Whole genome sequencing (WGS) identifies novel genes regulating human reproduction in patients with "balanced" chromosomal rearrangements (BCRs)

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Introduction: WGS using large-insert jumping libraries has previously allowed high-resolution delineation of complex BCRs. We hypothesized that WGS analyses in patients with Isolated GnRH Deficiency (IGD) with BCRs will reveal novel genes that regulate human puberty.

Methods: Jumping-libraries based WGS was applied to IGD cases with novel BCRs identified by karyotype that were likely to be de novo events.

Results: The BCR of Case #1 (46XY, t(7;12)(q22,q24)) was refined by WGS to [t(7;12)(q21.13,q23.1)]. The breakpoint of chr12 disrupts the second intron of a long non-coding RNA (lnRNA) RMST [Rhabdomyosarcoma 2 Associated Transcript (OMIM#607045)]. RMST acts as an upstream regulators of the known IGD genes CHD7, SEMA3A and TUBB3, suggesting an important role of RMST in the GnRH neuronal development and/or function. An additional case of chromothripsis was detected in whom the event of 46,XY,inv(3)(q24q26.32),t(3;13;18), a karyotyopically undetected 2.2 MB piece from chr7 was shattered and inserted inbetween chromosomes 13 and 3, interrupting genomic regions in proximity to SEMA3A and SEMA3E (genes previously associated with IGD), whereas two novel genes were interrupted by the breakpoints of chr13 (DIAPH3, Diaphanous-related formin 3, OMIM# 614567)5 and the (DTNA, Dystrobrevin alpha, OMIM#601239).

Conclusions: This first report of WGS analysis in IGD patients harboring de novo balanced chromosomal rearrangements reveals disruptions of several strong gene candidates highly likely to represent novel genes regulating human puberty. Rare de novo structural abnormalities thus represent a unique opportunity for discovery of new genes underlying human genetic diseases.

Poster Number 74

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Treatment of Liver Disease With a GLP-1 Fragment

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The goal is to develop a novel peptide therapeutic, GLP-1-5, for the treatment of non-alcoholic fatty liver disease (NAFLD), the commonest cause of chronic liver disease associated with the world-wide epidemic of obesity. Novel effective and safe agents for the treatment of obesity and NAFLD are needed. We discovered a fat-burning pentapeptide, GLP-1-5, LVKGRamide, which increases basal energy expenditure, reduces weight gain and fat mass, and prevents the development of hepatic steatosis in diet-induced obese mice. These studies address two questions: 1) Does GLP-1-5 reverse established steatosis and/or NASH? 2) Does GLP-1-5 cause thermotoxicity?

In a mouse model of NASH, male C57bl/6J mice were fed a high fat diet (HFD) containing trans-fat and fructose for 8 weeks followed by subcutaneous infusion of GLP-1-5 for 12 weeks. Food intake, body weights, temperature, composition (EchoMRI), and liver weights and histology were assessed. Compared to vehicle control, GLP-1-5 reduced body weight (16.5%), body fat (78%), and liver size (42%). Liver histology of vehicle control mice fed HFD for 8 weeks, before GLP-1-5 treatment began, showed severe macro and microsteatosis, and after 20 weeks on HFD developed areas of inflammation, hepatocellular necrosis, and fibrosis characteristic of NASH. In contrast liver histology of 4/5 mice in the group treated with GLP-1-5 was nearly normal with only minimal microsteatosis. These preliminary findings suggest that GLP-1-5 ameliorates the development of NASH and reverses established hepatosteatosis. No thermotoxicity was observed. These studies show promise for GLP-1-5 as a treatment for obesity and NAFLD with a favorable safety profile.

Poster Number 75

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A Systematic Review of Strategies to Prevent Readmissions among Racially and Ethnically Diverse Populations

INVESTIGATORS: A. Tan-McGrory, K. Kenst, J. Betancourt

Introduction: Studies have shown that patient-level factors such as race, ethnicity, and language proficiency, when tied to certain costly and complicated medical conditions such as heart failure, pneumonia, and acute myocardial infarction, may be predictors of readmission risk. Some minority populations are more likely than their white counterparts to be readmitted.

Purpose/Methods: The Disparities Solutions Center conducted a systematic literature review and created a Guide to Preventing Readmissions among Racially and Ethnically Diverse Medicare Beneficiaries. Pubmed and Ovid Medline were searched for articles published between 1997 and 2015, and a web-base search was conducted to identify relevant sources from the gray literature. One hundred thirty-five sources were identified out of 450 returned by the search. These included 110 peer-reviewed articles, 18 reports and briefs, 4 web articles, and 3 commentaries.

Results: The guide fills a gap in the readmissions literature by addressing how barriers faced by minority populations may be addressed in the context of broader plans to prevent readmissions overall. Recommendations include collecting demographic data; identifying the patients, populations, and characteristics linked to readmissions; developing preemptive efforts to prevent readmissions; deploying multi-disciplinary teams; creating systems responsive to the needs of diverse populations; developing culturally competent strategies for addressing communication-sensitive, high-risk scenarios; and partnering with communities to improve care coordination.

Conclusions: The evidence on preventing readmissions, cross-walked with the barriers that disproportionately impact minority populations, offer hospital leaders a set of evidence-based approaches for addressing the root causes of avoidable readmissions for diverse populations.

Poster Number 76

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Seeing the Benefits While Fearing the Obstacles: Comparing Cancer Patients' and Nurses' Perceived Uses, Benefits, and Downsides of Patient Self-Advocacy

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Background: Understanding the unique and additive roles of nurses and patients in ensuring patients' needs and priorities are met is essential to promoting patient-centered care.

Purpose: To describe the relationship between nursing advocacy and patient self-advocacy among female cancer patients.

Methods: This study analyzes two cross-sectional survey studies of adult female cancer patients (N=317) and nurses (N=21) in order to describe: (1) the concept of self-advocacy and (2) how both patients and nurses describe the uses, benefits, and downsides of self-advocacy. Analyses included an exploratory factor analysis of the Female Self-Advocacy in Cancer Survivorship Scale as well as descriptive statistics of investigator-developed questionnaires.

Findings: Results demonstrate that self-advocacy is primarily defined as how female cancer patients overcome challenges related to their cancer and is comprised of three main components: (1) being able to make informed decisions, (2) balancing giving and receiving support from others, and (3) communicating effectively with the healthcare team. Nurses mostly agreed with patients on self-advocacy's benefits (e.g. self-management, patient satisfaction, and patient-centered care) but disagreed on several of self-advocacy's downsides (e.g. increased clinic time, worse relationship between patient and providers, difficulty developing treatment plans, and problems responding to increased patient questions and requests).

Discussion/Implications: Self-advocacy among female cancer patients is a multi-component concept that nurses agree stands to benefit patient care and health. Yet nurses' perceived downsides of self-advocacy may hinder the adoption of behaviors that promote self-advocacy. Nurses should be trained in ways to help women with cancer advocate for themselves.

Poster Number 77

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Inconsistent HIV care among Latino immigrants: Patient and provider perspectives on interventions

INVESTIGATORS: J. H. Levison, L. M. Bogart, I. F. Khan, D. Mejia, H. Amaro, M. Alegria, S. Safren

Background: Interventions to improve retention (consistent attendance) in primary HIV care have not been adequately studied in Latino immigrants.

Methods: Bilingual Spanish-speaking staff conducted qualitative semi-structured interviews with 51 individuals, including 37 HIV-infected Latinos (aged ≥ 18 years, born in Puerto Rico or a Latin American Spanish-speaking country) and 14 HIV care providers in a metropolitan area. We explored participants' views on barriers to and suggestions for improving HIV clinic attendance. Interviews were recorded, transcribed, and translated. We developed and applied a coding scheme based on the Andersen Model of Health Care Utilization. Data were analyzed using thematic analysis.

Results: Patients suggested three areas to improve retention in HIV care: 1) skills for patients to manage HIV disclosure and stigma; 2) self-care through linguistically and culturally acceptable HIV education; and 3) referrals to community services. Younger patients (≤25 years old) reported less familiarity with community programming and lower perceived need for attendance in HIV care. Providers highlighted the need for a multi-disciplinary healthcare team including HIV, mental health and substance abuse providers; case managers; and Spanish-speaking community health workers. Patients, more so than providers, detailed the complexity of barriers and the cultural elements that could be integrated to improve retention in care (e.g. family-oriented themes and cultivation of patient trust).

Conclusions: Interventions to improve inconsistent attendance in primary HIV care in Latino immigrants should emphasize an individualized assessment to appropriately address variable barriers to HIV care. Patient input will be critical to assure relevance and acceptability of these interventions.

Poster Number 78

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Are Boston Healthcare Center Websites Linguistically Accessible?

INVESTIGATORS: J. A. Rodriguez, S. Percac-Lima

The Internet has become a significant source of health information, with 87% of Americans having access to the Internet. The availability of linguistically appropriate content is critical to providing patients with access to health information. While accessible health content is important for English-speaking patients, it becomes paramount for Limited English Proficient (LEP) patients. Much like the health disparities among racial, educational, and economical lines, language disparities have been associated with negative health outcomes. The LEP population is growing, with 20% of the US population speaking a language other than English at home and 8.6% defined as being LEP. In Boston, 12% of the population is LEP. Furthermore, homepages serve as the online face of healthcare centers and access points for patients. Thus, with this context in mind, we sought to evaluate the language accessibility of healthcare center websites in Boston. We reviewed the homepages of Boston hospitals and community healthcare centers to identify if there was an option to translate the homepage, and if so, the type of translation method provided and the number of available languages. After we categorized the translation type of each health center, we mapped their translation capacity to the percent LEP population in their neighborhood. We reviewed 45 homepages, 20 of which were hospital websites and 25 were community health center websites. Only 44% of homepages had a translation option available. Ultimately, despite the linguistic diversity of Boston neighborhoods, many of the hospitals and community centers do not provide translated versions versions of their websites.

Poster Number 79

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Validity of Implementing a Self-Reported Global Health Measure in a Medicare Accountable Care Organization to Predict Healthcare Utilization

INVESTIGATORS: K. J. Blumenthal, Y. Chang, T. G. Ferris, J. C. Spirt, C. Vogeli, N. Wagle, J. P. Metlay

Health Care systems participating in shared savings contracts need to stratify their patients in order to provide them with appropriate levels of care coordination services. In the past systems have relied primarily on administrative and clinical data to identify those individuals at greatest risk for high healthcare utilization. Patient Reported Outcome Measures (PROMs) that assess self-reported global health may be more responsive to changes in health status and therefore better predict future healthcare needs. Partners HealthCare (Partners) recently implemented point of care collection of the PROMIS Global Health instrument (PGH) using electronic tablets at outpatient visits. We investigated the association between PGH scores and both patient demographic and clinical characteristics as well as subsequent health care utilization—including Emergency Department (ED) visits and hospitalizations—in our Accountable Care Organization (ACO) Medicare patients.

The PGH demonstrated construct validity in that better scores were associated with patient characteristics typically associated with improved health. Moreover, the self-reported PGH was significantly associated with future healthcare utilization in this population. Ongoing analyses will assess whether this self-reported health measure provides incremental value for predicting future utilization after adjusting for administrative variables including comorbidities and past utilization.

In conclusion, PROMs, such as the PGH, can be routinely collected at the point of care and may help provide unique information on patients to assist providers and health systems predict subsequent healthcare needs and better target higher cost services to higher risk patients.

Poster Number 80

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Certification Review Study Groups: Nursing Professional Development Specialists Role

INVESTIGATORS: C. Avitabile, R. Chisari, P. Crispi

Background Evidence based practice and nursing literature finds nurses who hold professional certification in their nursing specialty practice with increased autonomy; empowerment, higher self-esteem; and enhanced collaboration. Professionally certified nurses report feeling more confident in their ability to detect early signs and symptoms of complications in their patients; while supervisors report improved performance in areas of teaching/collaboration and planning/evaluation

Objectives: The objective of this study is to understand the value in Nursing Professional Development Specialist's (PDS) role in promoting life-long learning and clinical excellence by establishing, supporting and fostering learning opportunities for successful completion of the Medical Surgical Certification examination.

Implementation: Weekly in-person, Medical Surgical review sessions were offered to inpatient RNs for 45 minutes over an 8 week period. Sessions were located on clinical units and included systematic overview of certification examination topics, test taking strategies and knowledge assessment. The review sessions were recorded and made available along with lecture notes for those unable to attend live sessions. Eleven RNs attended the in-person study sessions over 8 weeks. Twenty seven percent (3 of 11) sat for and passed the certification exam. Qualitative data suggests those who have not yet sat for the exam do not meet eligibility requirements. Participants who responded to a brief survey found value in attending live review sessions.

Performance Improvement Outcome: Certification review sessions provide a cost effective, resource neutral alternative to traditional certification review courses.

Poster Number 81

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Multilevel determinants of positive outlier status in a childhood obesity intervention

INVESTIGATORS: L. G. Fiechtner, M. L. Fonte, N. Kamdar, C. Horan, R. Marshall, E. M. Taveras

Background: RCTs to reduce a child's BMI have had limited success. One approach to optimizing interventions could be to examine characteristics and behavioral changes of children considered to be positive outliers in RCTs, e.g. those who succeed in reducing their BMI to below the 95th percentile or decreasing their BMI z-score by ≥0.5 units during an intervention.

Objectives: To examine multilevel characteristics and behavior change strategies associated with children being positive outliers in a childhood obesity RCT over a 1-year intervention period.

Design/Methods: We examined changes at 1 year for 340 children, ages 6-12 years with obesity who were randomized to the intervention arm. Using logistic regression, we examined the independent effects of child and parent/household characteristics on child's positive outlier status. We also examined behavior changes (i.e. SSB intake, sleep) that predicted positive outlier status.

Results: At baseline mean (SD) child age was 9.8 years (1.9 SD) and mean (SD) BMI z-score at baseline was 2.0 (0.29). At 1-year, 65 children (27%) in the intervention met the definition for a positive outlier. In multivariable analyses, children with severe obesity had lower odds of being a positive outlier (OR: 0.25 [95% CI:0.09, 0.66]). Children who increased their sleep duration during the intervention had a higher odds of being a positive outlier (OR: 1.84 per 1-hour increase in sleep; 95% CI:1.13, 3.01).

Conclusions: The odds of being a positive outlier was lower among those with severe obesity at baseline and higher among those who improved their sleep behaviors over time.

Poster Number 82

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Readmission Rates Following Passage of the Hospital Readmissions Reduction Program

INVESTIGATORS: J. H. Wasfy, C. M. Zigler, C. Choirat, Y. Wang, F. Dominici, R. W. Yeh

Background: Whether passage of the Hospital Readmissions Reduction Program (HRRP) in 2010 was associated with accelerated reductions in risk-standardized readmission rates, particularly for the poorest performing hospitals, is unknown.

Methods: We classified hospitals in four performance groups defined by the penalty that they were slated to receive. Using a pre-post analysis stratified by these four hospital performance groups, we evaluated (1) whether passage of the HRRP was followed by acceleration in improvement in 30-day readmission rates (RSRRs) after hospitalizations for acute myocardial infarction, congestive heart failure, or pneumonia, and (2) whether the poorest performing hospitals experienced a faster acceleration in improvement post-law than hospitals that were performing well.

Results: Of 2868 hospitals serving 1,109,530 Medicare discharges annually, 30.1% hospitals were highest performers, 44.0% were average performers, 16.8% were low performers, and 9.0% were lowest performers. Compared to the highest performing hospitals, the lowest performers were more likely to be major teaching hospitals (11.2% vs. 6.1%, $p < 0.001$) and were less likely to be rural (22.9% vs. 29.9%, $p < 0.001$). Rates of decline accelerated post-law for all performance groups ($p < 0.001$ for all), with the greatest acceleration occurring in lowest performance hospitals ($p < 0.001$ for all comparisons of higher vs. lower performance groups).

Conclusions: After the passage of the law, hospital risk-standardized 30-day readmission rates for myocardial infarction, heart failure and pneumonia declined nationally faster than they had been declining before the passage of the law. Improvement was most marked for hospitals with the lowest performance.

Poster Number 83

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Expansion of Regulatory B-Cells and Their Potential Role in Transplantation Tolerance

INVESTIGATORS: B. Amundsen, H. Deirawan, J. Kim, J. F. Markmann

Regulatory B (Bregs) cells, identified by their expression of, IL-10, have been shown to play a critical role in models of immune homeostasis and autoimmunity. A clear role for B-cells or Bregs in the maintenance of induced transplant tolerance has not yet been firmly-established.

Experimental analysis of Bregs has been hindered because of their small numbers. Here we describe a protocol to induce Breg expansion *in vitro* and secretion of TGF-β and IL-10 under various conditions.

Splenocytes were harvested from C57BL/6 mice. B-cells were purified by negative selection and cultured with irradiated NIH/3T3-CD40L cells for 7 days. Cells were treated on day 0 and 3 with combinations of anti-Tim-1, IL-4, IL-21, and BAFF. Expansion between different treatment groups was compared using ANOVA statistics. P values <0.05 were considered statistically significant.

In-vitro growth of cultured B-cells resulted in nearly 30-fold expansion and increased percentage of Bregs. The difference in fold-expansion under various conditions was statistically significant with $p<0.0001$. Further analyses of expanded B-cells showed a high percentage of TIM-1+ B-cells expressing IL-10 and TGF-β, molecules essential for Breg activity. Delivery of B-cells co-expressing these two inhibitory cytokines may provide an effective anti-rejection strategy and promote transplantation tolerance induction.

The splenic regulatory B-cell subset with the functional capacity to express IL-10 (B10-cells) modulates immune responses. Expansion of Bregs *ex vivo* will facilitate experimental studies dissecting their mechanism of action and ultimately Bregs may be an effective cell therapy for prevention or treatment of rejection and as an adjunct in gaining transplant tolerance.

Poster Number 84

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Neural control of intestinal host defense by a Acetylcholine-Wnt neuroimmune axis

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A large body of evidence indicates that many aspects of immunity are regulated by the nervous system, but the underlying mechanisms and their physiological significance remain unclear. Using C. elegans as a pre-clinical model organism, we discovered that neuronal Acetylcholine induces the expression of host defense genes in the intestinal epithelium during infection. Neural control of such response is mediated by muscarinic signaling and Wnt signaling. We show that acetylcholine signaling results in enhanced Wnt pathway activation, which induces a transcriptional host response in the intestinal epithelium. Thus, we define a novel pathway that connects pathogen detection in the neurons with intestinal epithelium activation of host defense.

These observations have potential implications for human intestinal innate immunity and inflammation, because Wnt signaling is conserved in human neurons, because the enteric nervous system is poorly understood in relation to innate immunity, and because the human intestinal epithelium expresses receptors for acetylcholine. Therefore, it is highly likely that a similar neural Wnt-Acetylcholine axis may operate in humans for the control of intestinal immunity and inflammation.

IMMUNOLOGY/INFLAMMATION

Poster Number 85

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Secondary Wound Necrosis is Suppressed by Resolvin D2

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Burn wounds often expand in size and depth in the first few days post injury. We used a rat burn model to assess the effect of Resolvin D2 (RvD2), an endogenous derivative of docosahexaenoic acid, on the expansion of the area of stasis around burn wounds and the healing process. Ten Wistar rats were burned using a heavy metal comb to create four burn areas (10x20mm separated by 5x20mm spaces) on their backs. They were divided into 2 groups. One group was injected intravenously with RvD2 and the other with diluent. The wounds were evaluated immediately after the burn insult and on post-burn days (PBD) 1,2,3,5,7 and 14. The parameters for healing were assessed via daily photographs with analysis of necrotic and healing areas using ImageJ software analysis. Laser Doppler (LD) and Doppler Optical Coherence Tomography (DOCT) measured total blood flux and functionality of capillary network of the burn injuries. On PBD 14, euthanasia was performed and biopsies were taken for histology to compare inflammation and wound regeneration processes between control and treated groups. We show that RvD2 treatment preserved the interspace areas and blood flux throughout the interspaces and the wound borders. Histologically, the smaller ratios of neutrophils to monocytes suggest an accelerated regeneration process in the RvD2 treated group when compared to the control group. RvD2 treatment could enhance the blood flux around wounds, suppress secondary necrosis changes, and accelerate regeneration after thermal burn injuries.

Poster Number 86

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An Evolutionarily Conserved PLC-PKD-TFEB Pathway for Host Defense

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The precise transcriptional regulation of pro-inflammatory and host defense genes is crucial to controlling infections while avoiding damage to the host. Although transcriptional control of such genes is known to be tight, the mechanisms involved have not been fully elucidated. We previously identified TFEB as an important transcription factor in host defense, but the mechanisms involved in its regulation during infection were not known. Here we use C. elegans to identify a novel upstream pathway that controls TFEB during infection, and use pharmacological agents to demonstrate the existence of a similar pathway in murine macrophages. Nematodes infected with Staphylococcus aureus require gene dkf-1, which encodes a homolog of protein kinase D (PKD), for TFEB activation. Pharmacological activation of PKD was sufficient to activate TFEB in the absence of infection. Furthermore, phospholipase C (PLC) homologous gene plc-1 is also required in nematodes for TFEB activation, downstream of Gαq homolog egl-30 but upstream of dkf-1. Chemical genetics supports the existence of a similar PLC-PKD-TFEB axis in Salmonella- and Staphylococcus- infected mouse macrophages. RNA silencing revealed that PKD1 is the relevant PKD homolog in mouse macrophages. In addition, PKCα is required in macrophages but not nematodes. These observations reveal a previously unknown host defense signaling pathway, which has been conserved across one billion years of evolution.

Poster Number 87

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Analysis of Acute Skin Rejection in Non-Human Primate Models of Face and Hand Allotransplantation

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Background and Rationale: Almost 85% of patients develop acute rejection (AR) of the skin within a year after hand or face transplantation (i.e. vascularized composite allografts, VCAs) and require treatment to prevent allograft loss. However, the mechanisms underlying AR remain poorly defined.

Methods: 6 cynomolgus monkeys received hand (n=2) and face VCAs from MHC-mismatched donors after induction with anti-thymocyte globulin and post-operative maintenance on triple immunosuppression (tacrolimus, mycophenolate mofetil, methylprednisolone) for up to 120 days. Protocol biopsies of VCA and host skin were performed at 30-day intervals for flow cytometric analysis of resident skin leukocyte populations. Further biopsies were obtained for histopathology during clinical AR and steroid treatment was administered.

Results: Flow cytometric analysis revealed near complete turnover of passenger donor skin-resident leukocytes within the VCA to host-origin cells by 30 days after transplantation. Interestingly, this coincided with the first episode of AR in those animals with a complete MHC mismatch, although no AR developed in those animals that were haplomatched, despite the same immuosuppressive regimen. All but one episode of AR were successfully treated with steroids; no allo-antibodies were detected in all cases. Histological grading of AR was Banff I to II with corresponding higher ratios of CD8:CD4 T cells.

Conclusions: Here we show a clinically-appropriate model for studying AR in VCA with implications on long-term management and tolerance induction. Sharing of haplotype appears to confer additional protection against AR by mechanisms that are currently being investigated in our laboratory.

Poster Number 88

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PTEN Controls Warburg Metabolism in Regulatory T cells

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Foxp3+ Regulatory T cells (Tregs) act as “Liaisons” between immunity and metabolism, cellular processes that are essential for dictating the fate and function of immune cells. Tregs originate in the thymus (nTregs) as well as extrathymically in the periphery (pTregs). Although it is known that iTregs (induced invitro from Tconv cells and resemble pTregs) rely predominantly upon lipid oxidation more than glycolysis or glutaminolysis, the metabolic properties of nTregs are not fully elucidated. Here we show that in contrast to iTregs, activated nTregs like Tconv cells are capable of undergoing glycolysis and glutaminolysis. PTEN, a lipid phosphatase, that inhibits PI3K/Akt signaling and promotes Tregs stability and function, can negatively regulate glycolysis and glutaminolysis in cancer cells. We hypothesized that elevated PI3K/Akt signaling (via PTEN loss) in Tregs, enhances glycolysis and glutaminolysis leading to Tregs instability and autoimmunity. We found that PTEN deficiency in Tregs (PTENΔTregs) enhanced glycolytic flux without affecting glutaminolysis. Furthermore, PTENΔTregs showed enhanced expression of pyruvate dehydrogenase kinase isoform 4 (PDHK4) that blocks pyruvate dehydrogenase (PDH) and enhances glycolysis. Although, PAN Blockade of PDHKs by DCA (dicholoroacetate) decreased the glycolytic flux of PTENΔTregs and percentages of activated CD4+ T cells, PTENΔTreg mice still succumbed to autoimmunity suggesting that blockade of glycolysis alone is not sufficient to rescue autoimmunity in PTENΔTreg mice. Subsequently, we found that PTENΔTregs showed enhanced nucleotide and lipid synthesis associated with reduced AMPK signaling. Overall, these findings indicate that overactivation of PI3K/Akt (that occurs during inflammation) leads to metabolic dysregulation and disruption of Treg homeostasis.

Poster Number 89

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Barriers to care and 1-year mortality in newly-diagnosed HIV+ persons in South Africa

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Background: Only a fraction of those newly diagnosed with HIV enter care promptly in South Africa. Our objective was to evaluate the impact of self-perceived barriers to health care on 1-year mortality among newly diagnosed HIV-infected individuals in South Africa.

Methods: We surveyed adults (≥18y) prior to HIV testing. We used Cox proportional hazards models to determine the association between the number of perceived barriers to care and time to death within one year of HIV diagnosis. Perceived barriers included: 1) service delivery; 2) financial; 3) personal health perception; 4) logistical; and 5) structural. We assessed deaths via the South African death registry.

Results: Among 4,903 participants enrolled, 1,899 (39%) were HIV-infected. Mean age was 35 years (SD 10) and median CD4 count was 192/µl (IQR: 72-346/µl). 1,057 participants (56%) reported no barriers, 370 (20%) reported 1-3 barriers, and 460 (24%) reported >3 perceived barriers to care. By one year after enrollment, 250 (13%) of participants had died. Adjusting for age, sex, distance to clinic, TB status and baseline CD4 count, participants who identified 1-3 barriers (aHR 1.49, 95% CI 1.06, 2.08) and >3 barriers (aHR 1.81, 95% CI 1.35, 2.43) had higher risk of 1-year mortality compared to those without self-identified barriers.

Conclusions: HIV-infected individuals in South Africa who reported multiple perceived barriers to care were nearly twice as likely to die within one year. Structural interventions such as extended clinic hours, travel vouchers, and streamlined clinic operations may improve linkage to care and treatment initiation for these patients.

Poster Number 90

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Latency reversing agents and cellular activation affect antigen processing in primary CD4 T cells

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Strategies to purge HIV reservoirs commonly rely on reactivation of HIV provirus by latency-reversing agents (LRA) followed by death or immune clearance of reactivated CD4T cells. LRAs such as Histone deacetylase inhibitors or Protein Kinase C agonists showed variable efficacy *in vitro* and limited efficacy in clinical trials. While productive infection mostly occurs in activated CD4T cells, reactivation occurs in memory resting cells receiving LRA and anti-retroviral therapy. How these parameters shape HIV antigen processing and presentation by CD4T cells is unknown despite its critical role in immune recognition after reactivation.

We measured hydrolytic activities of cellular proteases involved in antigen processing in live primary CD4T cells that were either resting, CD3/28-activated, LRA-treated, or in conditions used in the Lewin or Planelles latency models. To assess how changes in activities affect antigen processing we compared the degradation of HIV peptides in extracts of primary CD4T cells treated as stated above. The degradation products were quantified by mass spectrometry and their antigenicity measured by Chromium release killing assay with HIV-specific CTL.

Here we show that resting primary CD4 T cells process antigens differently from activated cells; they display lower peptidase activities, produce longer, more antigenic peptides and unique cleavage sites. Additionally, the different classes of LRA affect peptidase activities differently and some LRAs cause discrete changes in degradation patterns.

These variations in peptidase activities and degradation patterns suggest that LRA-reactivated and productively infected CD4T cells might process antigen differently, thus requiring different CTL responses for effective clearance.

Poster Number 91

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Transcriptomics of the Mucosal Innate Immune Response to Vibrio cholerae

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Background: Vibrio cholerae, a cause of severe diarrhea, serves as a model of microbial-host interactions and immunity at the mucosal surface. While Vibrio cholerae does not lead to overt inflammatory diarrhea, it elicits a potent innate immune response. There is a limited understanding of the scope and pathways activated in the innate response to Vibrio cholerae.

Methods: We applied RNAseq to define the innate immune signaling pathways activated by infection with Vibrio cholerae. For each patient we obtained duodenal tissue at two time points: during acute illness and 30 days later in recovery. We extracted total RNA, and used RNAseq to identify genes up-regulated at the mucosal surface during acute illness. We then did pathway and network enrichment analysis to identify signaling pathways and gene networks up-regulated during cholera.

Results: To date, we have generated RNAseq data from the two time points for 11 patients. Amongst the up-regulated genes during acute cholera, there was a preponderance of genes involved in innate immunity including CFB, CXCL9, DUOX2, LCN2, FCGR3A, REG1A, and TLR8. Pathway analysis revealed enrichment of eIF2 signaling, acute phase response signaling, and NRF2-mediative Oxidative Stress Response signaling pathways.

Conclusions: An up-regulation of genes involved in the innate immune response was observed in the duodenal mucosa of patients with cholera. Employing RNAseq to study the mucosal immune response can yield important insights into mucosal immunity and the host-pathogen interaction.

Poster Number 92

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The value of confirmatory testing in early infant HIV diagnosis (EID) programs

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Background: Infant HIV EID assays have high specificity, but positive predictive value is poor when mother-to-child HIV transmission (MTCT) risk is low. False-positive (FP) results lead HIV-uninfected infants to incorrectly initiate antiretroviral therapy (ART), and later testing after ART initiation is inaccurate. Confirmatory testing at the time of EID, while costly, reduces incorrect ART initiation.

Methods: We used the CEPAC-Pediatric microsimulation model, with clinical, cost, and EID assay data from South Africa, to simulate 6-week EID testing for HIV-exposed infants. We projected clinical outcomes, FP (incorrect) ART initiations, costs, and cost-effectiveness for three strategies: no EID (comparator), EID with confirmatory testing, and EID without confirmatory testing. For uninfected infants with FP results, we assumed 25 years of HIV care and ART (varied in sensitivity analyses), and excluded toxicity and stigma. We calculated incremental cost-effectiveness ratios (ICERs) in $/year of life saved (YLS).

Results: For EID using confirmatory testing, 6 out of every 1000 ART initiations were incorrect, accounting for 0.2% of cohort lifetime HIV care costs ($1,770/infant). EID with confirmation was very cost-effective for South Africa, compared to no EID (ICER $1,200/YLS, <0.2x per-capita GDP). EID without confirmation led to similar clinical outcomes, but lifetime costs were greater ($2,090/infant). Without confirmation, 297 of every 1000 ART initiations were incorrect, accounting for 14.3% of lifetime HIV care costs.

Conclusions: Without confirmatory EID testing, nearly 30% of infants initiating ART could be truly HIV-uninfected in low-MTCT settings, and care for FP infants could comprise a substantial fraction of HIV program costs.

Poster Number 93

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Mathematical Model of Oxygen Transport in Tuberculosis Granulomas

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Pulmonary granulomas—the hallmark of Mycobacterium tuberculosis (MTB) infection—are dense cellular lesions that often feature regions of hypoxia and necrosis, partially due to limited transport of oxygen. Low oxygen in granulomas can impair the host immune response, while MTB are able to adapt and persist in hypoxic environments. Here, we used a physiologically based mathematical model of oxygen diffusion and consumption to calculate oxygen profiles within the granuloma, assuming Michaelis-Menten kinetics. An approximate analytical solution—using a priori and newly estimated parameters from experimental data in a rabbit model of tuberculosis—was able to predict the size of hypoxic and necrotic regions in agreement with experimental results from the animal model. Such quantitative understanding of transport limitations can inform future tuberculosis therapeutic strategies that may include adjunct host-directed therapies that facilitate oxygen and drug delivery for more effective treatment.

Poster Number 94

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Association of nasopharyngeal microbiota profiles with bronchiolitis severity in infants hospitalized for bronchiolitis

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Rationale: Although the human microbiota may influence immune responses, little is known about the relationship between specific composition of airway microbiota and severity of bronchiolitis.

Objectives: To identify nasopharyngeal microbiota profiles and to link these profiles to acute severity in infants hospitalized for bronchiolitis.

Measurements and Methods: Multicenter prospective cohort study of 1005 infants (age <1 year) hospitalized for bronchiolitis over three winters, 2011–2014. By applying 16S rRNA-based pyrosequencing and unbiased clustering approach to nasopharyngeal aspirates collected within 24 hours of hospitalization, we determined nasopharyngeal microbiota profiles and their association with bronchiolitis severity. The primary outcome was intensive care use (defined as admission to an intensive care unit or use of mechanical ventilation).

Results: Overall, we identified four distinct nasopharyngeal microbiota profiles—three profiles were dominated by either Haemophilus, Moraxella, or Streptococcus, while the fourth profile had the highest bacterial richness. The rate of intensive care use was highest in infants with Haemophilus-dominant profile and lowest in those with Moraxella-dominant profile (20.2% vs 12.3%; unadjusted OR, 1.81; 95%CI, 1.07-3.11; P=0.03). After adjusting for 11 patient-level confounders (including viruses), the rate remained significantly higher in infants with the Haemophilus-dominant profile (OR, 1.98; 95%CI, 1.08-3.62; P=0.03). These findings were externally validated in a separate multicenter study of 307 children hospitalized for bronchiolitis.

Conclusions: In this large multicenter cohort of infants hospitalized for bronchiolitis, we identified four distinct microbiota profiles in their upper airway. The Haemophilus-dominant profile was associated with higher illness severity. These data challenge the conventional virus-centric view of bronchiolitis.

Poster Number 95

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Serial procalcitonin measurements for improved prognostic assessment of patients admitted with bacterial pneumonia

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Procalcitonin (PCT) is a pro-hormone that rises in response to bacterial infections including pneumonia. While PCT has applications in the initial assessment of patients with pneumonia, data is inconclusive on its use for prognostication of clinical outcomes. We conducted a prospective, observational cohort study of patients who were admitted with pneumonia and measured PCT on hospital days one through four. Demographics, medical history, objective data, and outcomes were obtained through chart review. Final diagnosis of community-acquired pneumonia (CAP) or healthcare-associated pneumonia (HCAP) was determined by two or more physicians blinded to PCT values. The primary endpoint was a composite adverse outcome defined as all-cause mortality, ICU admission, and bacteremia. We calculated regression models with area under the ROC curve as a measure of discrimination.

Here we show that among patients meeting the primary endpoint versus not, there was no significant difference in age or underlying medical comorbidities. PCT values were significantly higher for patients meeting the primary endpoint, as well as for individual outcomes of bacteremia and ICU admission, but not mortality. Addition of PCT to a statistical model including the pneumonia severity index (PSI) improved the prognostic performance of the PSI with respect to the primary endpoint, bacteremia, and need for ICU level care. Finally, the ability of PCT to predict adverse outcomes is also effective in patients with PSI scores greater than 130.

In conclusion, serial measurements of PCT in hospitalized patients with bacterial pneumonia are a promising tool for predicting adverse clinical outcomes, including in high-risk patients.

Poster Number 96

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Miniaturized fluorescence anisotropy system for point-of-care diagnosis of healthcare-associated infection

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Healthcare-associated infections (HAIs) have become a major healthcare issue. In the US alone, it is estimated that more than 600,000 patients develop HAIs every year. Of these patients as many as 1 out of 9 will die from HAIs. The ability to rapidly and accurately detect HAI-causing pathogens is critical to improve patients' care. Here we present a new technology, named PAD for polarization anisotropy diagnostics, for streamlined pathogen identification. The PAD assay detects PCR-amplified pathogenic DNAs that are captured in a sequence-specific manner by carefully designed detection keys. This hybridization event causes the detection keys to lock DNA polymerase, which alters the fluorescence anisotropy of a separate reporter probe. Due to this unique feature of the detection keys, the signal readout can be performed in a simple homogenous format. By targeting the conserved and hypervariable regions of 16S rRNA, the universal and differential detection of HAI bacteria was achieved with sensitivity down to a single bacterium. In addition, drug-resistant pathogens were distinguished by detecting the drug-resistance gene markers. We demonstrated the clinical utility of the PAD system by detecting pathogens in patient samples within 2.5 h. The PAD system holds promise to become a sensitive, selective and low-cost platform for point-of-care pathogen detection.

Poster Number 97

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Characterization of Immune Response to Coxiella Burnetti using Mass Cytometry (CyTOF)

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Coxiella burnetii (Cb) is a highly infectious and stable bacterial pathogen that can cause acute and severe chronic Q-fever in humans. Exposure of troops deployed to Q-fever endemic regions prompted the Department of Defense to identify Cb as a pathogen of interest. Antibiotics are effective against Cb, though treatment can last for months. Therefore,vaccination is considered critical to disease control; however, the current vaccine can cause severe reactogenic side effects in patients previously exposed to Cb. T-cells are thought to provide greater protective immunity than antibodies, though both are believed necessary and the durability of Cb immune memory is unclear. The goal of the Q-VaxCelerate consortium is to develop an efficacious and less reactogenic Cb vaccine. To better characterize the immune response to Cb, our team at MGH developed an approach that incorporates Cytometery by Time Of Flight mass spectrometry (CyTOF) to measure >35 immune-markers simultaneously. Using CyTOF and traditional immunology assays, we will describe the immune response to Cb infection by interrogating samples from immunologically well-characterized donors (naïve, sub-clinical, or Q-fever recovered). In a similar manner, mice that present antigen using human HLA-DR3 will be used to characterize the development of the immune response to infection and vaccination. Together these data may reveal hallmarks of the immune response that correlate to patient outcome and successful vaccination. These studies will deepen our understanding of the immune response to Cb, facilitate the testing of candidate vaccines, and serve as a template for studies seeking to broadly characterize immune responses to pathogens.

Poster Number 98

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Ionic mechanisms of apoptosis in the epileptic hippocampus

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Post-traumatic accumulation of intracellular Cl- results in GABA becoming depolarizing, which reduces inhibition, enhances propagation of neuronal firing, and may contribute to early post-traumatic seizures. Charge balance dictates that increases in [Cl-]i may be accompanied by increases in cations, which could underlie cytotoxic edema and accompany epilepsy. Here, we tested for changes in intracellular Na+ concentration using organotypic hippocampal slice cultures from wild-type C57BL/6J mice, imaged with the Na+-sensitive dye SBFI. Immediately post-trauma, neurons had significantly higher [Na+]i than has been reported in undamaged neurons. After a brief recovery period, [Na+]i again rose to high levels and remained elevated for weeks. Elevated [Na+]i coincided with decreased synthesis of fluorescent proteins induced by viruses such as TurboRFP, morphological changes such as cell shrinkage and retraction of processes, and increases in membrane permeability (allowing for the passive influx of dyes and stains such as propidium iodide). The high [Na+]i was mitigated by the activity of Na+/K+ ATPases, cation/Cl- cotransporters, and Na+/Ca2+ exchangers in order to support high rates of transmembrane Na+ flux during epileptogenesis. Inhibition of COX-2 and the protein Bax significantly lowered [Na+]i, suggesting that an apoptotic pathway leading to the insertion of permeability pores in the cytoplasmic membrane may be responsible for the rise in [Na+]i and related changes. Overall, elevated [Na+]i is a promising new biomarker for neuronal compromise and apoptosis, as it precedes many traditional indicators of epileptic activity and ictal cell death.

Poster Number 99

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Therapeutic Hypothermia in Neonatal Hypoxic-ischemic Encephalopathy: A Description of the MGH Neonatal Cohort

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Background: Hypoxic-ischemic encephalopathy (HIE) affects 0.5 to 1 per 1000 live births. Therapeutic hypothermia (TH) has been shown to improve neurodevelopmental outcomes and is considered standard therapy. The MGH Neonatal Intensive Care Unit (NICU) implemented the TH protocol in February 2009; however, institutional HIE data have not been rigorously evaluated to date.

Methods: A retrospective, IRB approved review identified clinical cases of HIE from 2001–2016. Cases with ICD-9/ICD-10 codes associated with perinatal brain injury or risk factors (e.g. low Apgar score or seizure) were included. Data were collected by expert chart review.

Data/Results: To date, 70 cases of HIE have been reviewed (20 received TH and 50 without TH). Demographic measures were similar between the two populations. In the TH group, 5 infants (25%) had clinical and/or electrographic seizures compared with 43 (86%) in the no TH group. Additionally, there were no identified cases of cerebral palsy (CP) among infants who received TH, compared to 7 cases (14%) among the infants who did not receive TH.

Discussion: Our preliminary data, consistent with previous reports in the literature, show improved trends in outcome measures (lower seizure burden and fewer cases of CP) in the TH cohort.

Future directions: We aim to collect prospective data to evaluate associations between clinical factors and outcomes in the MGH neonatal population with HIE undergoing TH. Additionally, we hope to evaluate patterns in magnetic resonance imaging (MRI) of HIE to help build prognostication paradigms.

Poster Number 100

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X-Linked Dystonia-Parkinsonism (XDP) patient cells exhibit altered TAF1 expression and NFkB signaling

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X-linked Dystonia-Parkinsonism (XDP) is a hereditary neurodegenerative disorder involving a progressive loss of striatal medium spiny neurons. The mechanisms underlying neurodegeneration are not known, in part because there have been few cellular models available for studying the disease. The XDP haplotype consists of multiple sequence variations in a region of the X chromosome containing TAF1, a large gene with at least 38 exons, and a Multiple Transcript System, MTS, comprised of five unconventional exons. A previous study identified a neural-specific TAF1 isoform, N-TAF1, which showed decreased expression in post-mortem XDP brain, compared to control tissue. We generated XDP patient and control fibroblasts and induced pluripotent stem cells (iPSCs) in order to further probe cellular defects associated with this disease. N-TAF1, which incorporates an alternative exon 34', was not expressed in fibroblasts, but was detectable in iPSC-differentiated NSCs at levels that were approximately 3-fold lower in patient cells than controls. Transcriptional profiling of patient fibroblasts compared to controls resulted in a 53-gene signature distinguishing XDP patient from control cells. A bioinformatics analysis revealed a potential relationship between the XDP signature and the NFκB signaling pathway. Further analyses confirm aberrant NFκB signaling in XDP fibroblasts and NSCs, consisting of increased inflammatory gene expression in XDP vs. control cells following TNFalpha stimulation. Together, these results support the previous findings that N-TAF1 expression is impaired in XDP and raise the possibility that the NFκB signaling cascade could be important for understanding the biology of XDP.

Poster Number 101

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Development of an Image-Based High-Content Screening Assay for Tau Clearing Drugs in a Human iPSC-Derived Neuronal Cell Model of Frontotemporal Dementia

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Autosomal dominant mutations in the microtubule-associated protein gene (MAPT) encoding the protein tau cause frontotemporal dementia (FTD). At the level of neuropathology, these MAPT mutations are associated with abnormal tau phosphorylation levels and intracellular accumulation of aggregated protein. Recently, a rare variant of tau p.A152T located N-terminal of the microtubule-binding domain has been described. This variant decreases affinity of tau for binding microtubules *in vitro* and has been shown to increases risk for FTD, Alzheimer's disease, and synucleinopathies. Here we utilize human induced pluripotent stem cells (iPSC) from a FTD patient with this tau p.A152T variant as a genetically accurate cell model of FTD. To derive a rapid and highly reproducible system capable of supporting the discovery of novel therapeutics using high-content image-based screening, we have adapted strategies for the inducible expression of the transcription factor Neurogenin-2 in iPSC-derived neural progenitor cells (iNgn2-NPCs). We demonstrate the ability to efficiently and reproducibly generate excitatory, glutamatergic-like neurons from these iNgn2-NPCs in a 96-well plate format with abundant expression of tau with enhanced polarized distribution to axonal processes. In combination with automated confocal microscopy and an advanced image-processing pipeline optimized for analysis of morphologically complex neuronal cultures, we summarize the results of a screen for small molecules targeting autophagy and protein homeostasis pathways with an emphasis on clinically used drugs with potential for repurposing. Further expansion of these strategies to additional FTD patient-derived iPSC models holds promise for phenotyping of patients and elucidating novel targets for therapeutic intervention.

Poster Number 102

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Focal Temporal Pole Atrophy and Network Degeneration in Semantic Variant Primary Progressive Aphasia

INVESTIGATORS: J. A. Collins, V. Montal, B. C. Dickerson

The Semantic Variant of Primary Progressive Aphasia (svPPA) is a devastating neurodegenerative disease characterized by the progressive loss of semantic memory. Despite a wealth of neuroimaging research that has associated svPPA with a distributed pattern of cortical atrophy that is most prominent in the left anterior temporal pole, there is little consensus regarding which region within this heterogeneous structure is most damaged, which may indicate the putative origin of neurodegeneration. In this study, we localized the most consistent region of atrophy in svPPA using cortical thickness analysis and surface-based inter-subject registration in two independent patient samples. Across both samples the point of maximal cortical atrophy was located in same region of the left dorsolateral temporal pole. Individual subject analyses localized the point of maximal atrophy for 100% of patients in both svPPA samples to the same temporopolar region. Using resting state functional connectivity (rs-fcMRI) we showed that the focal atrophy point anchored a large-scale network in healthy young adults that closely resembled the distributed atrophy pattern in svPPA and included several brain regions that are commonly implicated in semantic memory. In both patient samples, the magnitude of atrophy within a brain region was predicted by that region's strength of functional connectivity to the focal atrophy point in healthy adults. These findings suggest that cortical atrophy in svPPA may follow connectional pathways within a large-scale semantic network that converges on the temporal pole.

Poster Number 103

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Cortical reactivation of memory-related gamma activity in human NREM sleep

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Models of memory consolidation posit a central role for reactivation of cortical activity patterns during sleep. Such 'replay' has been well-demonstrated in rodents, where it is orchestrated by the hippocampus. However, direct electrophysiological evidence of reactivation in human is still largely lacking. By using intracranial electroencephalogram recordings from patients with epilepsy and by employing a neural decoding approach, we tested the reactivation, in human sleep, of patterns of cortical activation specifically evoked by earlier motor learning.

Six participants implanted with electrode arrays for long-term epilepsy monitoring learned a sequential finger tapping task which was followed by sleep. Neuronal firing in widespread cortical areas was estimated from high gamma-band [70-120Hz] power. Decoders were first trained to classify between finger-movement and control periods on wake data before being applied during sleep. The trained models classified samples from post-learning sleep as well as from a baseline pre-learning sleep (before the task) as motor- versus rest-classes.

For each participant, the proportion of time-period classified as motor-class was higher during post-learning sleep, demonstrating that the gamma-band patterns underlying finger movements were reactivated during sleep following motor learning. In addition, this increase in putative replay during the post-training sleep was highly correlated with performance improvement assessed after sleep. The results were consistent across three different classifiers. These data show the reactivation, in human sleep, of gamma-band patterns linked to task execution in wake and tightly correlated with behavioral evidence of learning, and thus confirm that a basic tenet of the replay theory does occur in humans.

Poster Number 104

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Effects of propofol-induced unconsciousness in thalamocortical circuits

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General anesthesia is a drug-induced condition, which includes reversible loss of consciousness. This clinical procedure allows thousands of patients to safely undergo both surgical and non-surgical procedures. One of the most common drugs used for induction and maintenance of general anesthesia is propofol. In humans, loss of consciousness induced by propofol is characterized by the appearance of alpha (8-15 Hz) oscillations, and slow/delta (0.1-4 Hz) oscillations observed in electroencephalogram. Propofol is a GABA-A agonists that increases neuronal hyperpolarization. Modelling studies have proposed simultaneous cortical and thalamic hyperpolarization results in thalamocortical synchronization in the alpha range. To test this hypothesis, we recorded local field potentials simultaneously from frontal cortex and several thalamic nuclei in awake, freely behaving rats, while slowly infusing propofol. We estimated loss of consciousness by assessing loss of the righting reflex. The frontal cortical signals reproduce the observed phenomena in the human EEG, with beta (15-30 Hz) oscillations before unconsciousness, alpha oscillations at the time of loss of consciousness, soon followed by a combination of slow/delta and alpha oscillations. A detailed analysis of the different layers of frontal cortex shows the greatest changes in layer VI. However, the different thalamic nuclei engage simultaneously in beta-alpha and slow/delta oscillatory modes. All the frontal cortex layers and all the thalamic nuclei become synchronous in the alpha range at the time of loss of consciousness, as the computational models predicted. Our results also point to a decoupling of the activity of the different cortical layers before and at loss of consciousness.

Poster Number 105

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Effects of Intranasal Oxytocin on Food Motivation Pathways in Overweight/Obese Men

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Background: Obesity is a major public health concern and there is need for novel therapeutic strategies. Preclinical research suggests that Oxytocin (OXT), a hypothalamic peptide hormone, has direct effects on food motivation neural pathways. We hypothesized that OXT would reduce obesity-related hyperactivation in relevant food motivation brain regions in response to a visual food stimuli paradigm.

Methods: We performed a randomized, double-blind, placebo-controlled crossover study of single-dose intranasal OXT (24 IU) in 10 healthy overweight/obese men. We investigated differences in blood oxygenation level dependent (BOLD) signal while participants viewed images of high-calorie foods vs. objects.

Results: Participants were 23-43 yrs old, mean±SEM age 31.5±1.8 yrs. BMI ranged from 25.7-33.9 kg/m2, with a mean of 28.7± 0.74 kg/m2. OXT reduced BOLD signal within the right caudate body of the basal ganglia ($p < 0.05$), bilateral VTA ($p < 0.05$), left hippocampus ($p = 0.017$) and left insula ($p < 0.05$) when subjects viewed high calorie foods compared to objects. Reductions in activation in the hypothalamus ($p < 0.2$), amygdala ($p < 0.15$) and OFC ($p < 0.1$) did not achieve statistical significance.

Conclusions: We show that a single dose of intranasal OXT reduces fMRI activation of the basal ganglia, VTA, hippocampus and insula, regions important for reward processing, during a visual food stimuli paradigm in overweight/obese adult men. This supports the hypothesis that OXT reduces caloric intake by modulating food motivation brain circuitry. Larger studies will be important to define the effects of single and repeated doses of OXT on neural pathways governing feeding behaviors.

Poster Number 106

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Evaluating the role of Hippo pathway in the onset and progression of Amyotrophic Lateral Sclerosis in the SOD1 mouse model

INVESTIGATORS: E. J. Granucci, K. E. Glajch, K. A. Mueller, G. Sadri-Vakili, K. Vakili

The Hippo/YAP signaling pathway has been implicated in mammalian organ size regulation and tumor suppression. Specifically, this pathway plays a critical role in regulating the activity of transcriptional co-activator Yes-associated protein (YAP), which modulates a proliferative transcriptional program by binding to the transcription factor TEAD. Recent studies have revealed that the Hippo/YAP pathway may also play a role in neurodegeneration. For example, mammalian sterile 20 (STE20)-like kinase 1 (MST1), a downstream pro-apoptotic protein kinase in the Hippo pathway mediates oxidative-stress-induced neuronal death. Additionally, homozygous deletions of MST1 in a mouse model of Amyotrophic Lateral Sclerosis (ALS)delayed symptom onset and improved survival of spinal cord motor neurons, implicating the Hippo/YAP pathway as a potential underlying mechanism in ALS pathogenesis. Therefore, weinvestigated the possible pathogenic role of the Hippo pathway in ALS by measuring the expression of MST1 in cortex, lumbar spinal cord, and muscle samples of transgenic and wild-type male and female G39A SOD1 mice using western blots. Our results revealed a significant increase in MST1 in muscle of both symptomatic and presymptomatic female as well as symptomatic male transgenic mice compared to wild-type littermates. These findings unveil a sex-specific increase in MST1 levels in muscle and spinal cord of transgenic presymptomatic female mice. Together, these results suggest that MST1 levels are altered in the SOD1 mouse model and that the Hippo pathway may play a critical role pathogenesis and disease progression in ALS.

Poster Number 107

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Novel Roles for RIPK1 and RIPK3 in the Pathogenesis of Traumatic Brain Injury

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Traumatic brain injury (TBI) is the leading cause of death in children and young adults. Our current treatments focus on managing the sequelae of injury to prevent secondary insults while the brain heals, but specific therapy targeting mechanisms of cell death are lacking. Receptor interacting protein kinases 1 and 3 (RIPK1 and RIPK3) induce regulated necrosis, termed “necroptosis” as well as pro-inflammatory signaling. We hypothesized that RIK1 and RIPK3 activity contribute to cell death and neurological dysfunction after controlled cortical impact, a model for cerebral contusion, in mice. Following CCI, RIPK1 expression decreased in injured cortex ($p < 0.05$) but was maintained in hippocampus, whereas RIPK3 expression increased at 24 and 48 h. Using genetically altered mice (RIPK1 kinase dead; RIPK1KD, and RIPK3 knock out (RIPK3KO) we demonstrate that following CCI, RIP3KO mice have similar acute neuronal death, permeable cells, and lesion volume as wild type (WT), but improved motor recovery and cognitive function compared to WT. RIPK1KD mice also had improved motor recovery but similar lesion size vs. WT, and no difference in cognitive outcome. Unexpectedly, protein expression of several members of the mTOR signaling pathway was markedly decreased in injured cortex of RIPK1KD and RIPK3 KO mice, suggesting a role for protein stability for these kinases. The data indicate that modulating RIP1K and RIP3K change functional outcome likely through mechanisms other that cell death and highlight these pathways as a potential therapeutic targets to improve functional outcome in patients with contusion TBI.

Poster Number 108

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Cortical Beta And Gamma Rhythm Networks Evolve along Distinct Maturation Trajectories

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Brain maturation from childhood through early adulthood is marked by large-scale changes in functional brain connectivity. Mapping these intricate developmental changes in connectivity in the typically developing brain is essential for understanding normal brain maturation, as well as for understanding what goes awry in developmental psychiatric and neurological disorders. Here, we studied resting-state magnetoencephalography data to map how distinct cortical networks mediated by the five fundamental fast (1-80 Hz) brain rhythms each change with maturation from age 7 to 29, in 162 participants including participants from an independent dataset, using graph theory metrics.

We found that maturation trajectories were dependent on the frequencies of the oscillations mediating the networks. Specifically, the gamma band mediated networks were characterized by increasing global efficiency, and followed the previously observed asymptotic growth curve. In contrast, the beta band mediated networks were characterized by increasing local efficiency, and followed a linear, rather than asymptotic, maturation trajectory. Additionally, the disparity in maturation trajectories for local versus global efficiencies resulted in significant changes through development in both small world property and network resilience, contrary to prior findings, and in opposite directions for the beta and gamma band mediated networks. Furthermore, the spatial distribution of growing and shrinking hubs was also markedly different for the two network systems.

These findings suggest that these two network systems are fundamentally distinct, have divergent roles in mediating normal cognitive maturation, and may be differentially linked to the etiology of developmental and psychiatric disorders.

Poster Number 109

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A non-human primate model of artificial vision

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Simulations of artificial vision have been used in human subjects to guide the design of visual prostheses. However, there is presently no animal model of artificial vision that permits both studying the viewing conditions experienced by human visual prosthesis implant recipients in addition to device development. To this end, we created a non-human primate (Macaca mulatta) model of simulated artificial vision. The animals (N = 3) controlled a simulation of artificial vision based on a retinotopic array of phosphenes (artificial points of light, each phosphene being a proxy for an implanted electrode). The model paradigm used a two-alternative forced-choice task to describe artificial vision performance. The stimuli to be recognized were the 26 letters of the Roman alphabet (Stelio typeface) presented on a computer screen at one of five letter size and decomposed by one of six simulated gaze-contingent phosphene patterns. The animals first visually explored a letter using changes in gaze location to shift the phosphene pattern (cue phase). They then chose by gaze fixation (choice phase) between the same letter (correct choice) and a non-matching distractor (incorrect), both presented in clear view. Here we show that non-human primates can learn to recognize letters using simulated phosphenes and can reach recognition performance levels close to humans. We are currently developing a visual prosthesis whereby the animals can recognize letters using only electrical stimulation delivered through a device implanted in the lateral geniculate nucleus.

Poster Number 110

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Microvascular abnormalities detected by DSC MR perfusion precede lesion progression in cerebral X-linked adrenoleukodystrophy

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X-linked Adrenoleukodystrophy (ALD) is a devastating neurodegenerative disorder that results from mutations in the ABCD1 gene and leads to rapidly progressive cerebral inflammatory demyelination in about 65% of affected males. The ABCD1 mutation is necessary but not sufficient to develop cerebral disease and a major challenge facing clinicians, now that newborn screening has become available, is determining which patients will convert to cerebral disease and qualify for highly toxic rescue treatments such as hematopoietic stem cell transplant. Blood brain barrier disruption, as indicated by a rim of contrast enhancement on MRI and ex-vivo histopathology has been associated with progression of cerebral ALD (CALD). While there is evidence that white matter hypoperfusion precedes blood brain barrier disruption and ABCD1 deficiency directly alters brain endothelial function white matter microvascular flow physiology has not been studied.

Here we show that alternations in microvascular flow pattern followed by increased endothelial permeability in cerebral white matter precede conversion to cerebral disease using advanced post-processing analysis of dynamic susceptibility contrast (DSC) MR perfusion. Further, these parameters normalize if disease arrests after treatment. Our findings indicate that ABCD1 deficiency causes microvascular flow changes in the white matter that contribute to the pathophysiology of cerebral inflammatory demyelination and that DSC MR perfusion may serve as a biomarker for risk stratification, treatment selection and monitoring of disease activity in ALD.

Poster Number 111

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Micro-magnetic stimulation of cortical pyramidal neurons

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Neural prosthetic implants that target the neocortex have the potential to dramatically improve a wide range of neurological and psychological diseases. Unfortunately, their long-term effectiveness has been limited, largely due to complex biological and chemical reactions that diminish the viability of the electrode and alter the local neural environment over time. Magnetic stimulation overcomes many of these limitations but coils small enough to be cortically implanted were not thought capable of neuronal activation. Here, we describe a new micro-coil design (for cortical implantation) and demonstrate its effectiveness via a series of electrophysiology experiments. For example, we show that L5 pyramidal neurons (PNs) respond strongly to magnetic stimulation and further, that the spatially asymmetric fields induced by such coils activate these neurons without simultaneously activating horizontally oriented axons or processes; as a result, the patterns of coil-elicited activity were more focal than those arising from electric stimulation. Further testing shows that not all locations of the coil elicit similar effects and that contrary to much previous work, the optimum site for positioning the coil may be in gray matter. *In vivo* testing demonstrated robust activation of whisker cortex including the ability to activate single whiskers or rows of whiskers, depending on the precise placement of the coil. Many of the other *in vivo* response properties arising from electric stimulation of whisker cortex were also matched or exceeded by the new coil; the enhanced reliability along with comparable or better performance suggests that this new approach may supplant conventional devices.

Poster Number 112

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Dual-gene CRISPR/Cas9 targeting to generate knockouts of potential Huntington's Disease modifier genes in the mouse

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Huntington's Disease (HD) is a dominantly inherited neurodegenerative disease, featuring motor signs, psychiatric disturbances and intellectual decline. The HD mutation is an unstable CAG trinucleotide repeat whose length is negatively correlated with the age at onset (AO) of diagnostic motor signs, accounting for ~65% of the substantial variance in AO. A genome wide association study (GWAS) by the GeM Consortium carried out with four thousand HD individuals has now identified two chromosomal regions that each harbor an HD modifier gene: one on chromosome 15 and one on chromosome 8. Each region contains two genes, only one of which is likely to be the true modifier: MTMR10 or FAN1 (Chr 15) and RRM2B or UBR5 (Chr 8). To enable functional prioritization of these alternative modifier candidate genes *in vivo*, we have used a dual-gene CRISPR/Cas9 blastocyst injection strategy to rapidly generate independent lines of HD candidate modifier gene mutant mice. This allelic mutation strategy generated a spectrum of mutant alleles for each gene, some of which have (through germline transmission) yielded independent lines of Fan1, Mtmr10, Rrm2b and Ubr5 mice with indels (in frame or truncating) mutations. Here we show the pros and cons of this dual-targeting approach.The lines of mice that we have created will provide an important resource for 1) genetic crosses with Hdh CAG knock-in mice to functionally prioritize each candidate as a modifier of CAG repeat instability and disease phenotypes and 2) detailed studies of the function of the true HD modifier genes.

Poster Number 113

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Investigating the Biocompatibility of Peripheral Nerve Cuff Electrodes through a 14 Month Chronic Implantation in the Rat

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The pathophysiology of neuromuscular injury or disease is characterized by an inability of the central nervous system to exert functional control over the peripheral musculature. In designing clinically-useful rehabilitative neural prosthetics for individuals with spinal cord injury or late-stage neuromuscular disorders, a fundamental question is: once a control signal is obtained from corticospinal tissue upstream of the lesion, how will artificial actuation of the intact downstream neuromusculoskeletal system take place. Muscles may be artificial stimulated through epimysial or intramuscular electrodes, or through peripheral nerve cuff electrodes. It is hypothesized that peripheral nerve cuff electrodes are superior to epimysial or intramuscular electrodes because of their potentially superior biocompatibility, tolerance and safety.

Here we investigate non-penetrating multichannel stimulating cuff electrodes that wrap around peripheral nerves. We tested the claims that peripheral nerve cuff electrodes do indeed exhibit better biocompatibility and are well-tolerated *in vivo*. We implanted a cuff electrode around the sciatic nerve of a rat for over 14 months, in a first study evaluating the biocompatibility and functionality of a peripheral nerve implant over such a long period.

Our histological, behavioral, and physiological data demonstrate that the cuff electrode was functional throughout without any biocompatibility issues. Our data support the hypothesis that peripheral nerve cuff electrodes are highly appealing for artificially actuating muscles when the normal spinal pathway is disrupted. This work has implications for chronically implantable neural interfaces for function restoration and rehabilitation in neuromotor disorders and injuries ranging from spinal cord injury to locked-in syndrome to limb amputation.

Poster Number 114

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Dysregulation of Auditory Cortex by Impaired Feedback in Autism Spectrum Disorder may Underlie Disrupted Auditory Perception in Concomitant Noise

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Autism spectrum disorder (ASD) is associated with increased difficulty in perceiving speech in the background noise. However, the underlying neuronal mechanism for this effect is poorly understood. We employed magnetoencephalography in an auditory frequency mismatch paradigm in the absence and presence of background noise. This paradigm is known to elicit a specific response to the deviant stimulus (mismatch field—MMF), through inferior frontal feedback modulation over temporal areas. In parallel, feedback information are usually mediated by beta band oscillations in general and in MMF in particular. We examined the neural sources of MMF and the spectro-temporal dynamics of the functional connectivity between temporal and inferior frontal areas, covering frequency ranges of 8-60 Hz, with and without a noisy background. In the absence of noise, common neural sources for both groups were located in right temporal and inferior frontal gyrus (IFG) underlying the MMF. However, in the presence of concomitant noise, the MMF response in the right IFG was diminished in ASD group, but preserved in controls. The MMF response in right IFG also correlated with severity of ASD. Lastly, in noise, we found a significant reduction in beta band (14-25 Hz) synchronization between left temporal and left inferior frontal sub-regions. Our findings suggest that feedback modulations are needed to mitigate the impact of noise on perception. Reduced fronto-temporal connections and consequent weak feedback influences in ASD, lead to dysregulation of temporal cortex, which is enhanced in noise, and likely contributes to impaired auditory perception in noise in ASD.

Poster Number 115

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Identification of IKAP responsive-genes as biomarkers for therapy of Familial Dysautonomia

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Familial dysautonomia (FD) is a congenital sensory and autonomic neuropathy caused by a "leaky" mRNA splicing defect that results in reduced levels of IKAP protein. IKAP, or ELP1, is a scaffolding protein of the Elongator complex which is required for efficient transcriptional elongation. We found that kinetin can correct the IKBKAP splicing defect and increase the amount of normal mRNA and protein. The identification of early disease-relevant pathways will permit to isolate biomarkers that can be used to assess the *in vivo* efficacy of therapies that alter IKAP levels. To identify IKAP-regulated pathways necessary for proper development of the nervous system, transcriptome sequencing analysis was performed in embryos expressing increasing amounts of IKAP. Consistent with the role of IKAP in transcriptional elongation, approximately 60% of all differentially expressed genes were down-regulated, and overall, down-regulated genes were longer than up-regulated genes. We identified 262 genes whose expression increased strictly as a monotonic function of IKAP levels. These genes highlight pathways involved in nervous system development, including synapse formation, neuron differentiation, axon guidance, axon growth and neuronal cell adhesion. Remarkably, the IKAP dose-responsive genes show strong enrichment of genes involved in neurotrophin signaling pathway ($p < 10\text{-}4$). Several genes in this pathway are known to be associated with clinically similar types of hereditary sensory and autonomic neuropathy, including NTRK1 for HSAN IV and NGF for HSAN V. Our findings emphasize the vital role of IKAP in embryo development and indicate that IKAP levels tightly regulate genes involved in neurodevelopmental pathways.

Poster Number 116

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Serum Calcium and Extent of Bleeding in Intracerebral Hemorrhage

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Background and Purpose: Calcium is a key cofactor of the coagulation cascade. We hypothesized that low serum calcium is associated with the extent of bleeding in intracerebral hemorrhage (ICH) as measured by baseline volume and risk of ICH expansion.

Methods: We performed a retrospective analysis of a prospective cohort of consecutive patients with primary ICH ascertained between 1994 and 2015. Subjects were included if ionized calcium measurement was obtained on admission. Hypocalcemia was defined as ionized calcium <1.13 mmol/L. Baseline and follow-up hematoma volume on non-contrast CT (NCCT) were measured using a computer-assisted semi-automatic analysis. Hematoma expansion was defined as increase>30% or 6 mL from baseline ICH volume. Association between serum calcium, baseline hematoma volume and ICH expansion were investigated in multivariable linear and logistic regression models respectively.

Results: 526 patients met the inclusion criteria (mean age 69.1+12.7 years, 57.8% males), of whom 348 (66.2%) had hypocalcemia on admission. Low ionized calcium levels were independently associated with higher baseline ICH volume (β= - 0.22, standard error =0.06, p=0.002). A total of 317 patients had a follow-up NCCT available and were included in the ICH expansion analysis. In this subgroup, the presence of admission hypocalcemia was associated with increased risk of ICH expansion (OR 2.4,95% CI 1.02–5.86,p=0.044), after adjusting for other confounders.

Conclusions: Hypocalcemia is common in ICH patients and correlates with extent of bleeding. Low calcium may be associated with a subtle coagulopathy predisposing to increased bleeding and might therefore be a promising therapeutic target for acute ICH treatment trials.

Poster Number 117

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Characterization of MLH1 as a candidate genetic modifier of Huntington's disease CAG repeat instability in patient brain

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Huntington's disease (HD) is a devastating neurodegenerative disorder for which there is no cure or disease-modifying treatment. It is caused by a CAG repeat expansion within the HTT gene, with larger CAG alleles associated with earlier disease onset. This repeat has a strong tendency for further expansion throughout the life of the patient, particularly in tissues/cell-types primarily affected in HD. This suggests that somatic expansion accelerates the disease, supported by our findings that longer expansions in HD cortex are associated with earlier disease onset.

We showed in HD mouse models that mismatch repair gene Mlh1 modifies somatic expansion and the pathogenic process. In a recent genome-wide association study we identified MLH1 as a candidate modifier of age of onset, suggesting that this gene may alter the disease course in patients via an effect on the HTT CAG instability. Here, we are testing the hypothesis that MLH1 is a modifier of somatic instability in patients by measuring CAG repeat length distributions in ~500 HD postmortem cortices and deriving quantitative measures of instability to test for association with MLH1 genotype. Preliminary results indicate that a common MLH1 genetic variant, associated with a later age of disease onset, is associated with reduced somatic instability.

These studies provide the first insight into a genetic modifier of CAG instability in HD patients. Importantly, they provide a means to understand the roles of disease modifiers in somatic CAG expansion, which may provide novel targets for therapeutic intervention directed at the mutation itself.

Poster Number 118

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The Hippo/YAP pathway: A novel pathogenic mechanism in Huntington's disease

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The Hippo signaling pathway has been implicated in mammalian organ size regulation and tumor suppression. Specifically, this pathway plays a critical role in regulating the activity of transcriptional co-activator Yes-associated protein (YAP), which modulates a proliferative transcriptional program. Recent studies have revealed that while the Hippo/YAP pathway is activated during tumorigenesis it may also play a role in neurodegeneration. For example, mammalian sterile 20 (STE20)-like kinase 1 (MST1), a downstream pro-apoptotic protein kinase in the Hippo pathway, mediates oxidative-stress-induced neuronal death. In addition, MST1 activity leads to caspase activation and impairment of autophagy in a mouse model of Amyotrophic Lateral Sclerosis. Importantly, homozygous deletions of MST1 in this mouse model delayed symptoms onset and improved the survival of spinal cord motor neurons. Finally, activation of the Hippo pathway has been linked to alterations in autophagy. Together, these findings implicate the Hippo/YAP pathway as a potential underlying mechanism in neurodegeneration. Therefore, we investigated the possible role of this pathway in Huntington's disease (HD) pathogenesis. Our results demonstrate that there is a significant increase in the active form of MST1, phosphorylated MST1 (pMST1), in post-mortem human cortex from patients. Additionally, pMST1 was also increased in the striatum and cortex of Hdh111/111 mice compared to control as well as STHdh111/111 cells compared to control STHdh7/7. There was also a significant, and concomitant, increase in YAP phosphorylation in Hdh111/111 striatum. Together, these results demonstrate that the Hippo/YAP pathway is altered in HD and may provide a novel therapeutic target.

Poster Number 119

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Novel characterization of CAG repeat instability in Huntington's disease (HD) patient-derived lymphoblastoid cell line and sperm DNA

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The CAG repeat expansion underlying Huntington's disease (HD) is highly unstable intergenerationally and somatically. To examine the relationship between somatic and intergenerational instability we first quantified instability in lymphoblastoid cell line (LCL) and sperm DNA. CAG expansion was positively correlated in LCL and sperm DNA with repeat length being a major driver of both.

We then investigated LCL instability in from a family in which the father with 51 CAGs transmitted repeats of 80, 107 and 115 CAGs to his offspring. LCLs were cultured for several months to characterize the repeat behavior repeat in the "intergenerationally unstable" father and his offspring. The CAG repeat in the transmitting father remained stable in LCLs; in contrast, the repeats from his offspring exhibited high instability with complex repeat length distributions reflecting different cell populations, and trends towards CAG expansion.

To gain insight into factors that might contribute to instability, independent of CAG repeat length, we modeled CAG instability in 24 HD LCLs as a function of repeat length and derived residual instability values that were correlated with the expression levels of a set of candidate DNA repair/DNA metabolism genes. Our results indicate two genes, FAN1 and TP73, whose expression levels correlate with residual instability in LCLs.

Overall, these results show that repeat length is the major determinant of instability, provide insight into the use of patient-derived LCLs as models for studying somatic instability and suggest candidate genes to be tested as CAG instability modifiers.

Poster Number 120

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Manganese Disrupts Amyloid Precursor Protein (APP) and Ferritin Translation Causing Neurotoxicity; Evidence for Therapeutic Intervention with Urate in Clinical Manganism

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Manganese (Mn2+) is an essential metal although occupational over-exposure to manganese causes neurodegeneration associated with gait and psychiatric disturbances. Manganism causes gait problems where clinical behavioral outbursts are discernible. This disorder is uniquely related to, but distinct from, Parkinson's disease. We demonstrated that Mn2+ dose-dependently inhibited translation of both the levels of ferritin and amyloid precursor protein (APP) as central iron homeostatic proteins that promote cellular survival, as exemplified by SH-SY5Y neuroblastoma cells. This action was manifested via iron-responsive element (IRE) RNA sequences in the 5'untranslated region (5'UTR) of the APP and ferritin-H transcripts. Levels of iron-regulatory protein-1 (IRP1) remained refractory to Mn levels, thus supporting this RNA binding protein to be key in Mn2+ interference of ferritin and APP translation. The consequences of the loss of the Mn-dependent protective axis of APP and ferritin expression against iron catalyzed oxidative stress caused a dramatic fall in cell viability after 48 hours and 72 hours metal exposure. We discovered the antioxidant urate offset this RNA-driven pathway to provide protection to neurons under toxic assault from manganese exposure. Increased urate offers clinical protection to PD patients and was found to overcome the toxic consequences of Mn exposure to neurons. We conclude the Mn2+ toxicity is partly attributable to translational inhibition of APP and ferritin and urate can be therapeutically adjusted to offset their absence to thus restore embargoed neuroprotective iron export and storage. Urate's action alters IRE activity to prevent an increase of iron-catalyzed reactive oxygen species (ROS), with subsequent neurorescue.

Poster Number 121

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Chronic Morphine Alters BDNF-TrkB Signaling in a Sex-Dependent Manner

INVESTIGATORS: K. E. Glajch, K. A. Mueller, G. Sadri-Vakili, E. H. Chartoff

Opioid addiction has been declared an epidemic in the United States. Emergency room visits due to opioid use increased 153% between 2004 and 2011. Additionally, the mortality rate from prescription-opioid overdose nearly quadrupled between 2000 and 2014. Given the rapid increase in opioid abuse and the rise in fatal opioid overdoses, there is an urgent need for a better understanding of the molecular events that may underlie this chronic, relapsing brain disorder. Brain-derived neurotrophic factor (BDNF) and its tyrosine kinase B receptor (TrkB) have been implicated in the rewarding effects of drugs of abuse. Therefore, we sought to determine whether exposure to chronic morphine would lead to alterations in BDNF-TrkB signaling. Male and female rats were treated with morphine (5 mg/kg) twice daily for 5 consecutive days. Protein levels were then measured in brain regions known to be important for drug addiction such as the nucleus accumbens (NAc) and prefrontal cortex (PFC) using western blots. Our results indicate no significant differences in BDNF levels in any of the brain regions tested. However, there was a significant increase in TrkB levels in the PFC and NAc of male, but not female, rats following exposure to chronic morphine. Additionally, TrkB phosphorylation/activation was decreased in the NAc of female rats. Our results are the first to demonstrate a sex-specific difference in BDNF-TrkB signaling in response to chronic morphine. Future studies are aimed at determining whether alterations in TrkB signaling underlie the rewarding effects of opioids.

Poster Number 122

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Staged anticonvulsant screening for chronic epilepsy

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Significant limitations are associated with studying seizures evoked in otherwise normal *in vitro* and *in vivo* preparations, which current anticonvulsant screening programs are predominantly based on. Although numerous anticonvulsants have been produced as a result of decades of drug screening and development efforts, one third of epileptic patients do not respond to these drugs. We present a drug discovery program utilizing a tiered compound screening platform based on chronic epilepsy and spontaneous seizures, with compounds advancing from high-throughput *in vitro* models to low-throughput *in vivo* models. The initial stage utilizes the reproducible, accessible and accelerated course of epileptogenesis in the *in vitro* organotypic hippocampal slice culture model of severe traumatic brain injury and subsequent post-traumatic epilepsy to conduct a blind screen of an array of compounds and conditions for anticonvulsant, antiepileptic and neuroprotective effects in chronic epilepsy. Lactate production and release of lactate dehydrogenase into spent culture media were used as biomarkers of seizure activity and cell death, respectively, to quantify epileptogenesis in slice cultures. Compounds exhibiting significant reducing effects were retested and subsequently advanced to a second stage comprised of wash-out screens to differentiate anticonvulsant from antiepileptogenic effects, as well as *in vitro* electrophysiological confirmation. The third stage was comprised of double-blind, crossover-controlled, *in vivo* continuous electrographic monitoring of spontaneous seizures in the kainate model of chronic epilepsy. We have screened over 500 compounds and conditions. The cyclooxygenase inhibitor celecoxib had no effect on chemically-induced acute epileptoform activity but exhibited robust anticonvulsant activity in all models of chronic epilepsy.

Poster Number 123

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Modeling the functional genomic impact of mutations in chromatin regulators on neurodevelopment

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Autism Spectrum Disorder (ASD) is characterized by deficits in social communication and repetitive patterns of behaviors. The inaccessibility of patient neurons for research has represented an obstacle in the elucidation of disease etiologies. The ability to generate neuronal progenitor cells (NPC) *in vitro* from induced pluripotent stem cells (iPSC) now allows examination of the molecular mechanisms underlying pathogenesis. To study the contribution of individual strong effect ASD risk genes in an isogenic background, an iPSC line derived from a healthy male subject was subjected to gene editing via CRISPR/Cas9 to knockout one allele of each gene—MBD5, EHMT1, METTL2B—to create a series of mutant iPSCs. These were then driven into the neuronal lineage by using STEMdiff™ Neural Induction Medium for subsequent transcriptional analyses by RNAseq.

Many genes associated with ASD, as the above, are involved transcriptional regulation and chromatin remodeling. In fact 2q23.1 microdeletions and mutations that disrupt MBD5 contribute up to 1% of individuals with ASD and the impact of this locus on neural differentiation and function has yet to be explained. By analyzing the transcriptional profile of MBD5 through neurodevelopment, we found that its expression increases during the differentiation process, indicating its potentially critical role at the post-mitotic stage. EHMT1 and METLL2B follow a different pattern, having expression remain stable or decrease, respectively. Therefore, these chromatin-associated genes may have critical roles at different stages of development, as part of a convergence of biological pathways in ASD.

Poster Number 124

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Progranulin-Deficient Frontotemporal Dementia: Modeling and Screening

INVESTIGATORS: A. A. She, S. Sheridan, T. Fu, K. Hennig, I. Kurtser, D. Lucente, J. Gusella, B. Dickerson, S. J. Haggarty, The Bluefield Project to Cure Frontotemporal Dementia

Frontotemporal dementia (FTD) is the second most common form of presenile dementia after Alzheimer's disease, accounting for 5-15% of all cases of dementia in individuals between 45 and 65 years of age. One proposed cause for the disease is the haploinsufficiency of the progranulin gene, which codes for a secreted glycoprotein widely expressed in the nervous system as well as epithelia, bone marrow, immune cells, and solid organs during both development and adulthood. In brain, progranulin is expressed in both neurons and microglia, and several of its granulin derivatives have been associated with neurite outgrowth and neuron proliferation, as well as inflammation control in the microglia. Because progranulin is a disease of haploinsufficiency, one course of therapeutic action could be to increase progranulin levels by upregulating GRN expression of the wild-type allele. The work below describes efforts to identify progranulin small molecule modulators in a human neuronal model with robust mRNA and protein assays, with future directions to further characterize effects of epigenetic modifiers of progranulin expression in human FTD neuronal models.

Poster Number 125

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Tauopathy: Discovery of Small Molecule Modulators of Tau Phenotypes in Human iPSC-Derived Neuronal Models of Frontotemporal Degeneration

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Frontotemporal dementia (FTD) refers to a group of neurodegenerative diseases characterized by the presence of potentially deleterious protein inclusions in the affected brain regions, but the molecular events leading to neuronal loss are yet not fully understood. Autosomal dominant forms of FTD are commonly associated with mutations in the MAPT gene encoding the microtubule-associated protein tau, and exhibit tau inclusions within neurons and glia. To identify early tau molecular events associated with FTD, we generated expandable neural progenitor cell (NPC) lines from induced pluripotent stem cells (iPSC) of individuals with the tau-A152T variant. Neurons derived from A152T-iPSCs showed rapid accumulation of total-tau and phosphorylated-tau, and differential tau solubility relative to control cells. Accumulation of tau in A152T neurons was coupled to increased neuronal vulnerability to proteotoxic, excitotoxic and mitochondrial stressors, suggesting that the accumulation of tau is an early event in pathology. To identify modifiers of tau-associated phenotypes, we performed a pilot screen with known bioactive compounds, including FDA-approved or under clinical investigation, which revealed three main classes of compound-hits targeting autophagy, neuronal signaling and the chaperone-protein folding pathways. Our lead compounds robustly downregulate steady-state levels of total and phosphorylated-tau, as well as insoluble tau, with less than a 24hr treatment. We anticipate that further dissection of the folding and clearance pathways as a mechanism to alleviate tau phenotypes will enable the discovery and validation of effective disease-modifying, targeted therapeutics, with relevance for a larger group of degenerative tauopathies including Alzheimer's disease.

Poster Number 126

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Role of Calcineurin in Alzheimer's Disease

INVESTIGATORS: M. Thaker, S. Hopp, E. Hudry, B. T. Hyman

Calcineurin (CN) mediates diverse physiological processes including neuronal differentiation, axonal transport, inflammation, and autophagy by dephosphorylating critical signaling proteins and transcription factors. The holoenzyme is composed of a catalytic and a regulatory subunit. Accumulating evidence suggests that CN inhibition is a specific target for alleviating symptoms associated with early synaptic changes in Alzheimer's disease (AD). Therefore we developed a series of assays to examine the mechanism of CN regulation in AD by comparing its three catalytic isoforms PPP3CA, PPP3CB, PPP3CC and two regulatory isoforms RCAN1 and RCAN2 using immunohistochemical and biochemical assays. Cytosolic, nuclear and synaptoneurosomal fractions from the frontal cortex of AD brains were compared to age and gender matched control brains. Strikingly, only the truncated forms of PPP3CA and PPP3CB were upregulated, while PPP3CC was reduced; both RCAN1 and RCAN2 were also reduced in AD brains implying enhanced activation of CN due to both loss of the autoinhibitory C-terminal domain, and a decrease in endogenous RCAN inhibitor. Notably the altered expressions of catalytic as well as the regulatory proteins were mainly in the cytosolic fractions. Immunohistochemistry demonstrated that the catalytic isoforms exhibit differential sub-cellular localizations in mouse brain; PPP3CA localized in the nucleus, PPP3CB in the cytoplasm, and PPP3CC is primarily axonal while RCAN1 and RCAN2 localize both in the cytoplasm and nucleus. Taken together, these data strongly suggest that both catalytic and regulatory isoforms of CN have altered expression and activity in AD brains which is certainly linked with the pathogenesis of AD.

Poster Number 127

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A Role for Genetic Variation in VKORC1 in Warfarin-Related Intracerebral Hemorrhage

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Background and Purpose: Warfarin remains in widespread use and increases intracerebral hemorrhage risk. Because roughly 1/3 of warfarin-related ICH (wICH) occurs in the setting of supratherapeutic anticoagulation, we examined whether common genetic variants that modify inter-individual differences in warfarin response (1) influence wICH risk and (2) account for higher degrees of anticoagulation among wICH patients.

Methods: We performed a genetic association analysis of two CYP2C9 variants and five VKORC1 variants associated with heightened warfarin response. 188 wICH cases and 184 ICH-free, warfarin-exposed controls were included. Using regression modeling and permutation, we tested for association between genetic risk scores and (1) wICH status and (2) International Normalized Ratio (INR) at presentation, controlling for known risk factors.

Results: wICH cases were older, more frequently hypertensive, with higher median INRs (2.60 vs. 2.40, p = 0.008) independent of warfarin dose. The VKORC1 score was associated with INR in cases (p = 0.038) and trended toward significance in controls (p = 0.091). Each additional VKORC1 variant increased INR by 0.092 (95% CI: 0.017—0.167) in cases and 0.035 (95% CI: -0.015—0.085) in controls. Permutation analysis confirmed a significant difference in strength of association (p = 0.002) between groups. No association was found between genetic risk scores and wICH risk.

Conclusion: VKORC1 variants have greater effect on INR in wICH cases than controls, suggesting that modulation of the effects of VKORC1 on warfarin response may influence wICH risk. Further studies are needed to characterize genetic contributions to INR and their relationship with wICH.

Poster Number 128

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[11C]Neuroflux: In vivo measurement of neuronal population flux

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Neurodevelopmental and neurodegenerative diseases often exhibit an aberrant neuron population flux, the time-dependent change in steady-state cellular counts of a neuron population. As the most dynamic adult neuron population, we propose that olfactory sensory neurons (OSNs) afford a sensitive locus to detect changes in neuron population flux following abnormal adult neurogenesis or neuron death. However, there are currently no tools to monitor OSNs on a population-wide basis. Here, we report a novel PET radiotracer, [11C]neuroflux, which quantifies steady-state OSN population by specifically binding mature OSNs. During the neurogenesis predominant periods of normative postnatal development and post-lesion regeneration, longitudinal [11C]neuroflux imaging provides OSN population flux measurements that are sensitive to individual variation. Meanwhile, during the neuron death predominant periods of normative aging and tauopathy-related neurodegeneration, [11C]neuroflux identifies OSN population deficits that, in the tauopathy model, occur prior to widespread tau deposition and at the age of behavioral symptom onset. These results demonstrate the first *in vivo* measurement of neuron population flux with specificity for a single, adult-neurogenic population with potentially wide applications for understanding neurogenesis and neuron death dynamics. The measured sensitivity of the OSN population to the life cycle alterations of aging and tauopathy-related disease lends *in vivo* support for involvement of OSN loss in the etiology of the olfactory dysfunction that predicts mortality in older adults and predicts conversion from mild cognitive impairment to dementia. We anticipate use of [11C]neuroflux to monitor OSN population across diseases with altered neuron life cycles will facilitate development of neurogenic or neuroprotective treatments.

Poster Number 129

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The limits of microbleed and microinfarct detection: high-resolution MRI-histopathology correlation

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Cerebral amyloid angiopathy (CAA) is a common neuropathological finding in the aging human brain, associated with cognitive impairment. Key contributors to cognitive impairment in advanced CAA are cortical microbleeds and microinfarcts. Here, we aimed to gain more insight in the pathological basis of these lesions, further define their neuroimaging properties, and establish the limitations of current microbleed and microinfarct detection. Brain slabs from 5 cases with pathology-proven CAA and multiple microbleeds on *in vivo* clinical MRI were subjected to high-resolution *ex vivo* 7T MRI. On the obtained high-resolution (200µm3) *ex vivo* MRI 171 microbleeds were detected, compared to 66 microbleeds on the corresponding *in vivo* MRI. Thirteen retrieved microbleeds on pathology were verified as acute or old microhemorrhages. Additionally, in 3 out of 5 cases, 48 microinfarcts were observed on *ex vivo* MRI. None of them were visible on *in vivo* MRI. Nine retrieved microinfarcts on pathology were verified as acute or old microinfarcts. Finally, we explored the burden of hemorrhagic and ischemic pathology that remains invisible, by scanning a smaller sample at ultra-high resolution, followed by serial sectioning. At ultra-high resolution (75µm3) MRI we observed an additional 48 microbleeds, escaping detection at high-resolution, which proved to be vasculopathies on pathology instead of frank hemorrhagic events. Assessing the serial sections yielded 9 additional microinfarcts of which 6 were retrospectively visible at ultra-high resolution. This study revealed that we have reached the limits of microhemorrhage detection in CAA, whereas current clinical *in vivo* MRI remains relatively insensitive for microinfarct detection.

Poster Number 130

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Synaptotagmin 1 in Alzheimer's disease—guard or partner in crime?

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Synaptic loss is the strongest correlate of memory deterioration in Alzheimer's disease (AD). Synaptic dysfunction is caused by local accumulation of amyloid β (Aβ), and in particular neurotoxic Aβ42. Aβ is a proteolytic product of a subsequent processing of the amyloid precursor protein (APP) by two enzymes - β-secretase and presenilin 1 (PS1)/γ-secretase. Continuous, default or experimentally induced neuronal activity causes an increase in Aβ production, which is strongly related to intracellular calcium flux and synaptic vesicle exocytosis.

To gain further insight into Ca2+-dependent regulation of the Aβ production, we performed a mass spectrometry screen and found synaptotagmin 1 (Syt1) as a novel PS1 interactor that binds directly to PS1 in high Ca2+. Syt1 is a calcium sensor in neurotransmitter release and is involved in trafficking of synaptic vesicles at the active zone of the synapse.

The interaction was confirmed *in vitro* and *in vivo* by co-immunoprecipitation and fluorescence lifetime imaging microscopy. The role of Syt1 in Aβ40 and Aβ42 production, and in the stability and trafficking of β- and γ-secretases was investigated using Syt1 knock-down and overexpression approaches. Our experiments demonstrate that PS1 interacts with Syt1 in a Ca2+-dependent manner and modulate maturation and trafficking of APP processing enzymes. Syt1 overexpression and knock-down result in increased and decreased levels of secreted Aβ, respectively.

In conclusion our study brings together important players in AD pathogenesis: synapse, Ca2+, PS1 and Aβ. The discovery that Syt1 affects Aβ at the synapse opens new avenues for therapeutic interventions focusing at the synapse.

Poster Number 131

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Single and multi-trait GWAS identify novel loci for sleep quantity, disruption and sleepiness, and highlight shared genetics with neuropsychiatric and metabolic traits

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Sleep disturbances chronically affect 50-70 million US adults and are significantly associated with higher risk for cardio-metabolic diseases, mood disorders and all-cause mortality. A wide range of inter-individual variation exists in sleep duration, timing and quality. Identifying the genetic basis for differences in self-reported habitual sleep quantity and quality should lead to better understanding of sleep function and causal relationships linking sleep to disease with potential for clinical translation. We performed genome-wide association analyses of self-reported sleep duration and disruption and daytime sleepiness in >100,000 subjects of European ancestry in the UK Biobank. We identified genome-wide significant ($p<5x10-8$) and suggestive ($p<5x10-7$) loci associated with sleep quantity (5 loci, including replication of PAX-8) and quality (5 loci including MEIS1; daytime sleepiness, 7 loci). Six genome-wide significant loci were discovered by multi-trait analyses, 2 of which were not found by single-trait analysis (near INADL and HCRTR2). Loci of interest were enriched for transcription factor binding sites, including MEIS1 binding sites (6.02 fold enrichment in sleep duration, $p=0.0096$). Significant genetic correlation was observed between sleep duration and schizophrenia ($r2=0.29$, $p=1.90x10-13$) and between daytime sleepiness and metabolic disorders (BMI $r2=0.199$, $p=3.12x10-09$; waist circumference $r2=0.199$, $p=2.12x10-07$). These results provide novel biological insights into regulation of sleep quantity and quality, reveal shared underlying biology with human health and disease, and offer potential new therapeutic avenues for sleep disorders and co-morbid conditions.

Poster Number 132

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Non enzymatic template directed oligomerization in aqueous buffer of RNA by phosphoimidazolide activated ribomononucleotides

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Contrary to fire that requires oxygen to release light and heat, RNA bring its own energy for polynucleotide assembly. Biologically, in the active site of polymerase, template sequence recognize the primer by Watson Crick base pairing then the 3' end of ribose moiety interact by nucleophilic substitution to phosphate entity of the following activated monomer. A molecular system for non enzymatic primer extension has been developped by Szostak team to give extended primers. Efficient non enzymatic synthesis of polynucleotides in aqueous buffer requires a common leaving group: phosphoimidazolide activated ribomononucleotide. The problem of rate template copying chemistry is chemical template copying occurs on the same timescale as template and substrate degradation. Hydroxyazabenzotriazole leaving group was found to lead to significantly improved rates of primer extension. The choice of leaving group is important. From experimental studies, electronic effects of substituents are involved in the mechanism of template directed oligomerization.

Poster Number 133

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The fasting glucose associated PPP1R3B locus maps to LOC157273, a long non-coding RNA which represses PPP1R3B expression in primary human hepatocytes

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Genome wide association studies identified a chromosome 8 locus associated with fasting glucose (FG), fasting insulin (FI) and lipid levels near PPP1R3B, which encodes the catalytic subunit of a serine/threonine protein phosphatase that promotes hepatic glycogen storage upon insulin signaling. The lead SNP rs4841132 lies in a long non-coding RNA, LOC157273, 175 kb away and not in linkage disequilibrium with PPP1R3B. We hypothesized that LOC157273 is the causal candidate that acts upstream of PPP1R3B to regulate FG and FI. We performed FG and FI association analysis on whole genome sequence data from 2876 nondiabetic European ancestry individuals on a contiguous 650kb region containing PPP1R3B and LOC157273. Using primary cultured human hepatocytes, we assessed LOC157273 expression by real-time PCR and full-length cDNA sequencing, localization with Stellaris RNA-FISH and regulation of PPP1R3B through LOC157273 knockdown with siRNAs. Our fine-mapping of the FG signal (rs4841132; P=0.004) uncovered a more significantly associated variant upstream of LOC157273 (rs983311; P=2×10-4) falling in a predicted transcriptional enhancer active in liver (P=4×10-31, Epigenome Roadmap data). Analysis of the FANTOM consortium data shows LOC157273 is expressed only in hepatocytes. We validated LOC157273 expression in primary human hepatocytes and showed localization to cytoplasmic punctate structures, suggesting regulatory functions of LOC157273-containing ribonucleoprotein complexes. LOC157273 knockdown using siRNA reproducibly increased PPP1R3B mRNA levels by 1.5- to 2-fold, demonstrating that LOC157273 represses PPP1R3B expression. Our results suggest that LOC157273 may be a key regulator linking genetic variants at the locus with PPP1R3B activity and with FG and FI levels.

Poster Number 134

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Genomic and Functional Stability of the Human Gut Microbiome

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Characterizing the temporal stability of the human gut microbiome is essential in identifying diagnostic biomarkers and modifiable microbiome components for disease prevention and treatment strategies. We have limited insight into the temporal dynamics of the microbiome and the determinants of stability.

We conducted metagenomic and metatranscriptomic sequencing of the human gut microbiome among 308 male participants enrolled in a sub-study of the Health Professionals Follow-up Study. Using a previously validated self-sampling collection method, participants provided up to four stool samples – one pair collected 24-72 hours apart followed by a second pair approximately 6 months later. DNA was extracted from 929 samples and RNA was reverse-transcribed to cDNA from a subset of 378 samples. Both were sequenced with the Illumina HiSeq platform. Metagenomic and metatranscriptomic data were profiled using HUMAnN2 and MetaPhlAn2.

Within-person variation in taxonomic and metagenomic composition over time was consistently lower than between-person variation at any given time point, unlike metatranscriptomic profiles. In exploring the determinants of stability, feature prevalence and relative abundance appeared to be highly correlated with feature stability. Metagenomic stability accounted for 70% of metatranscriptomic stability. 80% of differentially regulated pathways were consistently over- or under-expressed.

In one of the largest studies to date, we describe the major underpinnings of within- and between-person variation in the gut microbiome. Gut organismal composition and metagenomic profiles remain highly personalized over time unlike metatranscriptomic profiles. Thus, prospective characterization of the gut microbiome at a single time-point may provide a suitable measure for long-term follow-up of disease incidence.

Poster Number 135

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'Human Knockout Project' in a Pakistani population with high levels of consanguinity

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Experimental disruption of both gene copies ("knockout") in model organisms has proven useful to understand gene function. Here, we leverage naturally-occurring null mutations and consanguinity in the human population in order to: 1) identify humans knocked out for genes; and 2) characterize the phenotypic consequences of complete gene disruption. We performed exome sequencing in 7,078 individuals living in Pakistan, a region of the world with high levels of consanguinity, tested whether knockouts differed from wild-type participants across >200 cardiometabolic traits, and performed genotype-based recall for deeper phenotyping.

Among participants, the median excess homozygosity was 1.6%. 36,850 "high-confidence" null mutations, based on annotations, allele frequency, transcript position, splice sites, and conservation, across 12,131 autosomal genes were identified. 961 distinct genes were completely disrupted by homozygous high-confidence null mutations. 1,306 participants (18.4%) had at least one gene knocked out. Simulations using the excess observed autozygosity suggest that with the sequencing of ~200,000 Pakistanis may yield up to six-times greater knocked out genes compared to similar numbers from outbred populations. In a phenotypic screen, homozygosity for null mutations at PLAG27 was associated with absent lipoprotein-associated phospholipase A2 activity; at APOC3, with absent apolipoprotein C-III activity; at CYP2F1, with higher plasma interleukin-8 concentrations; and at either A3GALT2 or NRG4, with markedly reduced plasma insulin C-peptide concentrations. Oral fat tolerance testing of APOC3 knockouts demonstrated markedly blunted post-prandial lipemia compared to knockouts. These observations provide a roadmap to understanding the consequences of complete disruption of a large fraction of genes in the human genome.

Poster Number 136

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Genomic Landscape of Balanced Cytogenetic Abnormalities in Subjects with Multiple Congenital Anomalies

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Balanced chromosomal abnormalities (BCAs) represent a unique class of genomic variation associated to potential single-gene disruptions, yet their characterization has been reliant upon conventional cytogenetic methods. We performed whole-genome sequencing of cytogenetically detected BCAs in a cohort of 235 subjects with multiple congenital anomalies that either occurred de novo or segregated with phenotype in families.

Sequencing yielded to the precise mapping of 91% of included BCAs delineated by 699 breakpoints, revealing 164 simple chromosomal exchanges while 51 harbored additional cryptic complexity ranging from three breakpoints to over 57 breakpoints. Virtually all karyotypes misplaced breakpoints by at least one sub-band. Comparing these breakpoints to a random distribution of simulated breakpoints revealed that they are significantly enriched for genes associated with neurodevelopmental disorders, genes expressed early during embryonic development, and evolutionary constrained genes. Among 215 BCAs mapped, sequencing revealed the disruption of 50 pathogenic loci and 26 novel or recently described risk loci for congenital anomalies, suggesting that the phenotype is attributable to a single gene disruption in at least 23% of cases. Finally, a thorough analysis of BCAs with intergenic breakpoints hilighted 11 cases with possible positional effect involving genes associated to known syndromes and for which there was a strong phenotypic concordance. Specifically, six BCAs cluster at 5q14.3 and seem to phenocopy the associated MEF2C-microdeletion syndrome.

Our results suggest that 23-41% of BCAs associated with congenital anomalies likely represent pathogenic variations through single-gene disruption or deregulation, and emphasize that the impact of non-coding structural variations has been largely unexplored.

Poster Number 137

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Genome-wide analysis identifies novel loci for chronotype and a causal relationship with educational attainment

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Chronotype is associated with sleep disorders, cognitive and physical performance, chronic metabolic and neurologic disease, particularly when there is circadian desynchrony between internal and external timing. Using self-reported chronotype and genetic information from 100,420 subjects of European ancestry from the UK Biobank, we performed a genome-wide association study. Chronotype was reported as “definite morning”, “more morning than evening”, “more evening than morning” and “definite evening”. We measured heritability and performed single variant association tests adjusting for age, sex, principal components and genotyping array (n= 39,025,120 variants). Follow up analyses included gene-based association tests, gene-set analysis, heritability partitioning across tissues and functional classes, pair-wise genetic correlation to 19 traits, and Mendelian randomization. We identified 12 genetic loci, of which 9 are novel and 5 are in or near a gene with an established role in circadian rhythms. The 12 loci account for 4.3% of chronotype variation, and genome-wide genetic variation accounts for 19.8% of chronotype variation. Pathway analysis implicates a role for central nervous and ocular systems and fear-response related pathways. Heritability of chronotype is enriched in transcriptional enhancer and conserved genomic regions, as well as the CNS and adrenal gland/pancreas. In cross-trait analyses, significant genetic correlation was observed between chronotype and years of education, schizophrenia, and BMI, pointing towards shared biology, with a causal relationship between “night owl” chronotype and increased educational attainment. These results expand our knowledge of the human circadian system, and expose the influence of circadian characteristics over health and life-history variables such as educational attainment.

Poster Number 138

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An international, collaborative genome-wide association study of Tourette Syndrome identifies a non-coding RNA expressed early in human brain development as a candidate TS susceptibility gene

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Tourette Syndrome (TS) is a childhood-onset disorder with one of the highest heritabilities among non-Mendelian neuropsychiatric diseases. We have previously shown that TS is highly polygenic; however, to date the sample size of TS genetic studies has been too small to identify definitive susceptibility loci. Here, we present the largest TS genome-wide association study (GWAS) to date, totaling 4232 European ancestry cases and 8318 ancestry-matched controls. One SNP, rs2708146, achieved genome-wide significance (p=2x10-8) and lies within a 1Mb haplotype spanning an uncharacterized long, intergenic, non-coding RNA (lincRNA), LINC01122, that is expressed primarily in mid-fetal human brain development (15-16 pcw). Pairwise correlational analysis of 48,582 genes expressed in human fetal brain across 7 prenatal time points and 16 brain regions identified BCL11A, a gene 1 Mb centromeric to LINC01122 and known to harbor mutations in autism patients, as one of the top LINC01122 co-expressed genes. Subsequent analyses have integrated multiple sources of epigenomic data from human brain tissue and *in vitro* human stem cell models of developing cortical neurons, including 1) H3K27Ac marks of active gene enhancers, 2) developmental time-point-specific gene expression to identify regional genes that correlate with enhancer activity, and 3) biophysical models to predict changes in transcription factor binding site affinity caused by candidate causal GWAS SNPs within these active enhancers. These analyses, combined with efforts to replicate the association signal in independent cohorts, are in progress to characterize this locus and its potential role in the development of cortico-striatal-thalamic-cortical circuits thought to be abnormal in TS patients.

Poster Number 139

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Genetic risk of type 2 diabetes: Impact of chronotype and shiftwork

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Epidemiologic and experimental human studies suggest that shift-work and circadian disruption influence risk of type 2 diabetes. Previous genome-wide association studies (GWAS) have found >80 genetic variants that increase risk for type 2 diabetes. In this study, we tested whether shiftwork or circadian rhythm modify the effect of genetic risk variants on fasting glucose or type 2 diabetes susceptibility by performing genetic interaction tests. We used population data from three cohorts, the Sleep Heart Health Study (SHHS, n=4,300), Multi-Ethnic Study of Atherosclerosis (MESA, n=1,670), and the UK Biobank (n=96,945). Type 2 diabetes cases were defined using cohort specific guidelines including: elevated fasting blood glucose, medication use, and/or physician diagnosis. Objective (7-day actigraphy) and self-reported measures of chronotype were used. Shift work was assessed by self-report. Genetic data for up to 32 variants were available across all cohorts. We identified an interaction between genetic variation in IGF2BP2 (rs4402960) and sleep timing in all three cohorts. Results demonstrated that objectively measured sleep timing in SHHS modified the effect of the diabetes risk variant on fasting blood glucose in non-diabetic individuals (weekday sleep midpoint pint=0.054; weekend sleep midpoint pint=0.022), while self reported data suggested increased type 2 diabetes susceptibility based on chronotype in the UK Biobank (p=5.35E-04). Our results show an interaction between a type 2 diabetes risk variant and sleep and circadian rhythms and should inform future research on biology underlying the IGF2BP2 association and strategies for better risk stratification and treatment of type 2 diabetes.

Poster Number 140

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Lung Adenocarcinomas (ADC) with KRAS Mutations are Biologically Heterogeneous

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Background: We have reported the association of PD-L1 expression with KRAS mutations in ADC, suggesting that PD-1/PD-L1 blockade may be a treatment strategy for KRAS-mutated ADC (K-ADC). However, the majority of K-ADC does not exhibit PD-L1 and multiple studies have shown heterogeneity in therapeutic responsiveness and biology in K-ADC. Thus, we evaluated 139 K-ADC with a few biomarkers to classify them into biologically relevant groups.

Design: p53, HNF4a, PD-L1 and CD8 IHC were performed on tissue microarrays or representative sections of specimens. The cohort was classified into p53 abnormal (KP), p53 normal & HNF4a positive (KH) and p53 normal & HNF4a negative (KN) groups. Clinicopathological variables, PD-L1 expression, CD8+ tumor infiltrating lymphocytes (TILs) and progression free survival (PFS) were compared between these groups.

Results: The KP group (n=73) was associated with advanced stage (IV) (41%, p=0.023 vs. KN), PD-L1 expression (50%, p=0.010 vs. KH, p=0.018 vs. KN) and increased CD8+ TILs (29%, p=0.011 vs. KH, p=0.008 vs. KN), while the KH group (n=25) correlated with an invasive mucinous adenocarcinoma (IMA) morphology (48%, p=0.0003 vs. KP, p=0.0021 vs. KN). The KN group (n=41) showed a trend towards adverse outcomes. The 3-year PFS was 57%, 74% and 46% for the KP, KH, and KN groups, respectively.

Conclusion: K-ADC appear to consist of biologically distinct groups. While those with p53 alterations more likely present at an advanced stage but may be amenable to the PD-1/PD-L1 axis blockade, HNF4a positive tumors more likely exhibit the IMA morphology and follow a more indolent clinical course.

Poster Number 141

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Personalized Medicine for Diabetes by Controlling for Patient-Specific Variation in RBC Lifespan

INVESTIGATORS: R. Malka, D. M. Nathan, J. M. Higgins

The glycated hemoglobin assay (HbA1c) is the standard measure for managing diabetic patients because it provides the best estimate of a patient's average blood glucose (AG) level over the preceding 2-3 months and is the best predictor of long-term disease complications, including kidney failure and heart disease. However, there is a substantial unexplained glucose-independent variation in the AG-HbA1c relationship. The difference between a healthy (non-diabetic) and a diabetic patient that requires treatment can be as small as 10%, while variability in HbA1c for identical AG can be as high as 33%. This unexplained variation in the AG-HbA1c relationship compromises medical care for diabetes.

We dissect the factors responsible for inter-patient variation in the AG-HbA1c relationship by modeling both the chemical kinetics of hemoglobin glycation and statistical variation in average red blood cell (RBC) age. We find that inter-patient variation in mean RBC age is sufficient to explain all glucose-independent variation in the AG-HbA1c relationship. Other contributing factors may also vary from one patient to the next, but our analysis shows that any significant variation in these other factors must be strongly negatively correlated with variation in mean RBC age.

We show how a patient-specific estimate of mean RBC age can be derived from one pair of HbA1c and average glucose measurements. This derived mean RBC age can then be used to personalize future estimates of average glucose, improving clinical accuracy, and helping to realize the vision of precision medicine for diabetes.

Poster Number 142

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Optimization of the Positive Emotions after Acute Coronary Events (PEACE) Behavioral Health Intervention: A Factorial Design Study

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Background: Positive psychological (PP) constructs are associated with greater adherence to health behaviors. No study has assessed if PP interventions, which aim to boost positive emotions, promote physical activity (PA) in post-acute coronary syndrome (ACS) patients.

Method: The PEACE III study (planned N=128) uses the Multiphase Optimization Strategy to create a PP intervention for ACS patients. It will determine the feasibility of a targeted PP intervention. Using a 2x2x2 factorial design, it will assess the optimal frequency of exercise completion and the utility of both booster sessions and a motivational interviewing component. The primary outcome is PA at 16 weeks, measured via accelerometer. Three distinct between-group comparisons of PA (daily vs. weekly PP exercises, booster vs. no booster, PP vs. PP-MI) will be performed using random effects models, controlling for baseline PA, to identify ideal components of the PP-based intervention. To assess feasibility, descriptive statistics will assess the proportion of exercise completion and the engagement with self-report follow-up data and objective adherence devices.

Results: Thus far (n=61), 341/488 (70%) of assigned exercises have been completed by participants. Participants have rated highly both the ease (mean 7.8 +/- 2.2 out of 10) and utility (7.8 +/- 2.3) of the exercises. On-pre post ratings, participants reported significant improvements in both happiness ($p<.001$; effect size $d=.42$) and optimism ($p<.001$; effect size $d=.30$).

Conclusions: The PEACE III study will optimize a PP intervention in post-ACS patients. It is novel in its targeting of high-risk patients with a PP intervention and its factorial design.

Poster Number 143

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Fear Memories Strengthened by Rapid Eye Movement Sleep in Individuals with Primary Insomnia

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Insomnia increases risk of developing anxiety disorders, which may be associated with deficient fear extinction. We examined relationships between sleep and fear/extinction memories in individuals with Primary Insomnia (PI) and Healthy Sleepers (HS).

Ten PI (8 female, mean 37.9y) and 7 HS (5 female, 43.6y) underwent 2 weeks of home sleep monitoring then completed a 2-session fear conditioning/extinction protocol during fMRI. Sleep before both sessions was recorded using ambulatory polysomnography. During Fear-Conditioning, a shock established skin-conductance responses (SCR) to 2 differently colored lamps (CS+) but not a third (CS). One CS+ (CS+E), but not the other (CS+U), was then extinguished. After a 24-hr delay, all 3 CS were presented (Extinction-Recall). Because extinction memory opposes fear memory, higher SCR to CS+E at Extinction-Recall indicates poorer extinction memory and higher SCR to CS+U indicates greater recovery of unopposed fear memory.

Among PI, SCR to both CS+E and CS+U varied positively with between-session Rapid Eye Movement sleep (REM) percentage ($r=0.698$, $p=0.025$ and $r=0.630$, $p=0.051$, respectively) and REM minutes ($r=0.863$, $p=0.001$ and $r=0.776$, $p=0.008$) and varied negatively with REM latency ($r=-0.636$, $p=0.048$ and $r=-0.639$, $p=0.047$). No such relationships appeared in HS. Spectral analysis of F4 EEG across all subjects showed that increased theta power in REM predicted higher SCR to CS+E ($r=0.567$, $p=0.021$) and marginally greater SCR to CS+U ($r=0.431$, $p=0.09$).

In PI but not HS, greater amounts and more rapid entry into REM favors retention of fear memories both when opposed by extinction memory (for CS+E) and when unopposed (CS+U).

Poster Number 144

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Predicting Suicidal Behavior from Longitudinal Electronic Health Records

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Objective: To determine whether longitudinal historical data, commonly available in electronic health record (EHR) systems, can be used to predict patients' future risk of suicidal behavior.

Methods: Bayesian models were developed using a retrospective-cohort approach. EHR data from a large healthcare database spanning 15 years (1998–2012) of inpatient and outpatient visits were used to predict future documented suicidal behavior (i.e., suicide attempt or death). Patients with three or more visits (N =1,728,549) were included. ICD9-based case definition for suicidal behavior was derived by expert clinician consensus review of 2,700 narrative EHR notes (from 520 patients), supplemented by state death certificates. Model performance was evaluated retrospectively using an independent testing set.

Results: Among the study population, 1.2% (N =20,246) met the case definition for suicidal behavior. The model achieved sensitive (35%- 49% sensitivity), specific (90-95% specificity), and early (3–4 years in advance on average) prediction of patients' future suicidal behavior. The strongest predictors identified by the model included both well-known (e.g. substance abuse and psychiatric disorders) and less conventional risk factors (e.g. certain injuries and chronic conditions), indicating that a data-driven approach can yield more comprehensive risk profiles.

Conclusions: Longitudinal EHR data, commonly available in clinical settings, can be useful for predicting future risk of suicidal behavior. This modeling approach could serve as an early warning system to help clinicians identify high-risk patients for further screening. By analyzing the full phenotypic breadth of the EHR, computerized risk screening approaches may enhance prediction beyond what is feasible for individual clinicians.

Poster Number 145

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Genome-wide association studies of post-traumatic stress disorder in two cohorts of US Army soldiers

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Post-traumatic stress disorder (PTSD) is a prevalent, serious public health concern, particularly in the military. To discover genetic loci associated with lifetime PTSD risk, we conducted genome-wide association studies (GWAS) of PTSD in two cohorts from the Army Study To Assess Risk and Resilience in Servicemembers (Army STARRS), which includes the New Soldier Study (NSS, N=3167 cases and 4607 trauma-exposed controls) and Pre/Post Deployment Study (PPDS, N=947 cases and 4969 trauma-exposed controls). We performed association analyses within 3 ancestral groups (European, African, Latino) and then meta-analyzed the results. Heritability and genetic correlation and pleiotropy with other psychiatric and immune-related disorders were estimated.

We found a genome-wide significant locus in ANKRD55 on chromosome 5 (rs159572; odds ratio [OR] = 1.62, p-value = 2.43x10-8) in the African American samples from NSS. We also found a genome-wide significant locus near ZNF626 on chromosome 19 (rs11085374; OR =0.77, p-value = 4.59 x10-8) in the European American samples from NSS. These results were not replicated in PPDS, in the other ancestral groups from the NSS, or in trans-ancestral meta-analyses. SNP-based heritability was non-significant, and no significant genetic correlations were observed between PTSD and other mental and immune-related disorders. Significant evidence of pleiotropy was observed between PTSD and rheumatoid arthritis and psoriasis. In the largest GWAS of PTSD to date, involving a US military sample, we found limited evidence of association for specific loci. Further efforts are needed to replicate the observed population-specific association and pleiotropy between PTSD and inflammatory disorders.

Poster Number 146

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Subclinical and Clinical Skin Pickers in an Israeli Community Sample

INVESTIGATORS: E. E. Curley, V. Leibovici, E. S. Tung, N. J. Keuthen

Skin-Picking Disorder (SPD) is characterized by recurrent skin picking resulting in tissue damage. The disorder was included, for the first time, in the 5th edition of the Diagnostic and Statistical Manual of Mental Health Disorders. Despite this, little remains known about the differences between subclinical and clinical SPD.

4325 subjects were recruited from two SPD studies at the Hadassah-Hebrew University Medical Center. A questionnaire was used to diagnose subclinical and clinical SPD. Subjects completed self-report scales assessing skin-picking characteristics, perceived stress, obsessive-compulsive symptoms, depressive symptoms, and impulse control disorders. Additionally, individual questionnaire items measured perceived attractiveness, substance abuse, and eating disorders.

Of the 4325 subjects, 219 met criteria for subclinical SPD and 150 met criteria for clinical SPD. The two groups did not differ on demographic variables. Compared to subclinical skin pickers, clinical skin pickers had more severe skin picking ($p<0.001$), greater life impairment ($p<0.001$), were more likely to have a family history of SPD ($p=0.002$), had higher levels of perceived stress ($p<0.001$), had greater obsessive-compulsive ($p<0.001$) and depressive symptomatology ($p<0.001$), and were more likely to have a substance use disorder ($p=0.017$). No significant differences were found on perceived attractiveness, co-occurring impulse control disorders or eating disorders.

This study demonstrates that individuals with subclinical and clinical SPD differ on skin picking characteristics, psychological phenomenology and clinical correlates. Understanding these differences can help better define subclinical and clinical SPD, leading to improved diagnosis and more effective treatment. Future research should corroborate these findings in cohorts with larger sample sizes.

Poster Number 147

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Total Lifestyle Coaching: A Pilot Study Evaluating a Telephone Coaching Program on Weight Loss and Behavioral Eating Habits in Obese Adults at a Community Health Center

INVESTIGATORS: M. L. Dossett, E. Chad-Friedman, M. Pearsall, A. E. Wheeler, K. M. Miller, J. W. Denninger, D. H. Mehta

Obesity is a major public health concern and increases the risk of developing many chronic diseases. Preliminary studies suggest that mind-body interventions may improve weight loss and that telephone coaching may improve access to cost-effective care. We designed a program incorporating mind-body techniques and targeted nutrition education delivered by telephone for obese adults receiving care at a local community health center. Thirty patients receiving care at an MGH-affiliated community health center were enrolled in a single-arm, pilot study. Participants received an initial in-person visit with a nutritionist trained in mind-body medicine followed by biweekly telephone coaching sessions over six months. We collected anthropomorphic data and validated self-report measures at baseline and six month follow-up. Twenty-eight participants completed the in-person nutrition visit and 25 completed the 6-month intervention. In an intent-to treat analysis, participants' weight decreased significantly from 210 pounds at baseline to 205 pounds at follow-up, $p=0.04$. Systolic and diastolic blood pressures also decreased significantly (from 132/78 to 124/72, $p=0.004$ for Systolic and $p=0.037$ for Diastolic blood pressure). Additionally, scores on the Cigna behavioral eating questionnaire improved significantly (mean 12 to 14, $p=0.02$). Preliminary analysis of qualitative feedback demonstrated enthusiasm for the program. Here we show that participants in a six-month mind body and nutrition telephone coaching intervention demonstrated significant weight loss and improvements in blood pressure and disordered eating patterns. Our results suggest that this telephone coaching program is not only feasible, but potentially effective in improving health outcomes for obese community health center patients.

Poster Number 148

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Gene-by-environment effects (GxE) differ across development: An example focusing on FKBP5, sexual abuse, and depressive symptoms

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Background: Despite substantial efforts, gene-by-child maltreatment interaction (GxE) research has been inconclusive. A lack of attention to development, or the age when maltreatment occurs, may be one explanation for mixed extant findings. Consistent with this hypothesis, we used data from a large population-based sample of adult women to examine whether timing of exposure to sexual abuse, a known correlate of depression, interacted with variants in FKBP5, a gene we found to peak in expression between ages 1 and 11.

Methods: Data came from 3013 European-ancestry women in a sub-sample of the Nurses' Health Study II. Multivariate linear regression analyses assessed the association between sexual abuse timing and depressive symptoms. Timing of sexual abuse was categorized as: (1) never exposed, (2) exposure during childhood only (0-11 years), (3) exposure during adolescence only (12-18 years), and (4) exposure during both periods. We also tested for interaction between exposure and four functional FKBP5 polymorphisms (rs1360780, rs9296158, rs9470080, rs3800373).

Results: Exposure to sexual abuse during childhood (β=2.2; 95% CI: 1.3, 3.1), adolescence (β=2.2; 95% CI: 1.4, 3.1) or in both periods (β=4.1; 95% CI: 3.2, 5.0) was associated with elevated depressive symptoms relative to no exposure. All four FKBP5 variants interacted with sexual abuse exposure during childhood (all $p<0.05$). No significant GxE effects were observed for exposure during adolescence or exposure in both periods.

Conclusions: GxE effects appear to vary by development, with the effects pronounced during childhood when gene expression values of FKBP5 are greatest. Future studies are needed to replicate this finding.

Poster Number 149

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The Influence of Seizures on Language and Adaptive Functioning in Children with the Isodicentric Variation of Chromosome 15q Duplication Syndrome

INVESTIGATORS: A. Laffer, C. L. Evans, A. Morgan, A. Prasad, R. Thibert

Chromosome 15q duplication syndrome (dup15q) is a rare neurogenetic disorder with multiple chromosomal variations. Children with the isodicentric duplication (idic(15)) reportedly have lower cognitive functioning and an increased number of seizures as compared to other forms of dup15q. We sought to examine whether a presence of seizures was related to neuropsychological profiles of children with idic(15).

Twenty children with idic(15) (Mean age=7.87, SD=4.39) were evaluated at MGH. Children were separated into groups based on seizure presence (+SZR) or absence (–SZR). Adaptive behavior skills and receptive language abilities were evaluated through neuropsychological testing.

While both groups scored well below age-appropriate levels for overall motor abilities, the +SZR group scored significantly worse (p=0.046) than the –SZR group. A significant difference between groups' fine motor skills was found, with the +SZR group performing worse than the –SZR group (p=0.049). Gross motor skills approached significance at p=0.057, with the +SZR group exhibiting fewer gross motor abilities. No significant difference between groups was found on adaptive behavior or receptive language measures; though both were well below the mean.

Because both groups performed well below average, aggressive and appropriately targeted early interventions will be crucial to support the development of adaptive behaviors and receptive language skills in children with the idic(15) variation. Based on these findings, it's possible that the presence of the idic(15) duplication has such a profound effect on cognitive and adaptive functioning that the effect of seizures is not apparent on the administered measures; additional research is needed to investigate this further.

Poster Number 150

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Are autistic traits in youth meaningful?: A replication study in non-referred siblings of youth with and without ADHD

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Background: We previously reported on the high prevalence and burden of significant autistic traits (AT) in youth with ADHD that is associated with significantly greater impairment in psychopathological, interpersonal, educational, and neuropsychological functioning. Because the sample consisted of referred ADHD youth, uncertainties remain as to whether these findings generalize to non-referred populations of youths with and without ADHD.

Objective: The main aim of this study was to assess the prevalence and implications of autistic traits (ATs) in a non-referred population of siblings of probands with and without ADHD.

Method: Participants were non-referred siblings of ADHD (N=257) and Control (N=234) probands of longitudinal, case-control family studies conducted at the Massachusetts General Hospital. Assessments included measures of psychiatric, psychosocial, educational, and cognitive functioning. Presence of significant ATs were operationalized using the Child Behavior Checklist (CBCL) AT profile consisting of an aggregate score ≥ 195 for the sum of the Withdrawn, Social, and Thought Problems T-scores.

Results: ATs were significantly more prevalent in siblings of ADHD probands compared to siblings of Control probands (6% vs. 1%, p=0.02). Siblings of ADHD probands with a positive AT profile (N=15) were significantly more impaired than those without an AT profile (N=242) in psychopathological, interpersonal, educational, and neuropsychological functioning.

Conclusions: Consistent with previous findings on ATs in a referred sample of youth with ADHD, the current study reports a higher than expected prevalence of ATs in a non-referred sample of siblings of youth with ADHD. The presence of ATs is associated with greater morbidity and dysfunction.

Poster Number 151

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Clinical Correlates of Working Memory Deficits in Youth with and without ADHD: A Controlled Study

INVESTIGATORS: R. Fried, J. Biederman, L. Feinberg, A. Pope, J. Chan, S. Faraone, Pediatric Psychopharmacology Research Department

Working Memory is the ability to hold information in mind for the purpose of problem solving. Children with Attention Deficit Hyperactivity Disorder (ADHD) have been documented to have significant deficits in this area. The aims of this study were to analyze 1) whether the prevalent Working Memory (WM) deficits present in children with ADHD were the primary cause of the educational deficits documented in this population; 2) whether working memory deficits caused difficulties beyond academics and 3) whether working memory affected children outside the context of ADHD. Subjects were youth aged 6-18 of both sexes with (N=276) and without (N=241) ADHD ascertained from pediatric and psychiatric sources. Assessment included measures of psychiatric, psychosocial, educational, and cognitive functioning. Working memory was assessed using the WISC-R Freedom from Distractibility (FFD) factor. Here we showed that significantly more youth with ADHD had WM deficits than controls (31.9% vs. 13.7%, p< 0.05) and in ADHD children, their presence was significantly (p<0.01) associated with an increased risk for grade retention and placement in special classes as well as lower scores on reading and math achievement tests, relative to ADHD subjects without WM deficits. In contrast, no other differences were noted in other areas of functioning. Our results led us to conclude that WM deficits significantly and selectively increase the risk for academic deficits in children with ADHD beyond those conferred by ADHD. Screening for WM deficit may help identify children with ADHD at high risk for academic problems.

Poster Number 152

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Effects of Physical Health Burden on Stress via Fear of Recurrence among Cancer Survivors

INVESTIGATORS: D. L. Hall, I. T. Lennes, W. F. Pirl, E. R. Park

After treatment ends, many adult cancer survivors report having physical health symptoms and emotional distress. Uncertainty in Illness Theory posits that physical symptoms can trigger uncertainty among patients, leading to heightened subjective stress states. This study aimed to test whether survivors' physical symptoms are associated with self-reported stress, and if survivors' fear of recurrence partially mediates these associations.

Adult cancer survivors (n=66; median 2.2 years since diagnosis; 38% male) presenting at a hospital survivorship clinic completed measures assessing burden of physical health symptoms (Patient Health Questionnaire-15), perceived stress (Perceived Stress Scale-4), and fear of recurrence (Assessment of Survivor Concerns). Interrelatedness among variables was assessed using Pearson correlations. Indirect effects were modeled using 5000-iteration bootstrapping.

Survivors endorsed experiencing fatigue (79%), limb and joint pain (64%), and racing heart (24%). Physical symptoms, perceived stress (M=6.72, SD=3.66, range 0-14), and fear of recurrence (M=2.60, SD=0.95, range 0-4) were all significantly positively correlated (rs .21-.42). The indirect effects of physical symptoms on perceived stress via fear of recurrence were also significant. Specifically, fear of recurrence was an intermediary between physical symptoms and stress regarding: fatigue [B=0.31, SE=0.18 (95% CI: 0.03-0.79)], limb and joint pain [B=0.35, SE=.23 (95% CI: 0.03-0.98)], and racing heart [B=0.68, SE=.39 (95% CI: 0.09-1.68)].

This study shows that for cancer survivors, fear of recurrence explains a significant amount of covariance between physical symptoms and subjective stress. Findings support theoretical models highlighting fear of recurrence as a mechanism underlying stress in cancer survivors and implicate it as a target for psychosocial intervention.

Poster Number 153

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Prescribing Patterns in a Psychiatrically Referred Sample of Youth with Autism Spectrum Disorders

INVESTIGATORS: J. Shekunov, J. Wozniak, J. Biederman, N. Friedman, E. Pinsky, K. Conroy, G. Joshi

Objective: The aim of this study was to examine patterns of psychotropic medication use in a psychiatrically referred sample of youth with autism spectrum disorders (ASD).

Methods: We performed a retrospective chart review on a psychiatrically referred sample of 83 youths with ASD, collecting their psychiatric diagnoses and current medications. Clinicians identified the target disorder for each medication and any adverse events. They assigned baseline and current Clinical Global Impressions Scale-Severity (CGI-S) ratings and Clinical Global Impressions Scale-Improvement (CGI-I) scores to measure improvement with treatment. A CGI-I ≤ 2 constituted meaningful improvement.

Results: The most prevalent comorbidities were anxiety disorders (69%), ADHD (53%), mood disorder NOS (43%), and bipolar spectrum disorders (30%). The mean number of psychotropic medications per patient was 2.7 ± 1.5, and 74.7% of the sample was receiving combination therapy. Mean baseline GCI-S scores for specific disorders ranged from 4.2 to 5.6, consistent with moderate or marked levels of illness. 50 subjects (63.3%) met the criterion for meaningful improvement of at least one disorder.

Conclusion: This sample of psychiatrically referred youth with ASD had high rates of psychiatric comorbidity and combination pharmacotherapy. 63.3% of patients taking medication had meaningful improvement of at least one disorder. These findings highlight the challenges of treating youth with ASD and psychiatric comorbidity and the need for further research to guide treatment.

Poster Number 154

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Factors Impacting Resilience among Adolescents in Post-Conflict Liberia

INVESTIGATORS: E. J. Levey, C. E. Oppenheim, N. S. Plasky, B. C. Lange, B. L. Harris, D. C. Henderson, C. P. Borba

Between 1989 and 2003, Liberia experienced a brutal civil war characterized by ethnic killings, sexual violence and the use of child soldiers. Hundreds of thousands were displaced, productive capacity and physical infrastructure were destroyed, and family and community ties were eroded. Five years after the war ended, there were an estimated 340,000 orphans in Liberia, 18% of the total child population of the country. Given that children make up half the population and that these children experienced significant trauma and loss both through direct exposure to the war and then to the Ebola epidemic and indirectly as a result of the trauma experienced by their parents, the recovery of these children is essential to the recovery of the nation as a whole. The goal of this research was to identify factors contributing to resilience among children in post-conflict Liberia. The findings are based on in-depth interviews with 75 children.

Here we show that resilience is impacted by emotion regulation, cognitive flexibility, agency, social intelligence and meaning. Cognitive flexibility and emotion regulation together facilitate distress tolerance. They allow the preservation of one's sense of agency in the face of stress or trauma. From this, children derive self-worth and a sense of hope for the future, which further enhances their sense of agency. Separate but related is social intelligence, which encompasses an ability to connect with others, empathy and altruistic behavior. Finally, a belief system can promote a sense of agency or it can reinforce a sense of helplessness.

Poster Number 155

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The Impact of Transactional Sex with Teachers on Secondary School Students in Monrovia, Liberia

INVESTIGATORS: L. Parnarouskis, A. Stevenson, B. C. Lange, S. J. Pullen, L. J. Petruzzi, S. Dominguez, B. Harris, N. Quiterio, G. Lekpeh, B. Manobah, S. P. Slopadoe, V. C. Diandy, A. J. Payne, D. C. Henderson, C. P. Borba

Objective: Work with adolescents has shown that access to education is among the most influential social determinant of health worldwide. Relationships with teachers further influence adolescent health; those who feel connected to school and have healthy relationships with teachers have better academic, social, and emotional outcomes. Risky sexual behaviors of adolescent girls in low-income countries are particularly problematic because many of these countries have yet to narrow the gender disparities that put girls at risk for poor health outcomes, including access to education. This study sought to examine risky behaviors of in-school youth in Liberia.

Methods: Nine focus groups were conducted with secondary school students in Monrovia, Liberia in April 2012. The sessions took place in three public schools with N=72 participants aged 12-20 years old. A Liberian interviewer and MGH medical student led the focus groups using a semi-structured guide. After transcription, qualitative analysis was conducted by five coders, who identified themes in the data and met to reach a consensus on each code.

Results: The study found that transactional sex between girls and teachers often led to unintended pregnancies and inconsistent and coercive relationships with instructors. For other students, transactional sex between teachers and pupils facilitated negative attitudes about teachers and the school environment. Conversely, educators were not reported to experience any negative consequences from having sex with students.

Conclusion: Interventions to reduce the prevalence of transactional sex within the academic environment would likely help students' academic success, thus improving educational and economic outcomes in Liberia.

Poster Number 156

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The association of psychedelic use and opioid use severity among illicit users in the United States

INVESTIGATORS: V. Pisano, N. Putnam, H. Kramer, K. Franciotti, S. Holden

Background: Preliminary studies show that some compounds with psychedelic properties can treat substance abuse when used with adjunct psychotherapy. However, little is known about the association of psychedelic use in the general opioid using population. This study investigates the association between illicit psychedelic use and past year severity of opioid use within lifetime illicit opioid users.

Methods: While controlling for demographic and other substance use covariates, the relationship between lifetime classic psychedelic use and past year severity of opioid use was analyzed within 43,000 illicit prescription painkiller users and over 4,500 heroin users who completed the NSDUH from 2008–2013.

Results: Psychedelic use among painkiller users is associated with significantly reduced odds of meeting past year painkiller dependence criteria (weighted odds ratio (OR)=0.73(0.58-0.92)), abuse criteria (weighted OR=0.58(0.40-0.85)) and painkiller abuse or dependence criteria (weighted OR=0.69(0.56-0.85)). Among heroin users, psychedelic use is associated with decreased odds of meeting past year heroin dependence criteria (weighted OR=0.54(0.37-0.78)) and abuse or dependence criteria (weighted OR=0.54(0.38-0.76)), but does not have an association with meeting past year heroin abuse criteria (p>0.05).

Conclusion: Lifetime psychedelic drug use is associated with decreased odds of painkiller and heroin abuse or dependence. Conversely, lifetime use of other illicit drugs is largely associated with increased odds of opiate abuse or dependence. These findings suggest that psychedelics are associated with positive psychological characteristics and support prior reports of their efficacy treating substance use disorders.

Poster Number 157

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Reduction of placebo response in depression trials via independent remote (SAFER) patient interviews

INVESTIGATORS: J. P. Pooley, M. J. Flynn, M. Freeman, M. Fava, D. Mischoulon

Systematic approaches are required to increase the quality and precision of major depressive disorder (MDD) clinical trials. New treatment development for MDD and treatment resistant depression (TRD) is hindered by high placebo response rates that impair ability to identify signals of efficacy from promising new therapies. To increase precision in making a correct diagnosis, we created two clinical trial tools. The SAFER Interview and the Antidepressant Treatment Response Questionnaire (ATRQ) provide clinical researchers with user friendly tools to enrich the qualitative assessment of MDD and treatment resistance.

We performed a retrospective review of SAFER and ATRQ interviews conducted in eleven clinical TRD drug trials. In each trial, all subjects had passed screening at the site and were considered eligible. A structured severity interview, in addition to the SAFER and ATRQ, was performed remotely by MGH clinicians, who called patients directly.

Across eleven trials, 3,326 remote SAFER interviews were performed. 2,827 patients (85%) were deemed eligible for continued screening. 499 (15%) of the patients were deemed ineligible upon completion of the interview. In all the studies, placebo response rates were within a range of 18-28%, below the 30-37% average in studies of TRD approved treatments.

We showed that if SAFER interviews were not applied, many inappropriate patients would likely have been included impairing assay sensitivity and potentially resulting in a failed trial. These methods enhance the quality of trials and increase the likelihood of positive trials for efficacious compounds, based on low placebo response rates when the appropriate patients are enrolled.

Poster Number 158

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Sensitive periods for the effect of exposure to physical abuse on DNA methylation in late childhood

INVESTIGATORS: T. Soare, A. J. Simpkin, V. A. Fisher, E. C. Dunn

We hypothesized that effects of environmentally-induced epigenetic modification are strongest during developmental "sensitive periods." Specifically, we predicted that for genes expressed early in development, physical abuse during early childhood would be most strongly associated with subsequent DNA methylation. We examined this relationship in a large population-based birth cohort of children followed prospectively from birth through early adulthood (N=1,000). Exposure to physical abuse was measured at 7 potential "sensitive periods" (between 18 months and 6.75 years). DNA methylation in "sensitive period relevant gene sets" was measured in peripheral blood at age 7. These gene sets were identified by analyzing gene expression patterns in post-mortem amygdala samples (4 months to 82 years). The first gene set contained 276 genes displaying decreasing expression across age (i.e. highly-expressed in early life); the second set contained 248 genes increasing in expression across age. We also examined two control gene sets of more stable patterns of expression. Within each gene set, we evaluated the effect of exposure at putative "sensitive periods" on methylation at each CpG site using least angles regression. Preliminary results suggest the developmental timing of exposure explains more variance in DNA methylation than the number of time points exposed. We also observed differences based on the developmental timing of exposure which were consistent across gene expression profiles. These findings align with previous work showing exposure to adversity in the first five years of life elevates risk for psychopathology. Examination of specific gene sets is a promising approach to complement traditional Epigenome-Wide Association Studies.

Poster Number 159

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Neurobiological Evidence of Vulnerability to PTSD in ADHD: A Controlled MRI Study Assessing Fear Circuitry in Non-Traumatized Adults with and without ADHD

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A recent meta-analysis documented a robust statistical association between ADHD and PTSD, suggesting that individuals with ADHD may have a neurobiological vulnerability for PTSD. We used a well established fMRI fear conditioning and extinction paradigm to examine whether non-traumatized ADHD subjects have dysfunctional activation in brain structures that mediate fear acquisition and extinction, which could be neurobiological evidence of vulnerability for PTSD. The sample consisted of medication naïve, non-traumatized young adult subjects with (N=27) and without (N=20) ADHD. Subjects underwent a 2-day fear conditioning and extinction protocol in a 3-T functional magnetic resonance imaging scanner. Conditioning and extinction training were conducted on day 1 and extinction recall was conducted on day 2. Skin conductance response (SCR) was collected throughout the experiment as an index of the conditioned response. ADHD subjects showed significantly more impaired functional activation of brain structures involved in fear circuitry in every phase of the fear paradigm (conditioning, extinction learning, and extinction recall) compared with Controls. Specifically ADHD subjects had significantly lesser activation in vmPFC during both late extinction learning and extinction recall, as well as significantly lesser activation in hippocampus during extinction recall, similar to deficits previously documented in PTSD subjects compared to traumatized controls without PTSD. These findings suggest that lesser vmPFC activation during extinction learning and recall and lesser hippocampal activation during recall may represent an underlying neurobiological vulnerability to PTSD in non-traumatized individuals with ADHD. If confirmed in future research, these findings would have very important clinical, scientific and public health implications.

Poster Number 160

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Propensity for Exogenous Attention Capture is a Vulnerability Factor for Posttraumatic Stress Disorder (PTSD): A Monozygotic Twin Study

INVESTIGATORS: M. B. VanElzakker, S. Shomstein, L. K. Staples-Bradley, S. J. Dubois, P. Panic, N. B. Lasko, S. P. Orr, R. K. Pitman, L. M. Shin

PTSD's triggering trauma is emphasized by the DSM-5 diagnostic manual, which describes attention-related symptoms as "Marked alterations in arousal and reactivity associated with the traumatic event(s), beginning or worsening after the traumatic event(s) occurred." The current study questioned whether distracting cues must actually be associated with the traumatic event, and whether attention problems are triggered by trauma or are in fact part of what makes people vulnerable to PTSD. Using emotionally-neutral attention tasks (exogenous and endogenous versions of the Posner Cueing Task), we studied 31 male monozygotic twin pairs (N=62 individuals) in which one twin was combat-exposed (Ex) and the other was combat-unexposed (Ux). Participants were divided into 4 groups: 14 Ex participants with current PTSD (ExP+) and their 14 Ux cotwins (UxP+), as well as 17 Ex participants without current PTSD (ExP-), and their 17 Ux identical cotwins (UxP-). A significant mixed-model ANOVA interaction, (F(1,29)=8.921, p=.006, partial eta^2=.235) was driven by the difference in response times between P+ pairs and P- pairs during exogenous distraction. This is evidence that the propensity to have attention captured by external stimuli is a vulnerability factor for PTSD, and not the result of PTSD. Demonstrating that this vulnerability factor can predict PTSD severity, there was also a significant correlation between attention capture in Ux individuals and their corresponding Ex twin's hypervigilance symptoms, r(29)=.489, p=.005. These results reveal that a simple non-emotional attention task presented on a laptop can be used to test for pre-trauma vulnerability to PTSD.

Poster Number 161

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Natural Course of Post-Discharge Positive Psychological Attributes among Suicidal Inpatients

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Introduction: Low levels of positive psychological attributes (e.g., optimism) may be as important as elevated depression or hopelessness in predicting risk of self-harm. However, no studies have assessed the natural post-discharge course of positive emotional states among patients hospitalized for suicidality, despite the fact that this population is at exceedingly high risk for suicide after discharge.

Methods: Patients with mood disorders who were psychiatrically hospitalized due to suicidal ideation or attempt (N=44) underwent self-report assessments at discharge, and then 2, 4, and 8 weeks later. This included assessments for optimism (Life Orientation Test-Revised [LOT-R]), positive affect (Positive Affect Negative Affect Schedule [PANAS]), depression (Quick Inventory of Depressive Symptomatology), hopelessness (Beck Hopelessness Scale) and suicidal ideation (Concise Health Risk Tracking scale). Random effects models were used to estimate values at each timepoint to account for any missing data.

Results: Participants reported low and largely stable levels of optimism (LOT-R) at discharge (13.5+/-6.4), 2 weeks (15.5+/-8.1), 4 weeks (13.1+/-5.7), and 8 weeks (14.1+/-7.6). Similarly, positive affect was low and stable (PANAS) (baseline: 26.3+/-7.5, 2 weeks: 29.4+/-8.9, 4 weeks: 23.3+/-6.1, and 8 weeks: 23.9+/-5.1).

Conclusions: Post-discharge positive psychological attributes are persistently low in the 8 weeks after hospitalization in high-suicide risk patients. Given that low positive states may be associated with self-harm above and beyond the effects of negative emotions, interventions to target positive psychological constructs appear to be warranted in this cohort.

Poster Number 162

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Examining the Association between Attention Deficit Hyperactivity Disorder and Substance Use Disorders: A Familial Risk Analysis

INVESTIGATORS: A. Yule, M. Martelon, T. Wilens, J. Biederman

Background: The main aim of this study was to use familial risk analysis to examine the association between attention deficit hyperactivity disorder (ADHD) and substance use disorders (SUDs) attending to sex effects

Methods: Subjects were derived from two longitudinal case-control family studies of probands aged 6 to 17 years with (N=112 boys, N=96 girls) and without ADHD (N=105 boys, N=91 girls) and their first degree relatives (N=1,336). Cox proportional hazard models were used to estimate rates of ADHD and SUDs (any SUD, alcohol dependence, and drug dependence). Logistic regression was used to test co-segregation.

Results: ADHD and SUDs (any SUD, alcohol dependence, and drug dependence) in the proband were consistently associated with a significant risk for the same and the opposite substance use disorder (drug or alcohol) in relatives. There was evidence that ADHD increased the risk of SUDs independently of SUD in the proband. No significant interaction effects were observed between proband sex and the relationship with ADHD/SUD status and risk of ADHD and SUD in relatives.

Conclusions: Findings indicate a common familial risk between ADHD and SUDs. They also indicate that ADHD is a risk for SUDs in relatives independently of SUD in the proband.

Poster Number 163

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Association between renal disease and olfactory defects

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Malnutrition and cachexia are prevalent in renal disease patients and are associated with increased morbidity and mortality. We set out to investigate whether olfactory defects that could lead to food aversion exist in chronic kidney disease (CKD) and end stage renal disease (ESRD) patients, in order to assess their putative contribution to the pathogenesis of malnutrition. We quantified odor identification and threshold in CKD (n=28, 60.3±2.5 years old, mean±SEM) and ESRD patients (n=42, 57.4±2.5 y.o.) in comparison to healthy volunteers (n=15, 55.3±1.8 y.o.), using the standardized University of Pennsylvania Smell Identification Test (UPSIT) and Smell Threshold Test (Sensonics, Haddon Heights, NJ).

Here we show that renal patients have olfactory defects, as they obtained 70.5±1.9% correct answers on the UPSIT test, while healthy volunteers identified 87.0±2.8% of the odorants correctly (p=0.0004), thus exhibiting normal odor identification. The CKD and ESRD patient subpopulations scored 75.4±2.5% and 67.4±2.6% correct answers respectively, significantly less than healthy volunteers (p=0.0071 and p=0.0001).

Subjective assessments of smell and taste were similar among the control, CKD, and ESRD groups, thus not correlating with the UPSIT scores. No statistically significant odor threshold differences were observed among these cohorts.

The results of our study indicate that CKD and ESRD patients have olfactory deficits and that the UPSIT test is an appropriate tool to quantify them. Mitigating these olfactory defects may improve dietary intake and nutritional status in renal disease patients.

Poster Number 164

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Complementation of mitochondrial electron transport chain by manipulation of NAD+/NADH ratio

INVESTIGATORS: D. V. Titov, V. Cracan, R. P. Goodman, J. Peng, Z. Grabarek, V. K. Mootha

Compartmentalized control of NAD+/NADH ratio is central to cell physiology and to many disease processes. An important barrier to understanding the role of NAD+/NADH ratio in regulation of various biological processes is the absence of methods for direct compartment-specific perturbation of this ratio. Here, we introduce a genetic strategy for direct, compartment-specific manipulation of NAD+/NADH in human cells through the expression of a water-forming NADH oxidase from Lactobacillus brevis (LbNOX). Water-forming NADH oxidases catalyze the reaction between NADH and oxygen to make NAD+ and water. Kinetic and structural studies demonstrate that LbNOX is specific for NADH over NADPH, produces negligible H2O2 and has high catalytic activity. We show that LbNOX can be expressed safely in mitochondria and cytoplasm of human cells, and evaluate its impact on NAD+/NADH pools, coupled metabolic reactions, PDH phosphorylation, and gluconeogenesis. We then address the longstanding observation that human cells with a defective respiratory chain are not capable of proliferation in the absence of exogenously added pyruvate and uridine. By expressing LbNOX in these cells we are able to fully rescue this pyruvate auxotrophy and in doing so demonstrate that maintenance of NAD+/NADH ratio, and not proton pumping or ATP synthesis, is the essential mitochondrial function that is required for mammalian cell proliferation. The results underscore the role of reductive stress in mitochondrial pathogenesis and demonstrate the utility of targeted LbNOX for direct, compartment-specific manipulation of NAD+/NADH ratio.

Poster Number 165

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Autism Pathway Network Analyses: Convergence upon MAPK and Calcium signaling, Multisystem Involvement and Diverse Disease Overlaps

INVESTIGATORS: Y. Wen, M. J. Alshikho, M. R. Herbert

As of December 2014, the SFARI (Simons Foundation Autism Research Initiative) Gene Human Gene Module recorded 667 human genes implicated as relevant to autism spectrum disorders whose diversity challenges efforts at identifying coherent biological mechanisms. Here we present a systematic exploration of the contributions of these genes to autism pathophysiology utilizing pathway network analysis. We first identified 50 enriched pathways by investigating enrichment within the SFARI genes and KEGG (Kyoto Encyclopedia of Genes and Genomes) pathways. Redundancy Control in Pathway Databases (ReCiPa) was applied to minimize the impact of potential redundancy caused by some highly overlapped pathways, and this final yielded 40 pathways, which were then grouped as 10 disease and 30 function pathways: cancer (4/10), neurodegenerative (3/10), cardiac (2/10) metabolic diseases (1/10); cell signaling (9/30), cell structure/transport (5/30), immune (3/30), neural (5/30) metabolism (8/30). We then use KEGG pathway maps to identify the interactions among pathways and generate a pathway interaction network. Tabulation of the number of interactions each pathway had within the network showed MAPK signaling pathway and calcium signaling pathways interacting with the most other pathways (20/40 and 12/40 respectively) making them the interactive hubs in the network. These hub pathways also impact a large range of biological processes involved in many functions and diseases. In addition, we demonstrate marked overlaps between autism and other diseases, systemic as well as brain. These findings support the idea that autism may emerge from underlying vulnerabilities related to pleiotropic genes associated with pervasively important molecular mechanisms and multiple systemic comorbidities.

Poster Number 166

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Human enteric neural stem cells survive and differentiate following transplantation into embryonic and postnatal animal models

INVESTIGATORS: L. S. Cheng, N. Nagy, R. Hotta, H. K. Graham, A. M. Goldstein

Background: Neural stem cell therapy offers an innovative approach to treating Hirschsprung disease. Enteric neural stem cells can be isolated from human intestine, but the ability of these cells to survive, migrate, and differentiate within an aganglionic environment has not been demonstrated.

Methods: Enteric neural stem cells were isolated from the intestines of 17 patients undergoing bowel resections, including 5 patients with Hirschsprung disease. Cells were propogated as neurospheres, transplanted into embryonic day 5 chick aneural hindgut, and cultured on the chorioallantoic membrane of a host chick for 9 days (n=5). Neurospheres were also transplanted to the vagal neural crest of embryonic day 2 chick embryos and followed for 12-48 hours (n=3). Finally, neurospheres were transplanted to the aganglionic colon of 2-week-old Ednrb-/- mice (n=3), a model of Hirschsprung disease. Mice were sacrificed at 3, 5, and 7 days post-transplant.

Results: Human enteric neural stem cells transplanted into embryonic chick hindgut migrated in the intermyenteric layer, forming a plexus containing neurons and glia. Cells transplanted into the embryonic neural crest migrated to the foregut and formed neurons and glia. Cells transplanted into mouse colon survived and differentiated into neurons and glia.

Conclusions: Enteric neural stem cells can be isolated from human intestine, including ganglionic bowel of children with Hirschsprung disease. These cells can survive, migrate, and differentiate into neurons and glia following transplantation into embryonic aneural chick hindgut, embryonic neural crest, and postnatal aganglionic mouse colon. These results support the feasibility of cell-based therapy for future treatment of neurointestinal disease.

Poster Number 167

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A heterotopic model of lung transplantation in pigs

INVESTIGATORS: K. Kitano, H. Zhou, T. K. Rajab, D. J. Mathisen, H. C. Ott

Bioengineered lungs produced from patient-derived cells may one day provide an alternative to allogeneic donor lungs for transplantation therapy. The technique of perfusion decellularization provides acellular whole-organ scaffolds as a potential platform for organ-scale regeneration. Our group have demonstrated that human or porcine lungs can be decellularized to generate human-scale lung scaffolds. We also developed protocols to deliver and culture human iPSC-derived pulmonary epithelial or endothelial cells in the scaffolds, to obtain further cell maturation *in vitro*. One key aspect of taking these grafts towards clinical application is the development of a large animal model that enables functional testing of the bioengineered human grafts after transplantation. Here we show a porcine non-survival surgical model of heterotopic lung transplantation as a lung functional evaluation method. We anastomosed a fresh cadaveric, small porcine lung to the pulmonary circulation of an adult recipient pig, and observed the hemodynamics as well as mechanics and gas exchange function of the implanted graft. After reperfusion, gross pulmonary vascular resistance slightly decreased. Direct sampling of graft pulmonary arterial and venous blood showed oxygenation and CO2 removal. Chest of the recipient animal could be closed without drastic change in airway compliances. This model may be useful in studying *in vivo* function of bioengineered lungs. In addition, since the graft can be implanted and removed without compromising recipient's native lung function, this strategy of lung implantation may potentially be clinically translated as a transient lung support therapeutic system for end-stage respiratory failure patients.

Poster Number 168

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Derivation and Validation of a Novel Emergency Surgery Acuity Score (ESAS)

INVESTIGATORS: N. F. Sangji, J. A. Bohnen, E. P. Ramly, G. C. Velmahos, D. C. Chang, H. M. Kaafarani

Introduction: There currently exists no pre-operative risk stratification system for Emergency Surgery (ES). We sought to develop an Emergency Surgery Acuity Score (ESAS) that predicts perioperative mortality in the ES patient. ESAS could be useful for: 1) preoperative counseling; 2) identification of patients needing close postoperative monitoring; and 3) risk-adjustment for quality benchmarking.

Methods: Using the 2011 ACS-NSQIP database, we identified all operations classified as "emergent". Multiple logistic regression models were created to identify independent predictors (demographics, co-morbidities, and pre-operative laboratory variables) of 30-day mortality in ES. Based on the relative impact of each predictor (odds ratio), using weighted averages, a novel score was derived. This was validated using the 2012 ACS-NSQIP database.

Results: 18,439 ES cases were analyzed, of which 1,598 (8.7%) resulted in death at 30 days. Twenty-two independent predictors of mortality were identified. Based on the relative impact of each, ESAS was derived with a score range of 0-29, with a c-statistic of 0.86 for mortality. The observed probability of 30-day mortality increased from 0% at a score of 0 to 100% at a score of 22. In the validation phase, 18,146 patients were included, the mortality rate was 7.2% and the c-statistic was unchanged.

Conclusion: A novel score was developed and validated that predicts mortality in ES patients- ESAS.

Poster Number 169

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Treg-rich organized lymphoid structures (TOLS) in spontaneously accepted mouse kidney allografts

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In many MHC mismatched strain combinations (e.g., DBA/2 to B6) kidney allografts are spontaneously accepted without immunosuppression. This tolerance is regulatory, initially dependent Foxp3+ cells which are concentrated in the graft in distinctive periarterial structures we have termed TOLS. Depletion of Tregs causes dissolution of the TOLS and rapid onset of T cell mediated rejection. We further investigated the time-course by which various immune cells infiltrate in accepted mouse kidney allografts, and which immune cells could be involved in the formation of lymphoid like structures. H&E staining showed widespread infiltration of immune cells in the cortex of renal allografts at week 1, and by week 6, these various cell types were localized to TOLS . IHC staining showed TOLS contain Foxp3+, CD4+, CD8+, B220+, CD11c+ and CD11b+ cells. By FACS 18.8% ± 4.4 of T cells in the TOLS at week 6 were Foxp3+. IHC showed TOLS are podoplanin positive, indicative of the presence of lymphatics, but are MECA-79 negative, a marker for high endothelial structures (HEV). Similar TOLS with abundant Foxp3+ cells were seen in accepted kidney allografts from nonhuman primates (NHP). TOLS are distinctive structures in accepted kidney allografts in mice and NHP, that lack HEV, have abundant Foxp3+ cells and an admixture of T and B cells, in contrast to tertiary lymphoid organs arising from chronic inflammation. We propose that TOLS may be an intragraft marker and perhaps mediator of regulatory tolerance.

TRANSLATIONAL MEDICINE & EXPERIMENTAL THERAPEUTICS

Poster Number 170

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Olfactory ensheathing cell as a candidate for brain tumor therapy

INVESTIGATORS: L. A. Carvalho, B. A. Tannous

Over the last decade, several studies have evaluated the potential of stem cells as a vehicle to deliver gene products for the treatment of brain cancer. Although some level of success, one of the major concerns is the high risk of tumorigenicity. In this context, the olfactory ensheathing cells (OECs) is an adult glial cell that displays migratory properties and tropism for inflammatory environments commonly used to repair spinal cord lesions. Moreover, OECs can be easily obtained from patients allowing autologous transplantation, making them an excellent candidate for brain cancer therapy. Our aim is evaluate OECs to target deliver therapeutic genes to a human xenograft model of glioblastoma, the most aggressive type of brain tumors. We extracted/cultured OECs cells from mice olfactory bulb and confirmed their phenotype by the expression of two cell-specific antigens, the smooth muscle alpha actin (αSMO) and 2′,3′-Cyclic-nucleotide 3′-phosphodiesterase (CNPase). We then, engineered these cells by lentivirus vector to express a fusion of the E. coli cytosine deaminase (CD) and uracil phosphoribosyltransferase (UPRT; CD-UPRT) as the therapeutic gene, as well as the secreted Gaussia luciferase as a blood reporter. The CD-UPRT converts the exogenous 5-FC into the cytotoxic agent 5-FU. Upon intranasal administration, a simple route of delivery, these OECs were able to migrate toward the U87 glioma tumors, and surprisingly prolonged the overall mice survival rate, even without treatment with 5-FC. Our findings suggest that OECs could be used as a potential gene/cell therapeutic strategy for brain tumors.

Poster Number 171

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Suppression of Steroid-5-alpha-reductase 2 (SRD5A2) in Human Prostate is Regulated by Epigenetic Modifications

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SRD5A2, an enzyme that is critical for prostatic development, is utilized as an inhibitory target by finasteride for patients with bladder outlet obstrution secondary to benign prostatic hyperplaisa (BPH). However, we have found that many aging benign prostate tissues do not express the enzyme. Previously, we analyzed 96 human BPH samples from (TURP) surgeries and found that absence of SRD5A2 was closely associated with hypermethylation of the SRD5A2 promoter by DNMT1 and cytokines, such as TNF-alpha. We used MIRA to analyze the methylation status of SRD5A2 promoter. Here, we randomly selected 3 TURP samples with methylated SRD5A2 promoters and 3 TURP samples with un-methylated SRD5A2 promoters, and then bisulfide sequencing was performed to verify the methylation pattern of CpG dinucletides on SRD5A2 promoter. We found that bisulfide sequencing analysis of SRD5A2 promoter is consistent with MIRA assay. To further analyze the mediators of SRD5A2 promoter, we show that TNF-alpha up-regulates Snail expression, which is a central regulator of both DNA and histone methylation, and increases the binding of H3K9me to SRD5A2 promoter, but not H3K27me or H4K20me. Immunoprecipitation assay demonstrated that Snail protein interacted with DNMTs and G9a protein.

In summary, methylation of SRD5A2 promoter, which is regulated by DNMT1, cytokines and the histone methylase, H3K9me, account for suppression of SRD5A2 expression in many adult human prostate tissues. Bisulfide sequencing confirms the specific CpG dinucletides that are methylated enabling us to evaluate polyclonal vs. monoclonal pattern of SRD5A2 promoter methylation to demonstrate the functional significance of SRD5A2 methylation.

TRANSLATIONAL MEDICINE & EXPERIMENTAL THERAPEUTICS

Poster Number 172

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Bioadhesive metallo-hydrogel for surgical sealant application

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Surgical sealant is an emerging biomaterial as a successful alternative to surgical suture due to its minimal invasiveness and convenience in usage. Although several commercial products have been developed, it is still in challenge to achieve high levels of tissue adhesion under wet conditions with tunable mechanical properties and good biocompatibility. Here we describe a bioadhesive metallo-hydrogel formed by stoichiometric metal-ligand complexation between Iron (Fe3+) ions and chemically modified gelatin. We surprisingly found that the adhesion properties of gelatin are unexpectedly increased after the rapid complexation with Fe3+ occurred within seconds in a reversible manner, and the formed metallo-hydrogel showed negligible immune response and gradual degradation profile *in vivo*. In murine injury models of uterine and bile duct, the metallo-hydrogel performed as a surgical sealant effectively repaired the injury even in the presence of bio-fluids and remained attached up to several days while natural wound healing process is activated. Since two components can be delivered by microcatheters and rapidly complexed inside the body, we expect its wide use in interventional, endoscopic, surgical applications, and especially immediate closure of organ fistulas and leaks.

Poster Number 173

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Lysophosphatidic Acid as a Biomarker in Systemic Sclerosis

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Scleroderma (SSc) is a devastating disease with significant clinical and molecular heterogeneity. The development of effective therapies will require a thorough understanding of the molecular pathways underlying the biological processes that drive its progression. Novel biomarkers reflecting these processes, such as lysophosphatidic acid (LPA), will allow for more accurate prognostication and assessment of treatment efficacy. LPA is a small bioactive phospholipid produced by activated platelets and fibroblasts that acts through G protein-coupled receptors (LPA1-6). We previously implicated LPA and LPA1 in SSc pathogenesis using a mouse model of scleroderma fibrosis. Here, we evaluated LPA levels in the plasma of 12 SSc patients and 7 healthy controls (HCs) and compared LPA levels among different subsets of SSc patients.

Here we show that LPA levels are increased in the plasma of patients with SSc. Patients with diffuse cutaneous SSc (dcSSc, n=5) had elevated total plasma LPA levels (mean=561 pmol/ml) compared to those with limited (lcSSc, mean=348 pmol/ml, p=0.025) and HCs (mean=279 pmol/ml, p=0.012). Among individual species of LPA, the levels of the 18:2 species were highest and showed similar trends as total LPA levels between dcSSc (mean = 229 pmol/ml), lcSSc (mean = 115 pmol/ml), and HCs (mean = 127 pmol/ml). Additionally, patients with early disease (< 3 years duration) had increased total LPA levels (mean = 534 pmol/ml) compared to patients with late disease (mean = 368 pmol/ml) and HCs (mean = 279 pmol/ml), although the difference between early and late patients was not statistically significant given the small study size.

Poster Number 174

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Inhibition of the enzyme dihydroorotate dehydrogenase overcomes differentiation arrest in acute myeloid leukemia

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Acute myeloid leukemia (AML) remains a clinically devastating disease with a five-year survival rate of 30%. Furthermore, our chemotherapy standard of care has not changed in 40 years, highlighting the need for new and more effective therapies. AML comprises many distinct genetic subtypes, though one shared hallmark is that the leukemic myeloblast is arrested at an immature and self-renewing stage of development. Therapies that prompt a release from differentiation arrest, rather than cytotoxicity, represent a powerful treatment strategy.

Here we describe a novel AML screening system in which the degree of myeloid differentiation could be assayed by high-throughput flow cytometry. This system permitted an unbiased phenotypic screen of more than 400,000 small molecules to identify compounds capable of overcoming leukemic differentiation arrest. Successful target deconvolution of our lead compounds led to the unanticipated discovery that inhibition of the enzyme dihydroorotate dehydrogenase (DHODH) triggers myeloid differentiation.

This was confirmed in both murine and human models of AML *in vitro*. More importantly, in *in vivo* AML models, DHODH inhibitors also promoted myeloid differentiation, reducing leukemic cell burden and improving overall survival. This *in vivo* differentiation was confirmed functionally by correlation with a decrease in the leukemia-initiating cell potential.

DHODH may provide a new metabolic vulnerability for overcoming differentiation blockade in patients with AML. Given the availability of potent and well-tolerated small molecule inhibitors, we plan to advance these compounds swiftly into clinical testing.

Poster Number 175

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Characterization of T regulatory Type 1 (Tr1) cells in naïve and transplanted non-human primates

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Background: T regulatory Type 1 (Tr1) cells are peripheral CD4+FoxP3- regulatory T cells expressing CD49b and LAG-3 in mice and humans. Mouse Tr1 cells inhibit antigen-presenting cell (APC) activation via secretion of IL-10 and TGF-β and via cell-contact dependent mechanisms mediated through their expression of programmed cell death (PD)-1. However, the phenotype and functions of Tr1 cells non-human primates are still unknown.

Method: Mononuclear cells were isolated from the peripheral blood of cynomolgus monkeys(PBMCs). First, co-expression of CD49b and LAG-3 was compared among bona fide CD4+CD25highFoxP3+ Tregs and CD4+CD25-Foxp3-using flow cytometry. Second, cytokineswere processed after polyclonal activation. Next, we compared PD-L1 and CD45RO expression by Tr1 cells collected from the PBMCs of naïve monkeys, monkeys undergoing rejection of kidney allografts and monkeys which have been rendered tolerant of kidney allografts via donor mixed hematopoietic chimerism induction and leukocyte costimulation blockade.

Results: In cynomolgus monkeys, CD49b and LAG-3 were co-expressed on CD4+CD25-Foxp3- Tr1 lymphocytes but not on CD4+FoxP3+Tregs. After polyclonal activation, virtually all Tr1 cells secreted high levels of IL-10 anti-inflammatory cytokine but no pro-inflammatory IFN-γ or IL-17 cytokines. We observed a significant expansion of Tr1 cells expressing a memory phenotype (CD45RO) and the immunomodulatory receptor PD-L1 in tolerant but not other animals.

Conclusion: Our data show that the surface markers CD49b and LAG-3 can be used to distinguish Tr1 from "classical" FoxP3+ Tregs in cynomolgus monkeys. Tr1 cells are likely to inhibit T cell responses mediated by PD-1/PD-L1 interactions in non-human primates.

Poster Number 176

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Investigation of novel gamma-secretase modulators in the therapeutics of Alzheimer's disease

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Alzheimer's disease (AD) is a devastating neurodegenerative disease with no cure. Considerable genetic, biochemical and molecular biological evidence support the "amyloid-hypothesis" in the pathogenesis of AD, stating that the excessive accumulation of a small peptide, amyloid-β (Aβ), is the primary pathological event leading to AD. Aβ is generated through a sequential proteolytic cleavage from the amyloid-β precursor protein (APP) via β- and γ-secretase. One class of promising drugs for AD is known as γ-secretase modulators (GSMs), a group of small molecules that modulate the cleavage activity of γ-secretase in the processing of APP and specifically lowering Aβ levels without altering cleavage of other substrates, e.g. Notch. These GSMs bind directly to γ-secretase complex, decreasing the levels of longer Aβ species (e.g. Aβ42 and Aβ40) and increasing the levels of shorter Aβ species (e.g. Aβ38 and Aβ37). Now we have developed a series of novel GSMs and characterized those with desirable safety-profile and high aqueous solubility. We showed evidence that these GSMs significantly lowered both Aβ42 and Aβ40 levels. Importantly, these GSMs did not affect the processing of Notch, an essential protein involved in development. These data provide further in-depth support of the "amyloid-hypothesis" in the pathogenesis of AD and provide the mechanism-of-actions utilizing these novel GSMs to lower Aβ levels in the therapeutics of AD. Our results warrant follow-up characterization of these GSMs in animal-based neurobehavioral studies and further strongly support them as excellent candidates in clinical development.