ABSTRACTS
Molecular Signatures of Early CF Lung Damage Are Linked to Deregulated Hypochlorous Production by Myeloperoxidase
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Cystic fibrosis (CF) lung disease progressively worsens from infancy to adulthood. Early CF lung disease is associated with increased airway neutrophils, myeloperoxidase (MPO) and methionine sulfoxide. Neutrophils release MPO-rich primary granules in early CF, implicating hypochlorous acid (HOCl), an oxidant produced by MPO that generates methionine sulfoxide, in early CF lung disease.

We sought to identify significance and mechanisms of HOCl evolution in early CF. Infants at Erasmus MC or Emory were diagnosed with CF by newborn screening and enrolled in prospective studies (I-BALL and IMPEDE-CF, respectively) to collect bronchoalveolar lavage fluid (BALF) and chest computed tomography (CT) scans biannually from one to six years of age. Exhaled breath condensate (EBC) was collected at Emory. We analyzed 48 CF patients (mean age, 3.1±1.6 years) and 19 disease control patients (mean, 2.3±2.1 years). Lung damage was scored using PRAGMA-CF analysis of CT scans. A neutrophil transmigration model was used to test whether CF airway adaptation causes outcomes noted in early CF airway samples. Methionine sulfoxide and other small molecules were measured by high-resolution, accurate mass metabolomics, and MPO specific activity was quantified by an immunocapture method.

We confirmed prior observations by our group that increased MPO and methionine sulfoxide in BALF correlated with more severe lung damage in early CF; multiple peptidyl species and lysophospholipids were also significantly correlated with lung damage (p<0.05, q<0.05). Changes in methionine sulfoxide between visits correlated with changes in PRAGMA-CF, MPO and neutrophil elastase. Early CF BALF contained greater amounts of methionine sulfoxide compared to disease controls. Methionine sulfoxide and other metabolites were also detectable in EBC. Neutrophil adaptation to CF airway fluid increased MPO secretion and extracellular methionine sulfoxide, but decreased the cells’ capacity to generate superoxide.

In conclusion, lung damage in early CF is closely connected to MPO, methionine sulfoxide and the evolution of HOCl. Neutrophil aberrancy in CF appears to directly promote this pathway. Methionine sulfoxide may serve as a useful biomarker in EBC, pending further validation. Follow-up studies are needed to establish whether HOCl is a causative agent of disease progression in early CF, and to establish therapies to limit harm.

Metabolomic Pathways and Biomarkers Associated with Pediatric Patient-Reported Outcomes (PRO) During Childhood Cancer
Presenting Author: Janice Withycombe, PhD, RN, MN; Emory University

Withycombe, Janice; Mitchell, Rebecca M.; Castellino, Sharon; Sears, Dorothy; Lin, Yangjin; and Reeve, Bryce
INTRODUCTION: Up to 80% of childhood cancer patients experience side effects during cancer treatment. Identification of biomarkers associated with symptoms during childhood cancer therapy is needed to provide objective measurements for those who cannot self-report (very young or very ill) and to provide markers for intervention effectiveness. The purpose of this study was to explore associations between Pediatric PROMIS symptom measures, cytokines (IL-6, IFNγ, IL-1β, IL-10) and metabolites.

METHODS: Children 7-18 years were invited to participate while enrolled in a larger study assessing symptoms during cancer therapy (any diagnosis/stage). Serum samples and Pediatric PROMIS (Computer Adaptive Testing for anxiety, fatigue, depressive symptoms, mobility, and pain) scores were collected prior to and 7-14 days after identified cycles of intense chemotherapy.

Wilcoxon signed rank tests evaluated PROMIS score changes. Samples were divided by high/low PROMIS scores (<40 or >55). R (xmsAnnotator, xmsPANDA) and python (mummichog) were used to process data obtained from untargeted liquid chromatography-mass spectrometry analysis. Relationships among cytokines, metabolomic data, PROMIS scores and patient characteristics were visualized using xMWAS (0.54) and PLS analysis.

RESULTS: 40 children participated. Females 55%. Diagnoses: Acute Lymphoblastic Leukemia 38%, Hodgkin’s 32%, Other 30%. Ages (years): 7-12 (38%), 13-15 (32%), 16-18 (30%). Overall, PROMIS scores for individual domains did not change between time points (all p-values>0.05). IL-6 was negatively correlated with depressive symptoms (p=0.015) and IFNγ was negatively correlated with pain (p=0.03).

xMWAS analysis detected 5 communities in the dataset. Some communities had chemotherapy agents centrally represented (Vincristine, Doxorubicin, Methotrexate) while other communities contained central PROMIS score elements (Mobility and Psychological stress).

While 262 compounds had raw p-values <0.05 for Mobility score, FDR correction nullified significance of individual metabolites. Mummichog analysis of all metabolites with p<0.05 found differences in the androgen and estrogen biosynthesis and metabolism pathway (p=0.0005). Confirmatory analyses for pathways related to fatigue and depression are underway.

DISCUSSION: Findings of inverse relationships between IL-6/depression and IFNγ/pain were unexpected. One explanation is modulation of cytokine expression secondary to concurrent drugs (glucocorticoids, opioids, and selective serotonin reuptake inhibitors). Regarding metabolomics, this study offers preliminary evidence of pathways associated with pediatric patient reported symptoms during cancer therapy.

Comparative Metabolic Profiling of Metastatic vs. Non-Metastatic Tumors in a Medulloblastoma Mouse Model

Presenting Author: Danning Huang; Georgia Institute of Technology

Huang, Danning; Liu, Jingbo; Gaul, David; Eldridge, Ronald; Uppal, Karan; MacDonald, Tobey; and Fernández, Facundo M.

Medulloblastoma (MB) is the most common malignant pediatric brain tumor. Currently, the standard treatment for MB patients consists of tumor surgical excision, chemotherapy and radiation therapy administered to the entire brain and spine. Although 60-70% of children with MB will be long-term survivors employing this aggressive treatment, a majority of these patients will be left with permanent and
debilitating neurocognitive impairments as a result of the high-dose radiation. For those 30-40% patients that fail to respond to radiation, all will relapse with terminal metastatic disease. There is great clinical need to tailor the dose of radiation to patients with different risk stages and to understand the mechanisms of MB metastases, leading to more effective and less toxic future therapies. To date, an understanding of the underlying biology that drives MB metastasis is still lacking. To address this gap in knowledge, a robust SmoA1-Math-GFP mouse model that reliably reproduces human sonic hedgehog (SHH) MB was developed. This mouse model offers a unique opportunity to examine the metastatic biology of SHH MB, the majority subgroup of human MB with the worst clinical outcomes. Metabolic alternations were investigated by applying Ultra Performance Liquid Chromatography Mass Spectrometry (UPLC-MS) to primary tumor samples collected from mice with (n=18) and without (n=7) observed metastasis under positive ionization mode. 47 discriminating metabolites were selected based on VIP≥2 and log2 fold change +/-1 thresholds. Identified metabolites included ceramides (Cer), sphingomyelins (SM), triradylglycerolipids (TG), diradylglycerolipids (DG), monogalactosyldiacylglycerol (MGDG), glycerophosphocholines (PC) and glycerophosphates (PA). This study sheds new light on the metabolic alternations in the brain microenvironment associated with MB metastasis.

**Metabolic Changes in Pediatric Respiratory Viral Infection**

**Presenting Author:** Heather S. Smallwood, PhD; University of Tennessee Health Science Center

**Smallwood, Heather S.; Rezinciuc, Svetlana; Bahadoran, Azadeh; Devincenzo, John; Cormier, Stephania; and Shulkin, Barry**

Influenza (Flu) and respiratory syncytia virus (RSV) infections are a major cause of morbidity and mortality worldwide and pose increased risks to children. Despite considerable efforts, consistently effective vaccines remain elusive. The current therapeutic paradigm relies on early detection and treatment with viral targeting drugs resulting in variable efficacy. One way to circumvent some of these issues is to target the host response to infection. Despite growing evidence, from our lab and others, to support a role for targeting metabolism in respiratory viral pathogenesis little is known about it. Several brief clinical reports alerted radiologists that following Flu vaccination patients receiving PET/CT scans are prone to positive hypermetabolic lesions in draining lymph nodes near the injection site. This high metabolic activity was attributed to immune cells in the lymph nodes, while little was known cell specific responses in the respiratory tract. We performed a retrospective study of PET scans from immune compromised pediatric patients undergoing chemotherapy and found the patients with respiratory viral infections had hypermetabolic regions in their lungs. We collected upper airway cells from children with community acquired viral respiratory infections and found they had significant increases in glycolysis and mitochondrial respiration concomitant to changes in extracellular metabolites. We confirmed these findings *in vitro* using Flu or RSV infected pediatric human bronchial epithelial cells and murine dendritic cells (DC). Interestingly, during infection DC and epithelial cells acquired a similar bioenergetic phenotype through shared and distinct molecular mechanisms. However, DC retained metabolic plasticity that was not found in infected epithelial cells. Even so, some immune functions were altered when infected DC metabolism was restricted. Given we previously demonstrated oral treatment with metabolic targeting drugs has therapeutic potential for treating viral respiratory infections, these studies indicate cell specific responses to this line of therapy that need further delineation.
Developmental Trajectories of Social Vocal Behavior as a Biomarker for Autism During the First 24 Months of Life: Risk Status vs. Diagnosis
Presenting Author: Mitra Kumareswaran; Emory University
Kumareswaran, Mitra; Bailey, Jhonelle; Ghai, Shweta; and Ramsay, Gordon

Although autism is difficult to diagnose before age two, prodromal symptoms may emerge during the first twelve months. Due to natural variability in developmental timescales, cross-sectional measures of behavior usually fail to capture significant differences over this period. Current research suggests that early biomarkers of risk may be found instead by examining longitudinal trajectories of individual development. Experimental research shows the importance of contingent interaction between infant and caregiver in scaffolding vocal development, suggesting that developmental profiles of early vocal behavior and interaction may be candidate biomarkers for autism. Previously, we found evidence for a developmental cascade in vocal development discriminating high-risk and low-risk siblings, beginning at 12 months, with deficits in vocal contingency that were predictive of language outcome at 24 months. In this follow-up study, we re-analyzed the developmental profiles of the same children according to diagnostic outcome at 24 and 36 months, to determine whether deficits in vocal engagement appear earlier in infants who receive a diagnosis, and whether high-risk siblings who do not receive a diagnosis exhibit patterns that more closely resemble autism or typical development.

As part of an NIH Autism Center of Excellence, we tracked vocal development among 37 high-risk infant siblings and 35 low-risk controls. Utilizing automatic speech recognition technology developed by our laboratory, we identified the number of hourly vocalizations for infant and caregiver, and calculated the rate of contingent interactions. Using Functional Data Analysis, we determined developmental trajectories for each child and mean developmental trajectories by group—risk status and diagnostic outcome. The developmental cascade linking early declines in vocal interaction to later declines in adult volubility and subsequent declines in infant volubility is exaggerated in infants diagnosed with ASD. Differences that only became apparent at 12 months between high-risk and low-risk infants are present between infants with ASD and typically developing peers within the first year. High-risk infants without an ASD diagnosis resemble typically developing infants at birth, but transition towards developmental trajectories resembling infants with ASD by 24 months. Developmental trajectories of social vocal engagement over the first two years differentially predict diagnostic outcome at two years of age.

A Role for the SIX1 Homeobox Gene in CALM-AF10 Leukemogenesis
Presenting Author: Waitman D. Aumann, MD, MS; Emory University
Aumann, Waitman; Lavau, Catherine; Harrington, Amanda; Conway, Amanda; and Wechsler, Daniel

BACKGROUND: The CALM-AF10 translocation is detected in ~10% of T-cell acute lymphoblastic leukemias (T-ALLs), and in some acute myeloid leukemias (AMLs). CALM-AF10 leukemias are characterized by overexpression of proleukemic HOXA genes. Since HOXA genes are difficult to target, we hypothesized that identification of non-HOXA CALM-AF10 effector genes could potentially yield novel therapeutic targets. To this end, we took advantage of our prior observation that the nuclear export factor CRM1/XPO1 tethers CALM-AF10 to HOXA genes by interacting with a nuclear export signal (NES) in CALM. We used RNA-sequencing and microarray to determine that SIX1, similar to HOXA genes, is increased in CALM-AF10 leukemias and decreased in response to CRM1 inhibition.
OBJECTIVE: To evaluate the role of SIX1 in CALM-AF10 leukemias.

DESIGN/METHODS: RT-qPCR and Chromatin Immunoprecipitation were performed using both bone marrow progenitors and murine embryonic fibroblasts (MEFs) transduced with CALM-AF10 or an empty vector, with and without LMB. The ability of SIX1 to enhance self-renewal of hematopoietic progenitors was examined by measuring the colony-forming ability of transduced fetal liver progenitors. CRISPR-Cas9 was used to silence SIX1 in Human Embryonic Kidney 293 (HEK293) cells.

RESULTS: RT-qPCR confirmed overexpression of SIX1 in both CALM-AF10 transduced MEFs and CALM-AF10 leukemias, with decreased SIX1 expression observed in the presence of LMB. ChIP analysis showed that CALM-AF10 binds the SIX1 gene locus. Overexpression of SIX1 in fetal liver cells was sufficient to increase the self-renewal potential of these colony-forming progenitors. SIX1 was successfully knocked out in HEK293 cells, resulting in potentially slowed HEK293 proliferation.

CONCLUSIONS: SIX1 is a homeobox gene that is highly expressed during embryogenesis; expression is normally silenced post-embryogenesis. While increased SIX1 expression has been reported in numerous solid tumors, SIX1 involvement in leukemogenesis is uncertain. We have determined that SIX1 is upregulated in the presence of CALM-AF10, and increases the self-renewal potential of hematopoietic progenitors. Despite decreased proliferation rates in HEK293 cells with SIX1 knocked out, SIX1 is not critical for cell survival, and inhibition could be effective in impairing CALM-AF10 leukemia cell proliferation. Thus, SIX1 may play a pathogenic role in leukemogenesis and could be a novel therapeutic target in CALM-AF10 leukemias.

Poster Presentation Abstracts

Poster Competition Top 10

1. Clustering of Metabolomics, Clinical Biomarkers, and Ectopic Liver Fat in Children/Adolescents: An Integrated Network Analysis
   Cioffi, Catherine E.; Narayan, K. M. Venkat; Uppal, Karan; Pierpont, Bridget; Caprio, Sonia; Santoro, Nicola; and Vos, Miriam B.

Abdominal and/or hepatic fat accumulation are often associated with cardiometabolic disease (CMD) risk factors in children, but the mechanism is unclear. We measured untargeted metabolomics in plasma from 219 obese children/adolescents with (37%) and without (63%) fatty liver defined as hepatic fat fraction greater than 5% assessed by magnetic resonance imaging (MRI). Abdominal fat deposition as visceral, superficial subcutaneous and deep subcutaneous fat was measured by MRI also. Blood lipids and liver enzymes were collected by fasting blood draw and glucose and insulin metabolism were assessed by standard oral glucose tolerance test. An integrative network analysis, which involved several steps including pairwise association analyses and a multilevel community detection algorithm, was performed using xMWAS software to examine clustering patterns of metabolomics, clinical biomarkers, and abdominal and hepatic fat depots. From this analysis, we detected three communities consisting of significantly correlated variables (|r|>0.4, p<0.05). In the first community, hepatic fat clustered with the
majority of CMD risk factors, including measures of insulin resistance [i.e., fasting insulin, homeostatic model assessment of insulin resistance (HOMA-IR), and whole body insulin sensitivity index (WBISI)], dyslipidemia [i.e. high-density lipoprotein (HDL) cholesterol and triglycerides], and the liver enzyme alanine aminotransferase (ALT). Pathway analyses of the 170 metabolite features in this cluster using mummichog software showed enrichment in carnitine shuttle metabolism and glycerophospholipid metabolism ($p<0.05$). Aligning with these results from pathway analysis, individuals metabolites in the cluster included several acyl-carnitines, aromatic and branched chain amino acids, including tyrosine, tryptophan, and isoleucine/leucine, respectively, and linoleic acid. In contrast, the second community featured all three abdominal fat depots (i.e., visceral fat and superficial and deep subcutaneous fat) with 498 metabolite features, but none of the CMD risk factors. The third community featured low-density lipoprotein and total cholesterol with 237 metabolite features, but no fat depots. These results suggest that hepatic fat is more closely correlated with certain CMD risk factors compared to other abdominal fat depots. Further research is warranted elucidating the biological significance of the metabolites in this cluster as this may offer insights into mechanisms linking hepatic fat to CMD risk in youth.

2. Participant Behavioral Response Patterns and Outcomes in a Parent-Mediated Intervention to Address Food Selectivity in Children with ASD

Cohenour, Jessica; Burrell, T. Lindsey; Gillespie, Scott; McCracken, Courtney; Nuhu, Nadiratu; Wawrzonek, Addam; Scahill, Larry; and Sharp, William

INTRODUCTION: Behaviorally based intervention is the most researched and well supported treatment for food selectivity in children with Autism Spectrum Disorder (ASD) with parent-mediated training providing a less time and cost-intensive intervention option to treatment occurring in specialty clinics. The Autism MEAL Plan is a group-based intervention designed to teach parents techniques to expand their child’s mealtime variety. Previous studies have supported the feasibility and efficacy of this intervention program compared to no treatment conditions. The purpose of this study is to examine the unique behavioral patterns and outcomes associated with responders and non-responders of the intervention.

METHODS: Participants included children between the ages of 3 and 8 with moderate food selectivity and ASD who were enrolled in a RCT evaluating a manualized parent-mediated intervention, The Autism MEAL Plan. The current study evaluated results within the treatment condition to further assess differences in treatment responders and non-responders on the Brief Autism Mealtime Behavior Inventory (BAMBI). Data included in the analysis were those assessed at baseline, the end of the RCT (Week 16), and follow-up (Week 20).

RESULTS: Nineteen children (Mage=58.3 months, SD=14.6 months) participated in the study. Those considered responders (N=9) showed statistically significant differences between pre- and post-test scores, and pre-test and follow-up scores indicating a reduction in disruptive mealtime behavior and increase in sitting at the table and eating a variety of foods. Non-responders (N=10) also demonstrated improvements; however, statistically significant differences were not revealed until follow-up.

DISCUSSION: Results suggest that, while the intervention led to statistically significant differences between pre-, post-, and follow-up scores for both groups, responders showed a more immediate response to the intervention as evidenced by greater statistically significant differences between pre- and post-test scores. The current data also suggest that non-responders required more time to respond to the
intervention as scores showed a stronger statistical difference between pre-test and follow-up ratings compared to ratings taken at post-test.

3. Menstrual Function and Biological Aging in People Exposed to Polybrominated Biphenyl (PBB) Before Puberty
Curtis, Sarah; Kilaru, Varun; Terrell, Metrecia; Marcus, Michele; Conneely, Karen; and Smith, Alicia

In the 1970’s, Michigan residents were exposed to polybrominated biphenyl (PBB), an endocrine disruptor, during an agricultural accident. Children were exposed by directly eating contaminated food products or through breastfeeding, or were exposed in utero through placental passage. Studies in animal models have shown that PBBs can cause altered menstrual cycle length and altered hormone levels. Epidemiological studies show that people who were exposed before puberty report numerous developmental and endocrine-related health problems, like earlier age of menarche, even though those exposed as children typically have significantly less PBB exposure than those exposed as adults. In order to investigate whether PBB exposure during critical developmental time-points was associated with an increased risk for health problems, we evaluated 386 registry participants exposed before puberty. Epigenetic age acceleration, a measure of the rate of biological aging, was analyzed due to its known association with many age- and hormone-related health problems and all-cause mortality. In people exposed in childhood, increased current PBB levels associated with increased age acceleration (p=0.03), when adjusted for gender, and cell type estimates, suggesting that children exposed to higher levels of PBB may be at increased risk adverse health outcomes. Because age acceleration has been shown to be associated with estrogen-related conditions, and higher exposure to PBB has already been shown to be associated with lower E13G in this cohort, the association between age acceleration and hormone profiles was tested with in a subset of women (N=59) who participated in an in-depth menstrual function study that measured estrogen metabolites (E13G), progesterone metabolites (Pd3G), and follicle-stimulating hormone (FSH) in daily urine samples. To control for multiple samples per woman and day of menstrual cycle, nested mixed models were used for all association tests. Increased age acceleration was associated with lowered E13G levels throughout the cycle in univariate analyses, (p=0.03), and when controlled for PBB exposure level (p=0.01). This suggests that people who are exposed to environmental contaminants as children can have persistent adverse health outcomes, such as increased age acceleration, which can then put them at increased risk for altered menstrual function or other medical conditions.

4. Associations Between Changes in Social Visual Engagement and White Matter Microstructure During the First 6 Months of Life
Ford, Aiden; Li, Longchuan; Jones, Warren; Klin, Ami; and Shultz, Sarah

Attention to eyes—a critical skill that guides typical socialization—is already in decline by the second month of life in infants later diagnosed with Autism Spectrum Disorder (ASD), with steeper decreases in eye fixation associated with more severe social disability (Jones & Klin, 2013, Nature). In contrast, typically developing (TD) children increase their attention to eyes throughout infancy, establishing a foundation for continued social visual engagement (SVE) and brain specialization. The neural systems associated with this basic mechanism of social adaptive action are currently unknown, even in typical development. Therefore, identifying associations between trajectories of SVE and brain maturation in typical infancy is an important step towards understanding how deviations from these trajectories may lead to the emergence of social disability in ASD. Diffusion MRI and eye-tracking data were collected prospectively and longitudinally in
the same infants (n=31, 10 female) between 0 and 6 months. All participants were full-term, healthy infants with no family history of neurodevelopmental disorders or genetic conditions. Atlas-based tractography was used to delineate major white matter tracts. Eye-tracking data were collected while infants viewed scenes of actress caregivers engaging in naturalistic interaction and percentage of fixation time to each region-of-interest (eyes, mouth, body, and background) was calculated. Growth curves were fit using functional principle component analysis (FPCA) and Functional Linear Regression using Principal Component Analysis tested associations between longitudinal trajectories (Yao, Müller, & Wang, 2005, Ann. of Statistics). Functional regression analyses revealed a significant coefficient of determination (p<.05) between growth curves of eye-looking and fractional anisotropy in the inferior fronto-occipital fasciculus (IFOF). No other associations were found. This study provides the first demonstration of the relationship between trajectories of white matter development and SVE in the first months of life. Early attention to eyes is positively associated with the development of the IFOF, the structural foundation of pathways involved in voluntary goal-directed attention. These findings indicate that developing visual networks may be particularly responsive to experiential input involving the eyes of social partners and suggest a process by which early divergence from normative experience may lead to atypical patterns of white matter development in ASD.

5. Metabolome Wide Association Study of Serum DDT and DDE in Pregnancy and Early Postpartum

Hu, Xin; Li, Shuzhao; Cirrilo, Piera; Krigbaum, Nickilou; Tran, ViLinh; Ishikawa, Tomoko; La Merrill, Michele; Jones, Dean P.; and Cohn, Barbara

BACKGROUND: The advancement of high-resolution metabolomics (HRM) and the MWAS (metabolome wide association study) approach enables the readout of biological and metabolic effects by environmental exposures from human specimens.

Objectives: We used high-resolution metabolomics to improve our understanding of DDT-induced alterations of in utero environment and provide mechanistic insights for health effects of perinatal DDT exposures.

METHODS: Endogenous metabolites were measured in 397 maternal perinatal serum samples collected from the Child Health and Development Studies (CHDS) during 1959-1967 and in 16 maternal postnatal serum samples collected from DDT exposed and unexposed mice, by high-resolution Orbitrap mass spectrometers coupled with C18 reverse phase liquid chromatography. Metabolome-wide association study (MWAS) was performed to assess associations between metabolites and p,p'-DDT, o,p'-DDT and p,p'-DDE levels, using linear regression models. Pathway enrichment analysis was performed with Mummichog software to understand the biological effects of significantly associated metabolites.

RESULTS: Out of the 3121 metabolic features detected by high-resolution metabolomics, 278, 212 and 466 metabolic features were significantly (raw P < 0.05) associated with p,p'-DDT, o,p'-DDT and p,p'-DDE, respectively. Further enrichment analysis of these metabolites showed distinct metabolic profiles associated with p,p'-DDT and its metabolite p,p'-DDE. Amino acids such as serine, proline and arginine as well as urea cycle intermediates had a strong association with p,p'-DDT and o,p'-DDT in both CHDS women and in the mice, whereas fatty acids, lipids and acyl-carnitine intermediates were found exclusively associated with p,p'-DDE in CHDS women indicating impairment in mitochondrial fatty acid oxidation.

CONCLUSIONS: Changes in amino acid and lipid metabolism implicated by the association of these pathways with DDT and DDE in both women and in mice are consistent with an emerging literature
linking serine and fatty acid metabolism to diseases such as breast cancer and obesity. Given both of these diseases have been associated with developmental exposure to DDT and/or DDE as well, the potential role of serine and fatty acid metabolism on the causal pathway should be examined in the future.


Ivanova, Anna; Maner-Smith, Kristal; Boyd Barr, Dana; Dunlop, Anne L.; and Ortlund, Eric A.

Increasing evidence links maternal environmental toxicant exposures to the increased risk for pregnancy complications, which contribute substantially to infant mortality rates. African American infants disproportionately suffer from infant mortality and preterm birth, with rates 50% higher compared to those for other races/ethnicities. However, molecular mechanisms and biological pathways underlying the association between environmental toxicant exposures and poor pregnancy outcomes remain unclear. There is an urgent need to elucidate the factors contributing to these disparities and to identify biological markers to facilitate early diagnosis and treatment of affected pregnancies. Toward this goal, we integrated state-of-the-art lipidomics data analysis with the recent exposomic data collected for African American women recruited from prenatal care clinics in the Atlanta metropolitan area. We performed global untargeted lipidomics analysis on serum samples from 120 women enrolled in the prenatal cohort of the Emory Center for Children’s Health, the Environment, the Microbiome, and Metabolomics project. More than 450 lipid species across 10 lipid subclasses were identified providing a deep and comprehensive view of lipidome changes during pathological pregnancy. The determined lipid landscape was utilized to establish statistical models to predict different pregnancy outcomes. Partial Least Squares Discriminant Analysis (PLS-DA) and Random Forest models demonstrated applicability of the lipidomics approach to distinguish between term and preterm birth. The data analysis also revealed lipid classes with significantly different levels in samples from women with normal and pathological pregnancy.

Furthermore, we have identified specific lipid species that are highly sensitive to the organic pollutants, such as (polybrominated diphenyl ethers (PBDE). Together, our data revealed a strong connectivity between the environmental exposome, preterm birth pathology, and lipidomics profiles in African American women. These data build a basis for further development of lipid-based biomarkers for poor pregnancy outcomes.

7. Teaching an Old Dog New Tricks: Engineering Drug Synergy in Cocktail Chemotherapy for Pediatric ALL

Kelvin, James M.; Perdue, Lacey A.; Du, Yuhong; DeRyckere, Deborah; Graham, Douglas K.; and Dreaden, Erik C.

Combination chemotherapies have greatly improved treatment outcomes in leukemia patients; however, conventional approaches to their delivery often ignore ratio-dependent drug interactions that can either synergize with – or antagonize – cell killing when local concentrations of drug fluctuate following administration. One approach to overcome such variability is to administer a fixed ratio of drugs via an engineered nanoscale drug carrier. To this end, we have recently developed a high-throughput combinatorial screening approach that extends this strategy through the identification of triplet drug combinations in which tyrosine kinase inhibition enhances leukemia cell killing when administered with
Spotlighting Metabolomics and Child Health

11. Frontline Cytotoxic Chemotherapy. Among over 500 unique tumor-toxic drug combinations, we have identified and validated optimal ratios that augment leukemia cell killing in a panel of T cell and early T cell precursor acute lymphoblastic leukemia (ALL) cells. Further, we demonstrate simultaneous, combinatorial drug loading into nanoscale liposomes which we hypothesize will conditionally maintain drug synergy – both in circulation and following leukemia cell delivery – for prolonged periods in vivo. These novel drug formulations are expected to improve treatment outcomes and tolerability in patient-derived mouse models of leukemia.

8. Associations Between Socio-Behavioral Phenotypes and Genotypes of Relevance for Autism Spectrum Disorder (ASD) in Juvenile Rhesus Macaques (Macaca Mulatta)

Kovacs Balint, Zsofia; Gunter, Chris; Harris, Alan; Raveendran, Muthuswamy; Michopoulos, Vasiliki; Bachevalier, Jocelyne; Raper, Jessica; Sanchez, Mar; and Rogers, Jeffrey

Autism spectrum disorder (ASD) is a developmental disorder with high heritability and equal contributions of genetics and environment to the overall risk. Due to the limitations of experiments in human infants, we aim to develop a translational nonhuman primate (NHP) model. The rhesus monkey - with social behaviors, brain anatomy and development that closely resemble humans - provides critical alternative to study the origins of atypical social behaviors observed in complex social groups. The goal of this study was to identify 1) socio-behavioral phenotypes of relevance for ASD in juvenile macaques; and 2) genetic variants associated with those behavioral phenotypes and linked to ASD in humans.

To select social phenotypes, we validated the Social Responsiveness Scale (SRS, used in ASD diagnosis and research in humans, and adapted to chimpanzees and adult macaques) on 91 juvenile macaques in our breeding colony (YNPRC). We reduced the macaque SRS to 14 items and established the underlying factor structure using exploratory factor analysis (juvenile macaque SRS - jmSRS-). In addition, we collected 2 hours of behavioral observations per animal, using a well-established ethogram for rhesus monkeys. We also performed whole exome sequencing on enriched rhesus macaque DNA samples using the Rhexome v2 capture reagent and sequenced them using the Illumina NovaSeq system. Reads were aligned to the rhesus Mmul_8.01 (rheMac8) reference genome. We called genomic variants and made associations with social phenotypes with the Baylor College of Medicine pipeline and performed CADD analysis to predict the functional impact of variants of interest.

Analysis of the jmSRS revealed similarities to the human SRS, confirming its construct validity in juvenile macaques. Based on behavioral observations and the jmSRS, age- and species-atypical behaviors and extreme social phenotypes were identified. Exploratory exome sequencing yielded 1,350 single nucleotide variants (SNVs) and 95 indels in the 87 genes of interest for ASD. Single-hit SNVs were seen in several genes strongly associated with ASD risk in humans.

Our findings show a methodological advancement in the development of animal models of high translational value for ASD. We are further analyzing rhesus variants linked to atypical social behaviors.

9. Critical Role of ASCT2-Mediated Amino Acid Metabolism in Promoting Leukemia Development and Progression

Ni, Fang; Yu, Wen-Mei; Li, Zhiguo; Graham, Douglas K.; Jin, Lingtao; Kang, Sumin; Rossi, Michael R.; Li, Shiyong; Broxmeyer, Hal E.; and Qu, Cheng-Kui
Amino acid (AA) metabolism is involved in diverse cellular functions. However, it remains poorly understood how AA metabolism regulates normal hematopoiesis and leukemogenesis. Therapeutic strategies targeting AA metabolic pathways that are essential for leukemic cell growth but dispensable for normal hematopoietic cells remain to be identified. Here, we report that knockout of Slc1a5 (ASCT2), a transporter of neutral AAs (glutamine, cysteine, serine, threonine, and alanine), resulted in minor defects in bone marrow and mature blood cell development under steady state conditions. Importantly and in contrast, constitutive or induced deletion of Slc1a5 greatly decreased leukemia initiation and maintenance driven by the oncogene MLL-AF9 or Pten deficiency. Survival of leukemic mice was prolonged from ~50 days to ~300 days by Slc1a5 deletion, and pharmacological inhibition of ASCT2 also effectively decreased leukemia development and progression in xenograft models of human acute myeloid leukemia. Mechanistically, loss of ASCT2 generated a global effect on cellular metabolism, disrupted leucine influx and mTOR signaling, and induced apoptosis in leukemic cells. Given the substantial difference in reliance on ASCT2-mediated AA metabolism between normal and malignant blood cells, this in vivo study suggests ASCT2 as a promising therapeutic target for the treatment of leukemia.

10. Postnatal Zika Virus Infection Causes Persistent Abnormalities in Brain Structure, Function, and Behavior in Infant Macaques

Raper, Jessica; Mavinger, Maud; Kovacs-Balint, Zsofia; Gumber, Sanjeev; Sanchez, Mar; Alvarado, Maria; and Chahroudi, Ann

To date, most studies have focused on the impact of zika virus (ZIKV) infection in utero, documenting its association with microcephaly, fetal brain lesions, and other serious birth defects. Considering the impact that ZIKV infection can have on the developing nervous system and given that the postnatal period is also a time of rapid brain growth, it is important to understand whether ZIKV infection during infancy could have similar neurodevelopmental consequences. To address this question, we used a highly clinically relevant rhesus macaque (RM) model. Infant RMs were infected with ZIKV at 5 weeks of age and we longitudinally monitored the animals until 12 months of age with neuroimaging, as well as behavioral and neurohistopathology assessments. Postnatal ZIKV infection resulted in long-term behavioral changes, including increased emotional reactivity, decreased social contact, increased slips and falls, as well as visual recognition memory deficits at one year of age. Structural and functional magnetic resonance imaging demonstrated that ZIKV-infected infant RMs had persistent enlargement of lateral ventricles, smaller amygdalae, hippocampi, and putamen, as well as altered functional connectivity between brain areas important for socioemotional behavior and cognitive function. These structural and functional brain changes may explain the observed alterations in socioemotional behavior and learning and memory function. Neurohistopathology at 12 months of age did not show any signs of continued viral infection, yet brain lesions were observed. One infant RM showed persistent mild neuronal and perivascular calcification in the putamen and another RM presented distended lateral ventricle of the occipital lobe. These neurohistopathology findings confirm and validate the alterations in structural and functional neuroimaging, including weak functional connectivity between the putamen and inferior temporal cortex. Overall, this study demonstrated that postnatal ZIKV infection of infants in this model has long lasting neurodevelopmental consequences.
Poster Presentations

11. Case Series of Gastritis/Gastropathy Caused by Takis, Hot Cheetos or Hot Fries in Pediatric Population

*Allen, Sarah; Taylor, Morrisa; and Scheel, Lynn*

Gastropathy is a condition of the injury/damage to epithelium of the gastric mucosal with little to no inflammation. It is commonly secondary to endogenous or exogenous irritants such as bile acid reflux, alcohol, or medications such as aspirin and nonsteroidal antiinflammatory drugs. Additionally, spicy foods have been well documented as a cause of gastropathy. However, the correlation of popular snack foods such as Takis, Hot Cheetos, and Hot fries consumed by children has not been investigated.

Patient is a 10 year old female, with a past medical history of Gastric Reflux and constipation, who presented with a chief complaint of post prandial abdominal pain, differing from her reflux. The pain has been bothering her for a few months, occurs after eating, and has not affected her appetite. Commonly consumed foods include Takis/Hot Cheetos. She denies any fever, nausea, vomiting or diarrhea. Physical exam was benign except her elevated BMI of 29.1. Abdomen was soft, non-tender, with bowel sounds present in all four quadrants. A diagnosis of Acute Gastritis was given and instructions to discontinue eating Takis/Hot Cheetos, as well as, begin a trial of Ranitidine for 2 months.

Patient is a 6 year old male, with no past medical history, who presented complaining of vomiting and abdominal pain for three days. He complains of pain shortly after lunch, which includes Takis. Additional symptoms included decreased appetite, cough, nausea, and vomiting. Denies fever or any changes in bowel movements. Physical exam is unremarkable with normoactive bowel sounds, no distention or tenderness present. The patient was diagnosed with Acute Gastritis.

This case report highlights three patients who presented with acute gastritis after ingesting Takis/Hot Cheetos. Imaging studies or endoscopies were not performed in an attempt to mitigate the patients’ exposure to radiation or invasive testing. Additionally, since the patients improved after a month of dietary changes and anti-acid medication further workup was deemed unnecessary. A correlation between the consumption of spicy food in children has not been established in the literature. This cause serves to help identify causative agents and prevent gastritis in the pediatric population.

12. Using Infant Blink Rate to Quantify Infant Engagement During Social Interactions with Their Caregivers

*Ammar, Zeena; Zhong, Julia; Klin, Ami; Jones, Warren; and Shultz, Sarah*

Infant-caregiver interactions provide the ideal framework for social learning: as infants engage with their caregivers, caregivers, in turn, modify their behavior to the needs of their infant, creating cycles of contingency that scaffold infants’ emerging abilities. These reciprocal interactions are highly engaging to typically-developing (TD) infants and they often become distressed when the contingency is removed. By contrast, reduced social engagement is not only a defining feature of Autism Spectrum Disorder (ASD), but may also be a significant contributor to emerging social disability, as engagement during reciprocal exchanges is required for social learning. Unfortunately, few quantifiable measures of infant engagement exist, limiting inquiry into this important area.
The goal of this study is to determine if patterns of eye-blinking can be used to measure engagement during contingent and non-contingent interactions. This method is based on the fact the eye-blinks interrupt the flow of visual information. Consequently, the more engaged the viewer is, the more likely they will unconsciously inhibit blinking. This measure could provide new inroads for quantifying the subjective experiences of infants as they interact with their caregivers, enabling future research into disruptions of social learning in ASD.

To test this measure, eye-tracking data were collected in N=14 3-5-month-old TD infants while viewing 3 conditions: 1) a prerecorded non-contingent video of a stranger; 2) a live feed of the infant’s caregiver; and 3) a prerecorded non-contingent video of the infant’s caregiver. Mean blinks per minute (bpm) were compared across conditions. Paired samples t-tests revealed lower bpm during the contingent condition (mean bpm= 4.82, SD=4.89) compared with the non-contingent stranger condition (mean bpm=9.04, SD=8.40, p<0.05). A trend towards lower bpm during the contingent condition compared to the non-contingent caregiver condition (mean bpm=10.01, SD=11.08, p=0.10) was also observed. Immediate next steps include determining bpm in an additional N=24 TD infants and N=7 infants with ASD.

These preliminary findings suggest that eye-blink rates can provide an index of infant engagement, with TD infants blinking less during contingent than non-contingent interactions, and can be used in the future to investigate the influence of engagement on social learning in typical development and in ASD.

13. Targeting Metabolic Reprogramming of Respiratory Syncytial Virus Infection

Bahadoran, Azadeh; Rezinciuc, Svetlana; Bezavada, Lavanya; Skulkin, Barry; Kim-Hoehamer, Young-In; Devincenzo, John; Dinh Vu, Luan; Cormier, Stephania; and Smallwood, Heather S.

Respiratory syncytial virus (RSV) is a pervasive pathogen that infects virtually most children by 2 years of age. It is the leading cause of hospitalization of infants worldwide and reemerges later in life to be a serious lower respiratory tract illness in the elderly with no effective antiviral therapy. Recently, significant emphasis have been focused on changes in host cellular metabolism in response to viral infection. We performed a retrospective study of pediatric patients infected with respiratory viruses and found these infectious correlated with increased glycose uptake in the lungs. Eight of these patients were PCR positive for RSV, 5 of which had hyper metabolic lesions in their lungs. Next we characterized epithelial and immune cells from naturally infected non ventilated pediatric patients’ nasopharyngeal aspirates (NPA) and quantified their bioenergetics. We then will validate these changes in metabolism with primary human epithelial cells and determine the kinetics of RSV induced changes in epithelial metabolism. Additionally, we will develop an epithelial cell-dendritic cell co-culture to investigate how RSV infected-epithelial cell affect immune cells metabolism and characterization. We found a dramatic increase in glycolysis and mitochondria respiration in cells freshly isolated from patients’ nasal pharyngeal aspirates. RSV infection increases basal respiration, ATP production, and proton leak while reducing mitochondrial oxygen consumption and respiratory capacity in pediatric patients’ nasopharyngeal aspirate cells. Significant increases in metabolism in the infected patient’s upper respiratory cells ex vivo support our in situ findings in RSV infected patients lungs. Understanding and defining the metabolic changes in the host during RSV infection may lead to novel therapeutic approaches through targeted inhibition of specific cellular metabolic pathways.
14. Delivery of Particulate Gonorrhea Vaccine Using Laser Ablation
Bajaj, Lotika; Gala, Rikhav; D’Souza, Cherilyn; D’Souza, Nigel; Zughaier, Susu and D’Souza, Martin

INTRODUCTION: Gonorrhea is one of the most common sexually transmitted disease, caused by Gram negative diplococcus bacteria, *Nisseria gonorrhoeae*. Resistance has emerged to many antimicrobials; therefore, preventative vaccine for gonorrhea can be of great importance because of wide occurrence of the infection. Langerhans cells in skin, are phagocytic cells that signal T-cells. Upon activation, T cells and macrophages drain into nearby lymph nodes causing an increased immune response. We report on a rather novel ablative laser method of delivering particulate vaccines transdermally.

METHODS: Microparticles loaded with antigens, were prepared using spray drying method. The particulate vaccine formulation contains a biocompatible and sustained releasing polymer component containing cross-linked albumin matrix and formalin-fixed inactivated whole-cell gonococci and adjuvants. The microparticles were characterized for percent yield, size, charge and poly dispersity index (PDI). The microparticulate vaccine was delivered via transdermal route using ablative laser (P.L.E.A.S.E.®). In-vivo efficacy of this vaccine was checked in 6-8 weeks old Swiss Webster mice. Mice were administered one prime dose and two booster doses. There were five groups (n=6) in this study and animals were challenged with Gonorrhea bacteria at week 10 and sacrificed at week 12. Their lymph nodes and spleens were collected and levels of the immune cells such as CD4+ and CD8+ T cells in the collected spleens and lymph nodes were measured using BD Accuri™ C6.

RESULTS: The percent yield for vaccine particles was 89 % w/w. Vaccine particles were 3.5 um and PDI was 0.34 with a charge of -25 mV. The group that received the complete vaccine via transdermal route, showed significantly higher CD4+ and CD8+ T cells as compared to the negative and positive controls (p<0.05).

CONCLUSION: Since the bacteria is formalin fixed, all the surface proteins, antigenic domains are conserved in their native form that are presented by antigen presenting cells, to the immune cells of the body. The vaccination produced both CD4+ and CD8+ T cell based immune response, which is an important factor for the success of a vaccine. The transdermal vaccine delivery system can be a promising strategy for the delivery of particulate gonorrhea vaccine.

15. Long-Term Outcomes in Children Treated via Transcatheter Approach for Mild Congenital Heart Defects
Batsis, Maria; Thomas, Amanda; Perlow, Cabriel; Knight, Jessica; Oster, Matthew and Kochilas, Lazaros

BACKGROUND: Transcatheter interventions in children with mild congenital heart defects (CHD), such as isolated patent ductus arteriosus (PDA) and atrial septal defect (ASD), have become a common practice in the US in contrast to surgical procedures. Such patients may be exposed to unique risks such as late tissue erosion and arrhythmias. Data comparing outcomes of transcatheter versus surgical approach for similar lesions are scarce.

OBJECTIVE: To evaluate the long-term transplant-free survival of patients undergoing transcatheter interventions against surgical treatments for mild CHDs.
METHODOLOGY: Data from the Pediatric Cardiac Care Consortium, a US-based registry of patients undergoing interventions for CHD, were extracted for children (<21 years) with mild CHD procedures between 1987 and 2003. The extracted cohort was linked with the National Death Index and the Organ Procurement and Transplantation Network through 2014 for analysis. Mortality was compared with the general population using standardized mortality ratios (SMRs) matched on age, year, and sex. Kaplan-Meier survival plots were created from the date of index procedure until end of follow-up.

RESULTS: A total of 6,657 patients were treated by surgical and 3,606 by transcatheter procedures for mild CHD. Transcatheter approaches accounted for 5.8% of those treated for ASD or PDA in the early era (1987-1991) to 45.1% in the late era (1999-2003). The median age for surgical vs transcatheter procedures was 3.8 (IQR 1.8-7.0) vs 3.7 years (IQR: 1.8-7.2) respectively (P = 0.2833). The 20-year transplant-free survival was 97.3% vs. 97.7% (P=0.075) for PDA closure and 97.9% vs. 98.2 (P=0.755) for ASD closure by surgical vs transcatheter approach respectively. Adjusted SMRs for surgical vs transcatheter interventions were: 2.09 (95% CI: 1.596-2.582) vs 2.96 (95% CI: 0.766-5.144) for ASD and 2.21 (95% CI: 1.417-2.995) vs 3.86 (95% CI: 2.314-5.401) for PDA. Late deaths in patients of transcatheter and surgical procedures are primarily attributed to causes unrelated to their initial CHD.

CONCLUSION: In this large, U.S. cohort, long-term outcomes of children undergoing transcatheter interventions for mild CHD are excellent and comparable to those undergoing surgical procedures for similar lesions. Continued follow-up into adulthood is crucial for determining late serious morbidity in this population.

16. Increasing Access to Effective Intervention for Improving Postpartum Parenting Mood and Practices: A Case Examination of Maternal Engagement in a Mobile Health Intervention

Beacham, Chloe; Patterson, Alexandria; and Baggett, Kathleen

Mothers with postpartum depression are at an increased risk for ineffective interactions with their infants, which compromises infant development, particularly social communication development. Mothers who are poor are disproportionately at risk for postpartum depression, yet they rarely receive effective depression treatment and often struggle to engage in parenting interventions. Accessible interventions with an integrated focus on reducing depression and improving parenting are crucial for overcoming barriers that impede mothers from low-income communities from accessing depression treatment. This poster highlights innovative applications of m-health technology for increasing access to and engagement in an integrated intervention to improve maternal mood and parent practices that scaffold social communication among depressed mothers and their infants affected by poverty-driven health disparities.

Case examples are drawn from a formative sample of 45 mothers enrolled in a randomized controlled trial examining effects of an m-health intervention with an integrated focus on reducing postpartum depression and improving parenting practices (NIH RO1 HD086894). Mothers are provided a mobile phone, access to a mobile self-help app with structured video-based learning opportunities and video-based remote coaching for improving mood and parenting practices that scaffold infant social communication. Participants enrolled in the program are poor; most self-identify race as Black/African American (86%); highest level of education program completion is high school (73%); and many are single mothers (54%).
Mothers enrolled in the program face multiple challenges, which make clinic and home-based intervention extremely challenging if not impossible. These include multiple jobs, unpredictable work schedules, and limited support in caring for multiple children, including fragile infants and older children with special needs. In the face of overwhelming barriers, maternal success in intervention completion requires access to self-help learning material as well as access to flexible, video-based coaching with high implementation fidelity in targeting and reinforcing skill acquisition for improving mood and parenting in a safe and supportive environment.

Rigorous evaluation of m-health technology-based intervention are needed to reduce barriers often experienced by mothers struggling with postpartum depression, poverty, and the challenges of parenting an infant. Realistic solutions for promoting maternal access to and engagement in effective intervention are highlighted.

17. Doxorubicin Conjugation to Reovirus Enhances Tumor Cell-Directed Oncolyis

Berry, Jameson; and Mainou, Bernardo A.

Triple-negative breast cancer (TNBC), characterized by the lack of estrogen, progesterone, and HER2/Neu receptors, constitutes 10-20% of all breast cancer cases. Treatment is largely limited to cytotoxic chemotherapy. Combination therapy can increase treatment efficacy. Reovirus, an oncolytic virus, preferentially infects and kills transformed cells. In a high-throughput screen of small molecule inhibitors, we identified doxorubicin as an enhancer of reovirus infectivity in the TNBC cell line MDA-MB-231. To better control doxorubicin delivery and enhance reovirus oncolytic potential, we chemically conjugated doxorubicin to reovirus (reo-dox). Reo-dox attachment to MDA-MB-231 cells is slightly impaired, but viral replication is largely unaffected. Reo-dox induces cell death with faster kinetics than virus alone. MDA-MB-231 cells infected with reo-dox, but not virus alone, induced DNA double-strand breaks and activation of DNA damage response. Interestingly, reo-dox, but not virus plus drug, activates STAT1 and STAT2 at higher levels than virus alone. qRT-PCR amplification of mRNA further suggests that reovirus immune response may be Type-III, but not Type-I, interferon-associated, and is augmented with drug combination and conjugation. Together, our findings show that reo-dox exhibits superior toxicity in TNBC cells than virus alone. Future studies will define the mechanism of enhanced cytopathicity of reo-dox and oncolytic efficacy of dox-conjugated reovirus in vivo. Delivery of small molecule inhibitors via conjugation to reovirus particles may provide an effective new method to directly target and kill cancer cells while minimizing toxicity to healthy cells and tissues.


Bidwell, Jonathan; Sookyong, Koh, Escoffery, Cam; and Mynatt, Elizabeth

Many patients with epilepsy and their caregivers struggle to document seizure events, sleep, stress, activity and other health-related behaviors that are essential for effective patient self-management and informing clinical care.

In this study, we investigated the role that mobile surveys, health tracking devices, and financial incentives can play in improving the consistency and reliability of patient and caregiver data collection between clinical appointments.
Mobile survey questions were administered to 30 families to document patient seizures, mood, sleep quality, and physical exercise activities. The families were recruited at the Children’s Healthcare of Atlanta (CHOA), North Druid Hills clinic in Atlanta, GA. Inclusion criteria were being a child or young adult, aged 10-18, who was being treated for epilepsy or a caregiver who each spoke English and already had an Android or Apple smartphone.

The families were randomly assigned to one of the following three study conditions: 1) mobile surveys, 2) mobile surveys, and health tracking devices or 3) mobile surveys, health tracking devices, and financial incentives. The mobile survey condition included only the daily surveys. The health tracking devices included a patient Fitbit for measuring sleep and exercise, a pair of Tricella pill boxes for measuring patient AM/PM medication intake, and a Bluetooth key-chain that enabled us to simultaneously send the patient and caregiver text message reminders when they were in close proximity to one another. The financial incentives included a $5 per day bonus for completing a personal health goal such as “get at least 8 hours of sleep each night”. Intake and exit surveys were administered to all participants. Self-reports could be reviewed on a set of patient and clinician dashboards.

The key finding was that mobile surveys are indeed a feasible and effective approach for collecting self-reports during epilepsy treatment. Intake survey responses showed that among patients who keep track of seizures, most patients already use an electronic method (e.g. website or tablet). Introducing health tracking devices and financial incentives led to more frequent and reliable participant survey responses throughout the study.

19. Oral TLR7 Agonist Administration Induces an Immunostimulatory Response in SIV-Infected ART-Suppressed Infant Rhesus Macaques
Bricker, Katherine; Obregon-Perko, Veronica; Hesselgesser, Joseph; and Chahroudi, Ann

Globally, 2.1 million children are living with HIV-1 and the majority of new infections occur postnatally through breast milk transmission. The major obstacle to HIV/AIDS cure is the presence of a reservoir of latently infected cells that persists even under ART treatment. Recent studies have demonstrated that a toll-like receptor 7 agonist can reverse viral latency and alone or with use of a therapeutic CD8-inducing vaccine may facilitate reduction of the viral reservoir. In this study, two dose levels of an orally delivered TLR7 agonist (GS-986) were administered to SIV-infected ART-suppressed 7-month old rhesus macaques (RMs) to evaluate tolerability and pharmacodynamic responses. Two 5-week-old RMs were infected with SIVmac251 orally in two doses 24 h apart and placed on daily ART beginning at 4 wks post infection. Both animals were virologically suppressed for over 3 months before administration of GS-986. At 7 months of age, RMs received 0.1 mg/kg GS-986 via oral gavage (o.g.). Complete blood count (CBC), serum chemistry, plasma viral loads, plasma cytokine concentrations, and immune cell activation were monitored prior to administration, 24 h, and 1 wk post administration. Plasma was collected prior to and 30 min following administration for pharmacokinetic (PK) analysis. Following 4 wks of rest, animals received a second dose of 0.3 mg/kg (o.g.) and analyses were repeated. GS-986 was well tolerated at both administered doses with no adverse clinical observations and normal CBC and chemistry at 24 h and 7 d post administration. Both RM’s maintained undetectable viremia following administration. Concentrations of IFN-y, IL-1RA, IL-6, IP-10, and I-TAC were elevated in the plasma at 24 h post-administration and returned to pre-dosing levels by 7 d post-administration. Increases in monocytes (CD3-CD4int CD14+ CD16+) and circulating (CD169+) macrophages was observed 24 h following GS-986 administration with a return to baseline by day 7. In summary, we have demonstrated that oral administration of GS-986 is tolerated in infant RMs,
with induction of expected immune parameters. Future work will involve investigating the effect of GS-986 with a therapeutic vaccination on viral reservoir and viral rebound following analytical treatment interruption.

20. RSV Infection in Infancy Alters Development of Regulatory T Cells
Chirkova, Tatiana; Costello, Kaitlin; Hartert, Tina V. and Anderson, Larry J.

Respiratory syncytial virus (RSV) constitutes a significant burden for infant and young children’s health. Severe pneumonia and bronchiolitis induced by RSV result in high hospitalization rates and mortality each year in children <5 years of age. Host responses to RSV play important role in disease pathogenesis and immunopathology of viral infection. Animal studies demonstrate that regulatory T cells (Treg) play critical role in controlling RSV-induced pulmonary inflammation and disease severity. Only a limited number of clinical studies investigated the role of Treg in RSV infection in children. These studies showed a marked depletion of circulating Tregs in infants during RSV infection, particularly in children with severe RSV disease. Our project is focused on the delayed effects of infant RSV infection on the regulatory T cell responses. About 2000 children were enrolled at birth and followed up throughout the first years of life. RSV infection was observed between 0–6 months of age and confirmed clinically, by PCR and serology at 1 year of age. Blood samples were later collected at 2 years of age from the nested cohort comprised of 100 subjects who did or did not contracted RSV infection in infancy. PBMC were isolated from blood samples and stimulated with various clinical RSV strains. Circulating Treg and RSV-induced differentiation of suppressive activated, suppressive resting and non-suppressive Tregs were evaluated. Children with infant RSV displayed lower levels of circulating total Treg comparing to subjects who were not infected with RSV in infancy. RSV stimulation in all samples, irrespective of the history of infant RSV infection, induced increase in percentages of non-suppressive Treg with marked decrease of suppressive resting Treg. Interestingly, only in children who had RSV infection in infancy, stimulation with RSV induced significant decrease in activated suppressive Treg. This decrease in activated Treg was uniform for all children with infant RSV, regardless of the severity of the original infection. Our data suggest the RSV infection in infants below 6 months age can alter host Treg development which is reflected in the significantly dampened Treg responses to new RSV strains later in life.

21. Serum Cytokine Biomarkers from PANS/PANDAS Patients Directly Open the Blood-Brain Barrier: A General Mechanism for Antibody Entry During Autoimmune Encephalitis
Cutforth, Tyler; Ampatey, Nicole; Makani, Dylan; Swedo, Susan, Agalliu, Ilir; Vargas, Wendy; and Agalliu, Dritan

BACKGROUND: The crucial issue underlying neuroinflammatory diseases of the central nervous system (CNS) is how antibodies and sera components initially gain access to the brain through the blood-brain barrier. At a cellular level, pathogenic molecules must breach either the transcellular (i.e. transcytosis via caveolae) or paracellular (i.e. tight junctions between endothelial cells) barrier in order to exert their effects on neural targets.

METHODS: We tested sera from 28 PANS/PANDAS (two varieties of post-infectious basal ganglia encephalitis, or BGE) patients and 14 controls in vitro for direct effects on the human blood-brain barrier. Our assays included structural characterization of tight junctions, measurement of trans-endothelial
electrical resistance (TEER) and caveolar transcytosis. The presence of autoantibodies against the D1R and D2R dopamine receptors was also measured using a live-cell binding assay.

RESULTS: No significant effects of patient sera were observed on the paracellular pathway, using either structural or functional methods. The transcellular pathway, however, showed dramatic stimulation of caveolar transcytosis. These effects are likely due to 19 serum cytokine/growth factor clinical biomarkers we hereby identify for PANS/PANDAS by multiplex profiling.

CONCLUSIONS: PANS/PANDAS patient sera contain high levels of numerous inflammatory cytokines and growth factors that also stimulate transcytosis in brain endothelial cells. These proteins are the first serum biomarkers for basal ganglia encephalitis and should improve diagnosis and treatment monitoring for all autoimmune encephalitides.

22. Defining the Influence of the RNA Binding Protein Musashi 2 on the Neuroblastoma Metabolome

Cuya, Selma M.; Smith, Matthew Ryan; Pilgrim, Adeiye; Rathi, Komal; Chen, Dongdong; Jones, Dean P.; and Schnepp, Robert W.

BACKGROUND: Patients with high-risk neuroblastoma endure an extremely intense, treatment regimen and yet, approximately half of them die, mandating a better approach to this cancer. Our laboratory investigates the overarching hypothesis that RNA binding proteins (RBPs), which play key roles in balancing self-renewal, differentiation, and cell proliferation, are deregulated in neuroblastoma and constitute novel therapeutic targets. In this work, we focus on Musashi 2 (MSI2), an RBP shown to play a critical role in the maintenance of stem cell populations and in formation of aggressive adult tumors, but that is unexplored in pediatric tumors. Our preliminary data suggest that MSI2 positively influences the expression of isocitrate dehydrogenase 2 (IDH2), a mitochondrial enzyme that is deregulated in multiple diseases and promotes oxidative decarboxylation. Given this novel finding, we sought to use unbiased means to ask how MSI2 shapes cellular metabolism within neuroblastoma.

METHODS: We used in silico analysis of clinically annotated neuroblastoma gene expression datasets. We employed shRNAs to manipulate transcripts of interest in neuroblastoma cells and measured effects on cell proliferation, cell cycle, and apoptosis. To assess the influence of MSI2 on metabolism globally, untargeted high-resolution metabolomics (HRM) was utilized. Briefly, control and MSI2-depleted neuroblastoma cell line models were lysed and analyzed on an Orbitrap Fusion™ mass spectrometer, with data extraction and analysis performed using previously established methods.

RESULTS: MSI2 is robustly expressed in neuroblastoma cell lines, tumors, and patient derived xenografts and its expression is positively correlated with higher stage and worse prognosis. Moreover, depletion of MSI2 using four independent shRNAs led to 2 to 3-fold decreased proliferation across multiple models, partly due to increased apoptosis. HRM revealed that MSI2 knockdown led to perturbations in the TCA cycle, amino acids synthesis, and pathways involved in oxidative stress, consistent with mitochondrial energetic disruption.

CONCLUSIONS: MSI2 is robustly expressed in multiple models of neuroblastoma and appears to drive increased proliferation/survival. Preliminary HRM analysis implicates disruption of mitochondrial metabolism as a possible mechanism of these outcomes. This study provides an initial foundation for
understanding how MSI2 shapes metabolism within neuroblastoma and may form the groundwork for future therapeutic interventions.

23. Burnout in Pediatric Intensive Care: A Quantitative Analysis of Organizational Determinants
Dalal, Nupur; Gaydos, Laura; Calamaro, Christina; Cunningham, Charlene; and Basu, Rajit

BACKGROUND: Clinician burnout is an epidemic, impacting approximately half of physicians and one-third of nurses in critical care. Workers with higher burnout scores show higher rates of suicidal ideation, perceived medical error, job dissatisfaction and turnover; their patients experience less satisfaction and more nosocomial infections. Growing evidence reveals interventions targeting organizations are more effective than those targeting individuals. To date, these organizational interventions have treated burnout as a homogenous entity without delineating its group or location specific causes. We hypothesize specific burnout profiles comprised of varying organizational factors exist and are specific to professional role and ICU setting. No assessment of organizational contributors to burnout in pediatric intensive care environments (ICUs) have been published.

METHODS: We performed a multi-dimensional quantitative prospective analysis of staff within five ICUs at two hospitals in a quaternary care pediatric health system. We designed a novel survey instrument, integrating Likert scale based validated measures of organizational culture, organizational support, and an abbreviated burnout inventory; these were paired with measures of relationship quality, workplace conflict, work hours and schedules. The survey was distributed across units to physicians, advanced practice providers, nurses and respiratory care professionals. The primary outcome of interest was prevalence of burnout. The primary analysis was association of organizational culture with burnout.

RESULTS: 355 (36%) staff completed the survey with an overall burnout rate of 42%. Amongst the entire sample, those who had less burnout reported a greater constructive organizational culture rating [14.2 vs 12.8 on (range: 4-20) p<0.001], greater perceived organizational support [16.3 vs 12.9 (range 0-24) p<0.001], higher quality of relationships [7.5 vs 7.0 on (scale: 1-10) p<0.001] and fewer conflicts (1.4 vs. 2.2 per person per 7 days p<.001). Physician burnout rate was 30%, while nurse burnout rate was 40%. Profession based analysis revealed conflict with staff outside the unit impacted physician burnout (p<0.041), while conflict within the unit impacted nurses (p<0.011).

CONCLUSION: Preliminary data suggests specific organizational determinants correlate with patterns of burnout. An expanded analysis will identify individual burnout phenotypes specific to organizational drivers, ultimately facilitating targeted design and testing of personalized interventions.

24. Designing Cancer Care for Kids By Kids Part 2: Digitizing Methods for Engaging Patients and Their Families
Denham, Megan; Bushehri, Yousef; Mitchell, Russell; Harris, Kamyrn; Harvey, Samuel; and Wasilewski-Masker, Karen

The experience of children receiving cancer treatment is unique and different from that of an adult receiving treatment. A cancer diagnosis impacts a child’s ability to go to school, disrupting their education and social development. Children with cancer may not fully understand what their body is going through and can lack the necessary vocabulary to communicate their experiences in the same way adults do.
Parents have to take time away from work and their other kids so that their diagnosed child can receive the treatment needed. Patients and family members express frustration with the care received at pediatric cancer clinics and report low patient satisfaction. To address this problem, researchers often employ adapted tools which are designed to study adults, or they rely on reporting from adults which leaves low patient satisfaction and frustration unresolved.

To address these problems in children’s cancer treatment, and identify opportunities for improvement, a multidisciplinary team (comprised of clinicians, researchers, child-life specialists and architects) partnered with often neglected stakeholders – the patients.

Through an iterative process of mapping out care-processes, and understanding how patients and clinicians move through the clinic’s spaces, and by engaging patients and their families early in the process, the team developed a unique “passport” data collection tool that examined the experience of children by teaming up with them as researcher partners. The novel “passport tool” used language accessible to kids by focusing on how they were experiencing the clinic. The kids collected time-motion data and reported qualitative information expressing how they felt in each space.

Building on the work from the “passport tool”, the research team has developed an iOS application which allows for more flexibility than the original paper-based format. The app includes games to engage the children with positive distractions during their waits and has the capacity to adapt its contents and data points to the user’s age and clinic.

A multidisciplinary approach for the application of modelling, systems engineering and patient-centered design to improve the efficiency and experience of care will be presented. Lessons learned from working with outpatient pediatric oncology/infusion clinics and patients will be discussed.

25. The Effect of Low-level Lead Exposure in the Bioavailability of Progenitor Cell Populations in Metro Atlanta Children

Dickinson-Copeland, Carmen M.; Kendrick-Allwood, Salathiel R.; Pemu Priscilla E., Stiles John K.; Boyd Barr Dana.; and Dunlop Anne L.

Children are more sensitive to Pb toxicity than adults, and exposure results in long-term neurobehavioral and cognitive effects. In 2012, the CDC decreased the threshold level of concern for blood Pb level (BLL) concentration to 5μg/dL; although the Environmental Protection Agency stresses that there is no safe level of exposure in children. Associations between subthreshold exposures and poor health outcomes, including cognitive deficits, blood disorders, and vascular dysfunction have been reported. There is some evidence that blood Pb affects hematopoietic and endothelial cell precursors, or progenitors, but the susceptibility of these cell types to chronic, low-level Pb exposure is, as yet, undetermined. The objectives of this study were to 1) improve screening efforts to identify children with low-level lead exposure and 2) target hematological and cardiovascular system dysfunction upstream. To increase Pb screening sensitivity, we will use ZRT Laboratories in Oregon to investigate the prevalence of low-level blood lead in children ages 2 to 6 years in urban Atlanta counties to identify children with sub-threshold blood lead. ZRT is CLIA certified laboratory that specializes in sensitive ICP-MS technology capable of measuring Pb at the lowest limit of detection. To examine the effect of low-level lead exposure in the hematopoietic and cardiovascular systems of children, we will assess the bioavailability and functionality of CD34+
hematopoietic and endothelial progenitor cells. We hypothesize that sub-threshold BLLs are associated with sociodemographic risk factors and progenitor cell bioavailability in urban children.

26. Association of DNA Methylation with Clinical and Environmental Correlates of Crohn’s Disease
Doan, Sylvia; Somineni, Hari; Venkateswaran, Suresh; Kilaru, Varun; Hyams, Jeffrey; Denson, Lee; Cutler, David; Conneely, Karen; Smith, Alicia; and Kugathasan, Subramanium

BACKGROUND: Crohn’s disease (CD) is a chronic inflammatory intestinal disease of which pathogenesis is thought to involve underlying genetic risk, dysregulated immune system and environmental exposures. Epidemiological evidence implicates a role for environmental factors including smoking and breastfeeding in the development and progression of CD, though the underlying mechanism behind these remain unknown. DNA methylation has been shown to play a role in mediating the impact of environmental exposures on the risk of various complex diseases. Here we investigated whether environmental exposures and clinical markers of CD are associated with variation in DNA methylation, using a subset of subjects from the RISK pediatric CD inception cohort.

METHODS: We generated genome-wide DNA methylation data using the Illumina HumanMethylationEPIC 850K array in blood DNA samples of 74 controls and 164 pediatric patients with CD at two-time points – at diagnosis and follow-up within 36 months after diagnosis. Our primary exposures of interest were breastfeeding, mode of delivery, exposure to maternal, active or passive smoke, and therapy. Clinical markers tested for association include albumin, hemoglobin and erythrocyte sedimentation rate (ESR). Linear regression models were used to assess the relationship between DNA methylation and exposures; with age, gender, estimated blood cell-proportions, and genotype-based principal components as covariates.

RESULTS: We found differential methylation at 47 CpGs (annotated to 31 genes) associated with maternal smoking during pregnancy (FDR <0.05). Of these, 17 CpGs were hypermethylated and 30 were hypomethylated in subjects exposed to maternal smoke. The effect of maternal smoking on methylation changes at these CpG sites remained consistent at the two-time points examined – at diagnosis and during the 3-yr follow-up period (R=0.77; P<2.2x10-09). DNA methylation levels at 18, 2, and, 16 CpG sites showed association with albumin, hemoglobin and ESR, respectively. Conversely, we did not find any significant associations between DNA methylation and different classes of CD medication.

CONCLUSION: These data suggest that DNA methylation may serve as a molecular mechanism in mediating the risk of environmental exposures in CD. Our longitudinal investigations provide a rationale for the stability of environmental influences on DNA methylation modifications.

27. The Impact of Birth Month on the Development of JIA in the United States
Estroff, Brandon; McCracken, Courtney; Dave, Ishaan; Gergely, Talia; Ponder, Lori; and Prahalad, Sampath

BACKGROUND: Like many autoimmune diseases, the development of Juvenile Idiopathic Arthritis (JIA) is thought to be multifactorial, with both genetic and environmental factors playing a role. In other autoimmune diseases, such as type 1 diabetes mellitus, autoimmune thyroid disease and celiac disease, a month of birth effect has been found. Using the Childhood Arthritis and Rheumatology Research Alliance (CARRA) Registry, the largest multicenter observational registry for pediatric rheumatic disease, we investigated if there was a similar month of birth effect in JIA.
METHODS: A nationwide cohort consisting of 11,345 children with JIA who were enrolled in the CARRA registry were studied. Birthdate, gender and JIA category were collected at time of enrollment. Using a Chi Square goodness-of-fit test, the birth months of the children with JIA were compared to the birth months of the general population (109,066,226 births), which was obtained from CDC’s National Vital Statistics Reports. The children with JIA were stratified further by gender and disease category.

RESULTS: There was a significant difference in month of birth distribution between the cases and the controls. Children with JIA had a significantly increased likelihood of being born in January (p<0.001) and a significantly decreased likelihood of being born in August (p=0.027). JIA females alone were also found to have a significantly increased likelihood of being born in January (p<0.001) compared to the general population. JIA males were found to have a significantly decreased likelihood of being born in August (p=0.045). When JIA categories were investigated, Oligoarthritis (p=0.003), Polyarticular RF (-) (p=0.008), and Undifferentiated JIA (p<0.001) were all also found to have a significantly increased likelihood of being born in January.

CONCLUSION: Using a nationwide cohort, we found that month of birth is associated with the development of JIA, discovering an increased likelihood of JIA in those born in January. These findings support the hypothesis that seasonal variations, such as exposure to perinatal viral infections, seasonal hormonal changes or vitamin D deficiency, may contribute to the development of JIA.

28. Randomized Control Trial to Compare Sedation Medication on Infants and Toddlers Requiring Brain Magnetic Resonance Imaging Studies (MRI)
Fagin, David; Mustefa, Aziza; Mallory, Michael; Delgado, Carlos; Banks, David; Williams, Abby; Geng, Rena; and Immergluck, Lilly

BACKGROUND: Pediatric Emergency Medicine Associates (PEMA), LLC provides deep sedation to ~7,000 patients annually at Children’s Healthcare of Atlanta. Propofol has been the sedation medication of choice for uncomplicated radiological procedures. Routine use of Dexmedetomidine (Dex) is limited in this setting. Our pilot study addresses the lack of experiential data comparing the two medications, especially among younger aged children. We hypothesized that deep sedation can be achieved similarly between Dex and Propofol for uncomplicated brain MRIs, and that efficiency and effectiveness are similar between the two sedation drugs.

METHODS: This is a prospective, observational study comparing Dex to Propofol, using randomization scheme. Power analysis (α=0.05 and β=0.8) determined 60 patients (30 in each group), stratified into two age categories (< 12 months, > 12 months) were needed. Primary outcome measures include efficiency, defined as time to and recovery from sedation and effectiveness, defined as successful deep sedation of study participants to carry out the MRI procedure. Inclusion criteria: Children ≥3 months to ≤36 months of age, scheduled for uncomplicated brain MRI. Exclusion criteria: Allergies to medications, hypersensitivities to inert egg or soy products, history of unstable cardiac or respiratory status, or receiving digoxin. Descriptive statistics were performed using SAS 9.4, and the average time differences between Dex and Propofol for uncomplicated brain MRIs, and that efficiency and effectiveness are similar between the two sedation drugs.

PRELIMINARY RESULTS: Among 48 patients approached, 25 have enrolled. Mean age was 18 (SD=9.4) months, with 11 females and 14 males. Dex was randomized in 10 and Propofol in 15 patients. Average total time for induction through recovery was 79 min (SD=10.7) for Dex, compared to 56 min (SD=4.2) for
Propofol, \( p=0.06 \). Mean scan time for Dex was 44 min (SD=14.5) and for Propofol was 45 min (SD=15.7), \( p=0.9 \). Most common adverse effect included hypotension (2 events).

CONCLUSION: The total medication administration time may be longer for Dex as expected, but not statistically significant. However, the results reveal that the total scan time for both medications are similar. These preliminary results support the hypothesis that there are no significant differences between the two medications.

29. LGR5: Candidate Cell Surface Marker for Human Cardiac Progenitor Cells Committed Toward Cardiomyocytes
Forghani, Parvin; Li, Dong; Maxwell, Joshua; and Xu, Chunhui

Cardiovascular disease remains the greatest threat to health worldwide, especially in developed countries. The progression of human induced pluripotent stem cell (hiPSC)-derived cardiomyocytes \textit{in vitro} toward therapeutic applications can be facilitated by detailed understanding of cell-surface markers of cardiac progenitors. A combination of mesoderm-related markers has been identified during hiPSC differentiation towards cardiomyocytes. However, not all cells expressing these markers are committed to the cardiovascular fate. These evidences emphasize the need for improving sorting strategies to isolate cardiomyocyte-committed lineages among heterogeneous populations of hiPSC differentiation culture. We have recently identified transient upregulation of LGR5 (Leucine-rich repeat-containing G protein-coupled receptor 5) during the early stage of cardiomyocyte differentiation. Additionally, knockdown of LGR5 significantly reduced the proliferation of differentiated cells toward cardiomyocytes. Here, we show efficient differentiation of LGR5 (+/hi) cardiac progenitors towards cardiomyocytes. We found correlation in expression pattern of LGR5 with other identified mesoderm-related markers at different time points using multicolor flow cytometry. Additionally, expression level of LGR5 increased at differentiation Day 4 and 5 compared with those at Day 0. We also selectively separated LGR5 (+/hi) cardiac progenitor cells from early-stage of differentiated hiPSC culture using both FACS and MACS approaches and monitored their differentiation potential toward cardiomyocytes. The enriched LGR5 (+/hi) cells showed potency toward cardiomyocytes in a cardiomyocyte-specific environment. Our results support a key role of LGR5 as a biomarker at early stages of cardiomyocyte development from hiPSCs. We anticipate that understanding more details of LGR5 signaling and its role in an early stage of differentiation will greatly contribute to the establishment of a detailed cardiac lineage fate map for cardiac progenitor cell-based regenerative therapy.

30. Plasma and Serum-Based Metabolomics are Comparable in Children with Liver Disease
Frediani, Jennifer K.; Mitchell, Rebecca M.; Higgins, Melinda K.; and Vos, Miriam B.

OBJECTIVES: Given the variety of biological samples used for metabolomics analysis, we aimed to determine metabolite differences between serum and plasma samples in a convenience sample of children with liver disease.

METHODS: This secondary analysis was performed on 178 children with serum, collected in red top tube with clotting factor, and plasma, collected in EDTA tube with anticoagulant, samples recruited for various liver disease studies. Mean, standard deviation, and frequencies were used to describe demographics. Paired two-tailed student t-tests were performed to determine differences in metabolites.
RESULTS: Average age was 12.6 years (SD 4.8) and the children were 54% male. The sample was racially diverse with 31% African American, 38% Caucasian, 26% Hispanic, and 5% other. Average body mass index Z-score was 1.29 (SD 1.37). These subjects had various liver diseases, 44% nonalcoholic fatty liver disease. Serum and plasma samples were not inherently different in this cohort. Correlations were too high for traditional modeling. Student t-tests (p<0.05) produced 460 significant differences out of 12804 detected mass to charge ratios, less than 4%. The top 20 most significant differences included many pesticides, food additives and plant compounds and some lipid molecules.

CONCLUSIONS: There is no significant difference in metabolomics analysis between serum and plasma samples in children with liver disease. Most metabolites detected were comparable between samples. It can be concluded that studies with similar sample and data extraction but different blood samples can be compared. Researchers should be cautious when comparing lipids or environmental chemicals from serum and plasma-based metabolomics.

31. Polyamine Metabolism is a Modifier of Tuberous Sclerosis Complex Brain Pathology
Gambello, Michael; McKenna, James; Murray-Steward, Tracy; Kapfhamer, David; and Casero, Robert

Tuberous Sclerosis Complex (TSC) is an autosomal dominant disorder causing significant morbidity and mortality during the pediatric years. Children often suffer from both structural and functional brain pathology such as cortical tubers, subependymal giant cell astrocytomas, epilepsy, intellectual disability, autism and other neuropsychiatric disorders. TSC is caused by inactivating mutations in the tumor suppressor genes, TSC1 and TSC2, the protein products of which inhibit the mechanistic target of rapamycin complex 1 (mTORC1) signaling pathway. Hyperactivity of mTORC1 signaling due to loss of TSC1 or TSC2 induces an anabolic state with an increase in nucleotide, protein, lipid and other macromolecular synthesis to fuel cell growth and proliferation. In previous work using a mouse model of TSC in which the Tsc2 gene was conditionally targeted in most developing neurons and glial cells of the brain (Tsc2-RG), we performed unbiased metabolomic profiling and showed dysregulated polyamine synthesis in the brains of mutant mice. Specifically, we observed a 4-fold increase in levels of the polyamine putrescine and >10-fold increase in activity of ornithine decarboxylase (ODC), the rate-limiting enzyme in polyamine synthesis. Polyamines have been shown to play critical roles in cell growth, proliferation and migration, cellular stress, aging and neurodegenerative diseases. Moreover, multiple clinical studies investigating the therapeutic effects of the irreversible ODC inhibitor, 2-difluoromethylornithine (DFMO) on neuroblastoma, astroglioma and other cancers are currently underway. These observations led us to hypothesize that increased ODC activity and putrescine contribute to the neuropathology of Tsc2-RG mice. In the present study, we used genetic and pharmacologic approaches to reduce ODC activity and concomitant putrescine levels and assessed neurodevelopmental phenotypes in Tsc2-RG mice. To our surprise, ODC/putrescine reduction worsened neuronal growth and migration defects, astrogliosis and oxidative stress in Tsc2-RG brains, suggesting a protective effect of elevated putrescine in our TSC model. These data establish the polyamine pathway as an important modifier of TSC brain pathology that may be important for the development of new therapies.

32. Towards an HIV Cure: Screening for Agents That Selectively Disrupt Establishment of the HIV Reservoir in Primary Myeloid Cells
Gavegnano, Christina; Shepard, Caitlin; and Kim, Baek
BACKGROUND: HIV-1 infects both activated CD4+ T cells and non-dividing myeloid cells (macrophages) during pathogenesis. HIV-1 infection induces death of activated CD4+ T cells, leading to immune deficiency of infected hosts, but HIV-1 infected macrophages survive for a long period of time, becoming key long-living HIV-1 reservoirs contributing to HIV-1 persistence. While HIV-1 infected activated CD4+ T cells display robust viral production, including cell-cycle arrest and cell death, HIV-1 replication kinetics in macrophages is very slow. These delayed kinetics likely allows HIV-infected macrophages to survive, thereby facilitating a long-lived macrophage-derived reservoir. We screened a library of ~2,500 agents including FDA approved, late phase clinical agents, or natural products for their ability to stimulate the suppressed viral replication and production, which can interfere with the long-term survival of HIV-1 infected myeloid reservoirs. Agents with this unique profile can be developed as anti-HIV-1 therapies that can specifically target the long-living myeloid reservoirs for HIV-1 cure.

METHODS: Four donor pooled HIV-negative monocyte-derived-macrophage donors were pre-treated with 10µM library compounds for 2hr, and then transduced with DHIV-3 (HIV-1 single cycle vector system with a GFP reporter). Transduced cells were fixed (4% paraformaldehyde) and stained with DAPI (nuclei) at 48hr post transduction and analyzed by Cytation3 to quantify GFP expressing cells normalized to cell count. Controls were performed in triplicate per plate (negative control; no drug, vector only; positive control viral like particles (VLP)+vector, which increase dNTP substrates for the HIV-1 reverse transcriptase, thereby resulting in GFP expression at 48hr post transduction).

RESULTS: Of >1,000 compounds screened to date, agents demonstrating GFP increase above positive control (VLP) were: 46 demonstrated 2-5 fold increase; 21 demonstrated 5-10 fold increase; 14 demonstrated 10-20 fold increase; 9 demonstrated >20 fold increase without apparent toxicity (no significant reduction in total cell counts).

CONCLUSIONS: For the first time, we have identified safe, potent agents that modulate key events involved in establishment and maintenance of the HIV reservoir in macrophages. Agents will be further evaluated for their ability to modulate key events involved in HIV persistence and barriers to a cure in clinically relevant cells and eventually in humans.

33. The Effects of LSD1 Inhibition on Mouse Retinal Development
Gefke, Isabelle; Ferdous, Salma; and Nickerson, John

The enzyme lysine demethylase 1 (LSD1) has a major role in the development of the retina, aiding in the differentiation of cells and the expression of specific genes. LSD1 has been shown to be overexpressed in many cancers, particularly leukemia. The inhibition of this enzyme has been a promising therapeutic agent for these cancers to reduce tumor growth. Before LSD1 inhibitors are tested for diseases or cancers in the eye, it is important to learn the effects inhibition can have while the retina is developing. Wildtype mice were intraperitoneally injected with the drug tranylcypromine, an LSD1 inhibitor, while the retina is still being developed. Retinal changes were analyzed through in vivo imaging, immunohistochemistry, ERG analysis, and mRNA expression changes. From these analyses, it is expected that LSD1 inhibitors will stop the differentiation of rod photoreceptors, which will significantly decrease vision, but cone photoreceptor differentiation will be enhanced, and H3K4me2 and H3K9me2 will have unchanged levels. The retina will become thinner, and microglia will be present within the layers of the fundus. All of these changes will lead to many visual problems within the pups after LSD1 has been inhibited, that could lead to complete
blindness. This study can help further our knowledge of the LSD1’s role in the eye and will lead to testing the effect of LSD1 inhibitors on mice with retinoblastoma.

34. Urinary Biomarkers - CXCL9 and CXCL10 for Non-invasive, Early Diagnosis of Rejection in Pediatric Renal Transplantation
George, Roshan; Hanberry, Bradley; Dave, Ishaan; Lee, Brian; Sun, Elizabeth; Garro, Rouba; McCracken, Courtney; and Warshaw, Barry

BACKGROUND: Renal transplantation is the treatment of choice for End Stage Renal Disease, but requires strict medication adherence and lifelong surveillance for rejection, infection and allograft dysfunction. Renal biopsy remains the gold standard for diagnosing allograft injury but its invasive nature is challenging, especially in children. A compelling need hence exists, for validating non-invasive biomarkers, to detect subclinical rejection early, and allow safe modification of medications based on immunologic risk, to not only prevent rejection, but also reduce excessive medication burden or side effects. Urinary CXCL9 and CXCL10 are two such promising biomarkers, but prospective trials validating their clinical use, especially in children are limited.

METHODS: We performed a cross-sectional analysis of 46 urine samples obtained from 36 pediatric renal transplant recipients, who had kidney biopsies (for-cause or protocol) done at the same time. Urine was analyzed using a solid-phase bead-array assay for the interferon gamma-induced chemokines CXCL9 and CXCL10, in triplicate, using the commercially available CXCL9 and CXCL10 DuoSet ELISA (R&D Systems, Minneapolis, MN) as per manufacturer’s instructions. Urinary chemokine levels were matched to pathologic findings in renal biopsies, classified as acute rejection- borderline or more severe histologic findings (AR), BK nephritis (BKN), or interstitial fibrosis, tubular atrophy (IFTA).

RESULTS: In children experiencing AR, there was an elevation of both CXCL9 (p=0.041) and CXCL10 (p=0.024) but not in stable allograft recipients, or recipients with IFTA. Only 1 patient had biopsy proven BKN and showed increase in both biomarkers, as compared to patients with stable allograft. Using cut-point analysis for AR, BK viremia and donor specific antibody, we determined values for CXCL9 and CXCL10 to identify patients with allograft inflammation with 81% sensitivity and 84% negative predictive value.

CONCLUSIONS: These data show that urine chemokine monitoring, can help non-invasively screen and identify pediatric renal transplant recipients with renal allograft inflammation. In the future, we plan to use CXCL9 and CXCL10 cut-points as well as donor-recipient epitope mismatch, to stratify patients into immune-quiessent or immune-active groups to predict outcomes and either lower medication burden or escalate monitoring and/or therapy.

ACKNOWLEDGEMENT: Pediatric Research Alliance for grant support

35. Deep Phenotyping of Synovial Fluid from Patients with Juvenile Idiopathic Arthritis
Giacalone, Vincent; Gergely, Talia; Dobosh, Brian; Ponder, Lori; Cammarata, Alexandre; Vega-Fernandez, Patricia; Prahalad, Sampath; and Tirouvanziam, Rabindra
RATIONALE: Juvenile idiopathic arthritis (JIA) is an inflammatory autoimmune disorder affecting 1 in 1,000 children in the USA. JIA pathogenesis is driven by abnormal innate and adaptive immune responses and dysfunction of the joint tissue that together lead to the accumulation of synovial fluid and eventual destruction of the joint tissue. We hypothesize that deep phenotyping of synovial fluid will yield new targetable mechanisms of inflammation in JIA patients. To test this hypothesis, we recently initiated the Emory Multi Omics JIA Immunology (EMOJI) project that aims to enroll 30 JIA patients through Children’s Healthcare of Atlanta over the next two years. In preliminary studies here, we report successful proof-of-concept data for our deep phenotyping approach.

METHODS: Patients with JIA between the ages of 3 and 16 are consented and enrolled for collection of synovial fluid from the knee and venous blood, which are stored on ice for transport and immediate processing. Platelet-free plasma is obtained from whole blood and debris-free supernatant from synovial fluid by dual centrifugation, then banked for later analysis of free and extracellular vesicle (EV)–bound inflammatory mediators and enzymes, and metabolomics. Blood and synovial fluid leukocytes are directly analyzed by multi-color flow cytometry and separated/banked for later transcriptomic analysis and live cell culture.

RESULTS: In the preliminary studies conducted from February 2019 onwards, we enrolled three patients, all female, aged between 11 and 16 years. Of the seven described subtypes of JIA, our study currently includes one patient with oligoarthritis, one with rheumatoid factor negative polyarthritis, and one with psoriatic arthritis. All three patients underwent successful sample collection and processing for banking and direct analysis by flow cytometry. With the latter, we identified a large presence of leukocytes in the synovial fluid composed mostly of macrophages and lymphocytes, along with a smaller neutrophil population. We also achieved comparison of surface protein expression on leukocytes from synovial fluid vs. comparable populations in blood. Our preliminary findings offer valuable insights for analysis of future patients, including more in-depth characterization of the leukocyte populations identified.

36. Exhaled or Salivary Biomarkers? Results from an Air Pollution and Physical Activity Study in Adolescents

Greenwald, Roby; Finlon, Elizabeth; Hayat, Matthew; Jones, Dean P.; Panuwet, Parinya; Kartavenka, Kostya; and Brown, Lou Ann

The Study of Air Pollution and Physical Activity examined biomarkers in exhaled breath and saliva in adolescents participating in extracurricular sports. This study was conducted at high schools and playing fields in the Atlanta metropolitan area and included sites both near and far from major sources of air pollution. Study subjects wore biomonitoring devices to measure heart rate, breathing rate, and accelerometry, and lung function measurements were performed at the beginning and end of each approximately 2-hour exposure period using portable spirometers. Exhaled biomarkers included expired nitric oxide (eNO) and malondialdehyde (a marker of lipid peroxidation) and glutathione (both oxidized and reduced) measured in samples of exhaled breath condensate. In addition, a subset of subjects also produced saliva samples for the untargeted measurement of metabolomic profile. A time-resolved estimate of the inhaled dose of air pollution was calculated based on continuous air pollutant concentration and minute ventilation estimated from heart rate, breathing rate, and forced vital capacity measurements. We observed significant positive associations between eNO and both exposure to and inhaled dose for particulate black carbon; however, this was not conclusive for other pollutants including ozone. We similarly observed suggestive but not statistically significant associations for black carbon and
malondialdehyde; however, malondialdehyde was strongly associated with the previous day’s PM2.5 levels. Oxidized glutathione was negatively associated with the heat index during exposure, but was not associated with any pollutants. For salivary metabolomics data, we did not observe consistent meaningful results. Many metabolomic features were strongly associated with air pollution exposure metrics after performing multiple comparisons corrections, and many were also strongly associated with heat index, previous day’s PM2.5 levels, and physical activity intensity (from accelerometry). However, pathway analysis was largely consistent with the null hypothesis. This finding perhaps reflects the timing of sample collection immediately following the exposure period. Exhaled biomarkers reflecting airway chemistry might be expected to respond within this time frame to acute exposures, but salivary biomarkers reflecting systemic processes may be more responsive to exposures hours or more in the past.

Guerra, Karen; Kaiser, Eileen; Costo, Megan; and Stapel-Wax, Jennifer

One of the fastest growing autism spectrum disorder (ASD) populations in the United States is the Latino population (Baio et al, 2018), yet studies show that Latino children have less access, lower levels of utilization and worse quality of services when compared with White children (Liptak et al. 2008; Parish et al. 2012). A study examining Latino parents’ perspectives to barriers to diagnosis listed 3 categories as barriers-community knowledge and perception of ASD, parent and family factors and health care system barriers (Zuckerman et al. 2013b). Magaña et al. (2013) found that a lack of information in the community was an important factor that created a barrier to service use. As part of a funded research study, primary care practices who serve Spanish-speaking families used an online screening tool to screen their patients ages 9 to 20 months. After completing the screening, some families enrolled in a web-based portal giving them access to online resources in their primary language. Children with a positive result for autism on the screening were invited to participate in a no-cost diagnostic evaluation. In addition, a support group for families conducted in Spanish was offered at the center where the evaluations are conducted. This led to a funded study in Spanish on social communication for children with ASD. The objective is to provide an exemplar pathway for Spanish-speaking families to support the learning needs of young children with autism, looking at how the quantitative and qualitative impact the pathway. To date 333 families have completed the online screening in Spanish and 132 enrolled in the resource portal. Thirty of the children screened received a positive outcome for ASD. Eight to 14 Spanish speaking caregivers attend a support group. In addition, 6-8 Latino caregivers enrolled in a study on social communication for their children with ASD and at least 4 came in through a community based screener. Offering screening, evaluation and support services in the primary language could lead to narrowing the diagnostic gap for Latino children with ASD, increasing Latino parental engagement, improving understanding of ASD, and more focus on seeking EI services.

38. Missed Opportunities for Early HIV Diagnosis in the Pediatric Emergency Department
Gutman, Colleen; Morris, Claudia; Camacho-Gonzalez, Andres; Zmitrovich, April; Belay, Zena; and Middlebrooks, Lauren

CDC recommends universal HIV screening over age 13, which has been successfully implemented in adult healthcare settings. Although adolescents are thought to account for most undiagnosed HIV in the US, most pediatric providers perform targeted testing for HIV in high-risk patients only.
To 1) identify young adults (<25 years) diagnosed with HIV through adult-ED-based opt-out screening and 2) describe all visits made by these individuals to the pediatric ED (PED) in the 10-years before diagnosis.

Retrospective chart review of young adults diagnosed with HIV through adult ED-based opt-out screening (2013-17). Patients were identified through laboratory results and included if they had a HIV+ screen; patients with indeterminate screens were excluded, as were patients who had received treatment for known HIV. CD4 count at diagnosis was used to determine the potential window of infection before diagnosis. Records of PED visits in the 10-years prior to HIV+ were reviewed.

There were 193 included patients (median age 22 yrs, 90% male). Of the 132 patients with available CD4, 20% had stage-3 HIV on presentation. Thirty-eight patients had a PED visit in the preceding 10 years (median 5 years between most recent PED visit and HIV+), for a total of 109 PED visits. The most common reasons for PED visit were injury (22%) and non-GU/GYN infectious symptoms (18%). Sexual history was documented in 12% of PED visits; an STI test was sent in 6%. Two patients had HIV testing in the PED and one was positive; the patient was referred for outpatient care and did not follow-up. Ten patients had 26 PED visits during the potential window of HIV infection; sexual history was documented in 7 of these visits and STI testing was sent in 2, including the one HIV+ test.

Young adults who screen positive for HIV through adult-ED-based opt-out screening often present with late-stage HIV. Many of these patients have had visits in the PED, where sexual history is infrequently documented and HIV testing is rare. Adolescents with asymptomatic HIV will likely go unrecognized in pediatric healthcare visits; implementing CDC-recommended universal screening in the PED may save lives with earlier diagnosis.

39. Immunogenicity of Novel RSV M-based Virus-Like Particles
Ha, Binh; Jadhao, Samadhan; Ke, Zunlong; Yang, Jae; Wright, Elizabeth; and Anderson, Larry J.

RSV is the leading cause of lower respiratory tract infection inducing bronchiolitis and pneumonia in infants. Despite decades of research, a vaccine for RSV is still not available. Virus-like particles (VLPs) are good candidates for vaccines since they are immunogenic and have been used for licensed human vaccines. We have been interested in looking at RSV F and G interactions using VLPs as one system. For these studies, we felt that an RSV platform versus another viral protein was preferable. We developed two systems with stably transfected 293F cells expressing RSV M, P, F, G proteins and M, M2-1, F and G proteins. Sucrose gradient ultracentrifugation of supernatants from the induced cell lines and Western blot results suggested F+G VLPs in both systems, i.e. similar relative intensity for F, G, and M bands, observed in some fractions. Electron microscopic imaging showed VLP-like structures with surface projects consistent with the F protein on VLPs. Preliminary studies in a mouse model showed these VLPs were immunogenic with antibodies against the F and G proteins detected in sera of immunized animals. The successful generation of RSV platform based VLPs adds to the repertoire of potential candidate vaccines for RSV and provides tool to study F-G glycoproteins interactions.

40. Soluble PD1 as a Biomarker in Pediatric Immune Mediated Liver Disease
Hadley, Timothy; Espinoza, Hillary; Kolachala, Vasantha; Chandrakasan, Shanmuganathan; and Gupta, Nitika
BACKGROUND: Immune mediated liver disease entails a broad category of disease entities that result in increased morbidity and mortality amongst the pediatric population. Programmed death 1 (PD1) is an inhibitory receptor mainly expressed by T cells and with its ligands, PD-L1 and PD-L2 present on antigen presenting cells. Soluble PD1 (sPD1) is known to be shedded from activated T Cells and released into plasma. Autoimmune Hepatitis (AIH) is a chronic liver disease caused by impaired immune regulation while Primary Sclerosing Cholangitis (PSC) is considered a chronic, complex idiopathic disorder where inflammation and fibrosis leads to multifocal biliary strictures with causality potentially being immune mediated. The AIM of this study is to determine if the levels of sPD1 in plasma are different amongst AIH, PSC and AIH/PSC.

METHODS: We collected plasma samples from 36 pediatric patients. In regards to the AIH patients they were further categorized (Active, Incomplete Responders and Responders) based on response to accepted standardized therapy. These were compared with PSC and Overlap (PSC/AIH) patients. All samples were analyzed by Human PD1 ELISA Kit from R&D Systems.

RESULTS: In the AIH group, those with Active disease demonstrated a significantly higher sPD1 level in comparison to responders (8881±1119 versus 573±165.2, pg/ml, *p>0.0001). However, the Non responders didn’t display a reduction in sPD1 in comparison to Active AIH. Interestingly, patients with PSC showed a significantly lower level of sPD1 as compared to Active AIH (2363±1880 vs 8881±1119, *p<0.02). In addition, patients with PSC in conjunction with AIH (overlap syndrome) demonstrated a significant increase in sPD1 (9205±1795 vs 2363±1880, *p<0.01).

CONCLUSION: Our study shows that sPD1 is a valuable biomarker to distinguish amongst active AIH in the pediatric population which may suggest an impaired PD1 axis while PSC doesn’t appear to be mediated through the PD1 axis.

41. Exploring the Role of NUP214 in Mediating the Interaction Between CRM1 and the HOXA Gene Locus

Harrington, Amanda; Aumann, Waitman; Lavau, Catherine; Tope, Robert; and Wechsler, Daniel

BACKGROUND: Chromosomal translocations resulting from fusion of CALM and AF10 genes are recurrent abnormalities in 5-10% of T-cell acute lymphoblastic leukemias. These leukemias display elevated HOXA gene expression, although the mechanism by which CALM-AF10 transactivates the HOXA locus is unclear. CALM contains a Nuclear Export Signal (NES) that is required for CALM-AF10-mediated leukemogenesis. The NES mediates the interaction with the nuclear export receptor protein CRM1, which typically functions to translocate proteins from the nucleus to the cytoplasm through the nuclear pore (NUP). We have shown CRM1 can substitute for CALM – a CRM1-AF10 fusion protein is leukemogenic in vitro and in vivo. In traversing the nuclear pore, CRM1 interacts with NUP components, including NUP214. Additionally, NUP214 is involved in leukemogenic chromosomal translocations that also cause increased HOXA gene expression. Together, these observations strongly suggest that NUP214 may mediate the ability of CRM1-AF10 to activate HOXA gene expression.

OBJECTIVE: To investigate NUP214 as a candidate protein that mediates the interaction between CRM1 and HOXA genes.
METHODS: Using a CRM1-AF10 fusion construct in which the CRM1 NUP214 binding sites have been mutated to impair binding (CRM1NUP-AF10), we performed HOXA7-luciferase reporter and methylcellulose colony assays, and carried out in vivo mouse transplantation.

RESULTS: Both CRM1-AF10 and CRM1NUP-AF10 activate the HOXA7-Luciferase reporter assay. However, CRM1NUP-AF10 was unable to transform hematopoietic stem cell precursors in in vitro colony-forming assays, and mice transplanted with CRM1NUP-AF10 did not develop leukemia.

DISCUSSION: Investigating how CRM1 interacts with the HOXA locus will further elucidate a role for CRM1 as a transcriptional activator of leukemogenic HOXA genes. We have demonstrated the importance of interaction between CRM1 and NUP214 by demonstrating abrogated leukemia development in mice transplanted with a mutated CRM1-AF10 fusion wherein NUP214 can no longer bind to CRM1. We plan to synthesize a NUP214-AF10 fusion protein to evaluate the potential for NUP214-AF10 induced leukemogenesis, to further investigate this mechanism. Our finding that mutation of NUP214 binding sites on CRM1 interferes with leukemia development warrants further exploration of NUPs as candidate proteins for HOXA gene activation, and may establish a CRM1/NUP interaction as a novel therapeutic target.

42. Serum Lipidome Alterations in a Rodent Model of Mild Traumatic Brain Injury
Hogan, Scott; Milligan, Kyle; LaPlaca, Michelle; and Fernández, Facundo M.

Traumatic brain injury (TBI) is initiated by mechanical loading to the brain that alters neurological function. An estimated 70-90% of all TBIs are classified as mild (mTBI) with 250,000 children (under 19) per year treated in US emergency departments alone. mTBI diagnosis is challenging, especially in the pediatric population, due to inherent heterogeneity, as well as the reliance on subjective self-reported symptoms. mTBI-specific blood-based biomarkers may aid in refining diagnosis by providing objective information about changing metabolites. Here, using a preclinical rodent model and untargeted metabolomics, we report on the metabolites and lipids that are most altered acutely following a closed-head mTBI. Thirty-eight rats (male Sprague Dawley, 300-400 g; mTBI, n=30 and sham, n=8) were anesthetized and injured using an air driven pneumatic piston (silicone tip diameter=1 cm, velocity=3 m/s, duration=200 ms, head displacement=5 mm). Employing a matched pair study design, a baseline serum sample was taken, followed by 24 hour post-mTBI sample (or time-matched sham). A Waters Xevo G2 QTOF mass spectrometer with an electrospray ionization source operated in both positive and negative ionization modes was used. A total of 660 and 269 species were reliably detected (CV<20%) in positive and negative ion modes, respectively. Following Bonferroni correction to account for multiple comparisons, 33 features exhibited significant changes between mTBI and control groups. Initial principal component analysis (PCA) show trends toward separation of mTBI and control samples, but an accurate classification required further feature selection to isolate differences between classes. An interval partial least squares discriminant analysis (iPLS-DA) algorithm was utilized to select 13 features for binary classification capable of differentiating mTBI samples from controls with nearly 88% accuracy (92% sensitivity, 84% specificity). This panel contained a variety of phospholipids and triacylglycerol species. Exact identifications of these lipids are currently being pursued using MS/MS data matched to isotopically labeled standards. As with our previous study in moderate TBI, cholesterol sulfate (m/z=465.304) was significantly decreased across mTBI samples (p<5.0x10-9). These results indicate that serum lipidomic changes may be a sensitive tool for diagnosis in the acute period following mTBI.
43. Enzyme Linked Immunosorbent Assays to Detect and Quantify Antibodies to Respiratory Syncytial Virus Antigens in Human

Jadhao, Samadhan; Ha, Binh; McCracken, Courtney; Hartert, Tina V.; and Anderson, Larry J.

Respiratory syncytial virus (RSV) infection is a major cause of serious lower respiratory tract infections in infants and young children and causes repeat infections throughout life with persons with compromising cardiac, pulmonary or immune conditions and the elderly at greatest risk for serious disease. Antibody assays provide a means to determine which young children had infection with RSV, detect current infection if acute and convalescent phase serum specimens are available, and understand immune response to infection and immune correlates of protection. Enzyme linked immunosorbent assays (ELISA) to detect and quantify antibodies to RSV surface proteins have often been included in measures of the immune response to RSV with the two surface proteins F and G that induce neutralizing antibodies being especially important. We developed four ELISAs, one with the lysate of pooled RSV antigenic group A and B infected cells (lysate), recombinant expressed F protein (F), recombinant expressed group A G protein (Ga), and recombinant expressed group B G protein (Gb). With these assays, we detected antibodies and estimated titer in plasma specimens (1:200 dilution) from young children exposed to one RSV season, with known RSV infection, i.e. RT-PCR positive respiratory illness, or unknown infection status. The RSV lysate and F protein ELISAs detected antibodies in most (99%) of the 108 children with confirmed RSV infections while the Ga protein ELISA detected 81 (75%) and the Gb protein ELISA 43 (39%). In 96 children with unknown infection status 49% were positive by the lysate and F protein assays and 12 (13%) by the Ga protein ELSIA and 16 (17%) by the Gb protein ELISA. The lysate and F protein ELISAs were most sensitive and were 100% congruent. The Ga and Gb ELISAs were less sensitive but were able to distinguish between group A or group B primary infection. Serum neutralization assays using selected known RSV antibody positive and negative children plasma specimens will be done to establish the sensitivity and specificity of these ELISA assays. These assays provide a reliable way to detect past RSV infection and estimate the antibody titer.

44. Biofabrication of "Smart" Microcapsules for Treatment of Infantile Parkinsonism Dystonia

Joshi, Devyani; Chitre, Neha; D'Souza, Cherilyn; Murnane, Kevin; and D'Souza, Martin

INTRODUCTION: Infantile Parkinsonism-dystonia, also known as dopamine transporter deficiency syndrome (DTDS) is the result of the inborn errors of metabolism affecting the dopamine biosynthetic pathway. It is the inherited neurological syndrome that presents in early infancy with hypokinetic parkinsonism and dystonia that can be fatal. PC12 is a cell line derived from pheochromocytoma of the rat adrenal medulla. These cells upon exposure to the nerve growth factor extend neurites and acquire the morphology of the neurons, which then store and secrete a neurotransmitter dopamine.

METHOD: The PC12 cells were cultured in the complete Dulbecco’s Modified Eagle's Medium. For fabrication of the microcapsules, the cells were suspended in the trehalose – alginate solution and sprayed through 1.40mm nozzle using the buchi mini spray dryer into the calcium chloride solution. The microcapsules were coated with the chitosan glutamate solution and stored in the media. FTIR spectroscopy of the microcapsules was performed to confirm the crosslinking of alginate by calcium chloride. The viability of the microencapsulated PC12 cells was examined using fluorescent live dead cell staining and with trypan blue using the automated cell counting device. The rat model of the disease was developed using 6-Hydroxydopamine and the development was confirmed using various behavioral studies and neurochemical analysis.
RESULTS: The microcapsules were spherical in shape with no deformities. The average size of the microcapsules was 250µm. The FTIR spectra confirmed crosslinking of the alginate microcapsules with calcium chloride. Crosslinking promotes an increase in the polymer intermolecular hydrogen bonding and disappearance of the peak of hydroxyl group indicates the poor affinity for water. Results of the live/dead cell staining revealed that the cells were viable inside the microcapsules.

CONCLUSION: Microencapsulation is known to protect the transplanted cells from the host immune response. In this study, we fabricated the microcapsules using the simplistic device equipped with the specialized nozzle that allows manipulation of the microcapsule size. The microcapsules produced were reproducible, scalable and thus can be made in large quantity with the narrow size distribution. The microencapsulated cells produce a neurotransmitter dopamine and thus can be used as a long-term treatment for Infantile Parkinsonism-dystonia.

45. Revisiting A “Wait and See” Mindset to Improve Early Intervention Referrals
Kaiser, Eileen; Costo, Megan; Guerra, Karen; Edwards, Nicole; and Stapel-Wax, Jennifer

Advances in early screening and diagnosis make it feasible for experienced clinicians trained on validated tools to diagnose ASD by 18-24 months of age (Guthrie, Swineford, Nottke, & Wetherby, 2013). In spite of this, the median age for ASD diagnosis in the US is 4-5 years (Baio et al 2018), far beyond the window of opportunity for early intervention (EI). There is often a delay between suspecting concerns and receiving services (Harrison & Roush, 1996). Many believe children will outgrow concerns (Wall et al., 2005). Building on the literature, a quasi-experimental design is used for a study of Primary Care Providers’ (PCP) and parents’ views on a ‘wait and see’ mindset.

The objective is to determine whether and why some families and PCPs decide to wait before referring children with signs of delay for EI and to gather suggestions for increasing the willingness of PCPs and families to refer early.

Parents of children with autism were sent an online survey and asked to participate in a phone interview. PCPs in a metro area were sent an online survey to complete. Survey questions focus on the extent to which PCPs and families are using a ‘wait and see’ approach for developmental concerns and knowledge of/attitude towards EI.

Preliminary results for the Family Survey indicate that a majority of the respondents had concerns about delay prior to their child turning 2. A majority relied on their PCP to make the referral and believed their child would outgrow the problem. A large number of respondents on the Primary Care survey shared the view that families were more likely than PCPs to have a ‘Wait and See’ mindset. We are continuing to collect data from the surveys and interviews.

Family responses indicated that they might have sought a referral earlier if they had better understood the signs they saw were indicative of autism or a developmental delay and that getting help early could help their child gain important skills. PCP responses indicated a need for more training in early recognition of signs of autism and more time for developmental screening.
46. Innovative Nanoparticulate Formulation for Brain Targeting
Kale, Akanksha; Zaman, Rokon Uz; Braz Gomes, Keegan; Oppong Damoah, Aboagyewaah; D'Souza, Nigel; D'Souza, Cherilyn; Murnane, Kevin; and D'Souza, Martin

INTRODUCTION: The Blood Brain Barrier (BBB) is a major impediment in the delivery of drugs for the treatment of neurodegenerative disorders. Endogenous biological mechanisms can be utilized to cross BBB for delivering drugs and therapeutic peptides to the brain. Conjugation of nanoparticles with brain targeting ligands such as Transferrin (Tf) or Rabies Virus Glycoprotein (RVG) can enhance their delivery to the brain. In this study, we develop oxytocin loaded Tf/RVG conjugated polylactide-co-glycolic acid (PLGA) nanoparticles and bovine serum albumin (BSA) nanoparticles that can cross BBB.

METHODS: Oxytocin loaded PLGA and BSA nanoparticles were formulated by multiple emulsion solvent evaporation method and nanoprecipitation method followed by lyophilization. Terminal carboxylic acid groups were activated by 1-ethyl-3-(3-dimethylaminopropyl) carbodimide (EDC) and N-hydroxysulfosuccinimide (NHS) before conjugation with Tf or RVG. Particles were characterized in vitro for size, zeta potential, encapsulation efficiency, and release profile. Oxytocin specific ELISA kit was used to detect the amount of oxytocin released. Potential immunogenicity of particles was assessed by measuring nitric oxide released by dendritic cells in the presence of nanoparticles. Cytotoxicity of nanoparticles was assessed by MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. To determine brain penetrance, Tf conjugated indocyanin green loaded nanoparticles were administered to mice intraperitoneally. Unconjugated indocyanin green loaded nanoparticles and indocyanin green solution were used as controls.

RESULTS: Mean diameter of PLGA and BSA nanoparticles ranged between 197.7-278.3 nm and 100.1-197 nm respectively. Both particles were negatively charged with zeta potential ranging between -11.9 to -19.6 mV (PLGA) and -15.4 to -22.8 mV (BSA). No particles were immunogenic or cytotoxic. The encapsulation efficiency was ≥ 75% for all nanoparticles. Release studies demonstrated that BSA nanoparticles exhibited a faster initial burst release compared to PLGA particles, in addition to later sustained release. Bio-imaging data revealed that nanoparticles crossed the BBB within 30 minutes of administration.

CONCLUSION: In this study, oxytocin loaded Tf or RVG conjugated PLGA and BSA particles were successfully formulated. It was found that they were non-immunogenic, non-cytotoxic and showed sustained release of oxytocin.

TRANSLATIONAL RELEVANCE: This approach can be utilized to cross BBB for delivery of drugs and therapeutic peptides to the brain.

47. Elucidating the Mechanism of JAGGED1-Mediated Osteoblast Commitment During Maxillary Development
Kamalakar, Archana; Oh, Melissa; Ballestas-Naissir, Samir; Amanso, Angelica; Davis, Michael; Willett, Nick; Drissi, Hicham; and Goudy, Steven

Maxillary bone deficiency (MBD) results from changes in cell signaling mechanisms during maxillary bone development, trauma or surgery. While in adults, bone morphogenetic protein (BMP2) is commonly used to ameliorate MBD, in pediatric cases, it leads to significant pain, erythema and inflammation. Therapies
based on stimulation of bone formation without triggering painful inflammation in children with MBD are lacking. JAGGED1 (JAG1), a membrane-bound NOTCH ligand, is required for normal craniofacial development, and Jagged1 mutations in humans are known to cause maxillary bone hypoplasia and Alagille Syndrome, associated with bone demineralization and fractures. We previously demonstrated deficient maxillary osteo- and vasculo- genesis in Wnt1-cre;Jagged1f/f (Jag-1CKO) mice by conditional deletion of Jagged1 in maxillary mesenchymal stem cells. We hypothesize that a JAG1-NOTCH1 pathway is essential for maxillary development and for differentiation of neural crest cells (osteoblast precursors) during intramembranous ossification. In this study, we investigated the JAG1 signaling pathways in a cranial neural crest cell line, O9-1. Treatment with JAG1 induced an increase in expression of osteoblast differentiation and maturation markers, Runx2 and Ocn, respectively, ALP production, as well as classic NOTCH1 targets, Hes1 and Hey1. While JAG1-induced Hes1 and Hey1 expression levels were predictably decreased after DAPT treatment, JAG1-induced Runx2 and Ocn levels were surprisingly constant in the presence of DAPT, indicating that the JAG1 effects in the CNC cells are independent of the canonical NOTCH pathway. JAG1 treatment of CNC cells increased JAK2 phosphorylation, which was refractory to DAPT treatment, highlighting the importance of the non-canonical NOTCH pathway during CNC cells osteoblast commitment. Pharmacologic inhibition of JAK2 phosphorylation, with and without DAPT treatment, upon JAG1 induction reduced ALP production, Runx2, and Ocn gene expression. Collectively, these data suggest that JAK2 is an essential component downstream of a non-canonical JAG1-NOTCH1 pathway through which JAG1 stimulates expression of osteoblast-specific gene targets that contribute to osteoblast differentiation and bone mineralization in maxillary development. Currently, there are no FDA approved therapies for JAG1-associated bone loss but our identification of JAG1’s mode of action and its ability to independently induce bone formation could change the paradigm of treatment for maxillary hypoplasia in Alagille Syndrome patients.

48. Identifying Novel CRM1-Interacting Proteins in CALM-AF10 Leukemogenesis by Proximity Based Labeling

Kazi, Rafi; Aumann, Waitman; Tope, Donald; and Wechsler, Daniel

BACKGROUND: While progress has been made in the treatment of pediatric leukemias, certain leukemias still have a poor prognosis. CALM-AF10 leukemias, which account for ~10% of childhood T-cell Acute Lymphoblastic Leukemia (T-ALL) and a subset of Acute Myeloid Leukemia (AML), are particularly difficult to treat. Our laboratory discovered that the CALM protein contains a nuclear export signal (NES) that is critical for CALM-AF10-mediated leukemogenesis. The NES interacts with the CRM1-nuclear export receptor, and we have determined that CRM1 is essential for transcriptional activation of HOXA genes by CALM-AF10. How CRM1 interacts with HOXA genes is poorly understood, since CRM1 does not contain a recognized DNA binding domain. To identify proteins that mediate the interaction between CRM1 and DNA, we take advantage of a proximity-based labeling approach using BioID2, a biotin ligase, fused to CALM-AF10. This permits biotinylation of nearby proteins which may be identified using mass spectrometry.

OBJECTIVE: Identify novel proteins that interact with CRM1.

DESIGN/METHODS: We created plasmids in which BioID2 is fused to HA-tagged CALM-AF10 and a CALM-AF10 mutant unable to bind CRM1, (CALM(NES*)-AF10). To ensure that BioID2 does not stericly interfere with CALM-AF10 and inhibit its activity, we used luciferase reporter and colony forming assays, and confocal microscopy. Human Embryonic Kidney 293 (HEK293) cells were transiently transfected with
expression plasmids, followed by incubation with biotin. Purified biotinylated proteins will be analyzed using mass spectrometry.

RESULTS: CALM-AF10 and CALM(NES*)-AF10 fusion plasmids containing BioID2 were synthesized and confirmed via Sanger sequencing. Western blot demonstrated expression of fusion proteins containing BioID2. Similar to CALM-AF10, BioID2-CALM-AF10 activates the HOXA7 luciferase reporter 6-7-fold versus empty vector, transforms hematopoietic progenitor cells (HPCs) upon serial replating, and localizes primarily to the cytoplasm. Expression of biotinylated proteins in transfected HEK293 cells following biotin exposure was confirmed by Western Blot. Purified biotinylated protein samples have been submitted for analysis.

CONCLUSIONS: The presence of the BioID2 moiety does not interfere with CALM-AF10 activity. Furthermore, the BioID2 ligase is active when fused to CALM-AF10. Based on these findings, biotinylated protein samples have been submitted to the Mass Spectrometry Core and further results will be presented at the conference.

49. The Effects of Childhood Trauma on Developing Chronic Illness
Killingsworth, Kayla; and Fitch, Vincent

INTRODUCTION: Childhood trauma is an ongoing problem that has debilitating consequences, including the development of physical illness (Felitti et al., 1998). Trauma results in chronic stress, impairing the neuroendocrine and immune systems. This stress often leads to an abundance of cortisol which can have detrimental effects on the body, specifically a higher risk of developing chronic illnesses (Oral et al., 2015). This systematic review will evaluate research on the relationship between childhood trauma and chronic illness.

METHODS: A search was conducted on Galileo using the key terms “chronic illness,” “trauma”, and “child/youth” which resulted in N=891. The literature search was narrowed by using peer-reviewed articles between the years of 1998 and 2018.

RESULTS: A systematic review of the literature found a strong relationship between childhood trauma and chronic illness. Majority of studies found that participants with a history of childhood trauma had a greater cortisol release when under stress compared to a normative group. Overall, the majority of literature found that childhood trauma had a significant influence on the development of chronic illness, and in specific cases, that a type of childhood trauma predicted specific chronic illnesses.

CONCLUSION: This systematic review corroborated previous research that childhood trauma can influence developing chronic illness. Specifically, it found that screening for traumas could help in predicting chronic illnesses. The literature showed that trauma-informed care should be used in primary healthcare to provide physicians with the knowledge on gathering information of childhood trauma. Trauma-informed care focuses on recognizing and responding to trauma (Oral et al., 2015). This could prove very beneficial in the future of preventative medicine.
50. Trauma-Informed Care in Pediatric Healthcare Settings
Killingsworth, Kayla; and Stillman, Mark

INTRODUCTION: Childhood trauma can have many short- and long-term consequences that impact both mental and physical health. Trauma can result in chronic stress which impairs the neuroendocrine and immune systems. This toxic stress often causes a prolonged activation of the HPA axis response which leads to abnormal patterns of cortisol in the body. Along with this, toxic stress can also cause issues with immune responses, gene expression, and neurodevelopment of the brain which may result in poorer physical health. Trauma-informed care focuses on recognizing and responding to trauma and therefore, should be included in healthcare assessments from primary care physicians (PCPs). This systematic review will evaluate the utilization of trauma-informed care in pediatric primary care settings.

METHODS: A literature search was conducted on Galileo using the key terms “trauma-informed care,” “primary care,” “child, youth,” and “physicians, doctor.” Exclusion criteria included full text, scholarly, peer-reviewed articles written in English that were published between 1999 and 2019.

RESULTS: A systematic review of the literature found that PCPs should regularly screen for trauma as this is a crucial first step in identifying children at risk for developing a toxic stress response. PCPs can also be more aware of risk factors and can promote resiliency, both before and after trauma has occurred, as these can work to prevent negative health outcomes. It is important for PCPs to understand how to assess for and handle disclosures of trauma since they can oftentimes be the only medical professional seen on a regular basis. In addition, behavioral health consultants (BHCs) are essential in the process of training and communicating with PCPs about intervening with patients who have a trauma history.

CONCLUSION: The literature showed that trauma-informed care and brief interventions should be regularly employed by PCPs before referrals to psychological services. Additionally, PCPs should be aware of the detrimental health effects that can occur after trauma. This could prove very beneficial in the future of preventative medicine. Future research should focus on brief interventions useful for PCPs to utilize alongside providing trauma-informed care. Incorporating integrative assistance from BHCs within brief interventions will be vital for beneficial implementation.

51. Recovery of Consciousness Following Severe Acquired Brain Injury in Children
Kwong, Hiu Sze; and Blackwell, Laura

BACKGROUND: Limited research exists regarding behavioral evaluation or treatment for pediatric disorders of consciousness (DOC) including coma, vegetative state (VS) and minimally conscious state (MCS) following acquired brain injury. Our study aims to investigate clinical outcomes and predictors of recovery in sample of children with DOC in an inpatient rehabilitation setting.

METHODS: We conducted a retrospective chart review of an inpatient clinical database from 2014 to 2016. We identified 43 patients between 2 and 19 years old (51.16% female, 48.84% male; mean age 11.67±5.29 years) who were admitted to the pediatric rehabilitation unit for DOC. Patients had a range of diagnoses including traumatic brain jury (TBI) (53.49%), anoxic brain injury (20.93%) and others (25.58%). Patients were assessed using the Coma Recovery Scale, Revised (CRS-R). Scores range from 0 to 23, with higher scores indicating higher levels of consciousness. The primary outcome was the emergence from VS or MCS to conscious state during admission.
RESULTS: The mean CRS-R at admission was 7.56 (SD=2.99) while the mean CRS-R at discharge was 11.70 (SD=5.97). Ten (23.25%) patients emerged from MCS during inpatient rehabilitation. Patients in MCS at admission had higher CRS scores at discharge compared to other levels of responsiveness [t(35.88)=5.003, p=0.000]. Those who remained in VS longer had a lower discharge CRS-R score [r=-0.577, n=40, p=0.000]. Patients with a TBI diagnosis were more likely to emerge from MCS compared to those with non-traumatic diagnoses [t(37.866)=-3.037, p=0.004]. Age at the time of injury and gender was not significant predictors of CRS-R score at discharge or emergence.

CONCLUSION: Previous literature on pediatric DOC in an inpatient rehabilitation setting had limited sample sizes. However, our larger cohort allows us to further explore clinical variables associated with emergence to conscious state. Consistent with past studies, our patients demonstrated good recovery during rehabilitation. Among them, patients with TBI had higher CRS-R scores at admission and better prognosis for improvements in consciousness, emphasizing the importance of careful clinical examination as well as serial monitoring during rehabilitation to help impact care strategies.

52. The Obesity Paradox: Adipocyte-secreted Proteins Promote Metabolic Changes, DNA Damage, and Apoptosis in T-Cell Acute Lymphoblastic Leukemia
Lee, Miyoung Lee; Chandler, Joshua D.; and Henry, Curtis J.

T-cell acute lymphoblastic leukemia (T-ALL) accounts for 10-15% of pediatric and 20-25% of adult cases of ALL. The cure rates for patients with T-ALL is between 60-80% for adult and pediatric cases. These numbers further decline in patients with primary resistance and those who relapse from their initial diseases. Given the poor outcomes for patients with T-ALL, there has been growing interest in identifying risk factors that contribute to the pathogenesis of this disease. Recently, obesity (characterized by a body mass index > 30) has been shown to reduce the survival of patients with ALL. However, a closer examination revealed that obese patients with T-ALL exhibit superior survival outcomes.

To address the impact of adiposity on T-ALL function, we exposed human T-ALL cells to adipocyte-secreted factors to determine how metabolism, proliferation, and survival were impacted. Global gene expression profiling of T-ALL exposed to unconditioned media, stromal cell-conditioned media (SCM), and adipocyte conditioned-media (ACM) revealed that ACM exposure significantly suppressed genes involved in the oxidative stress response and those associated with the TCA cycle. Cell cycle analysis of human T-ALL exposed to ACM revealed an accumulation in the sub G1 population, suggesting that the adipocyte-secretome induces significant apoptosis in T-leukemia cells. This hypothesis was confirmed using Annexin-V/ PI analysis in which we also found that ACM-mediated apoptosis of human T-ALL occurred independently of Notch1 and p53 mutational status. Given that Notch1 is mutated in > 60% of T-ALL cases, we decided to further define the impact of ACM on this signaling pathway. Exposing human T-leukemia cells to ACM resulted in a significant downregulation of both surface expressed and global Notch1 protein levels. The decreased Notch1 levels in T-ALL exposed to ACM coincided with increased γ-H2AX activation (indicative of DNA damage).

Our studies reveal a surprising finding that adipocyte-secreted proteins are cytotoxic to human T-leukemia cells. Future studies focused on defining the cytotoxic component of the adipocyte-secretome could be informative in the development of novel, well-tolerated therapeutics to improve the survival outcome of lean patients with T-ALL.
53. Effect of Metabolic Regulation on Maturation of Human Induced Pluripotent Stem Cell-Derived Cardiomyocytes

Li, Dong; Gentillon, Cinsley; Jha, Rajneesh; and Xu, Chunhui

Heart diseases continue to be a major cause of death in developed countries despite the recent advances in medical treatments. Human induced pluripotent stem cell (hiPSC) has emerged as an attractive cell model for studying human heart diseases in vitro and further rendered cardiomyocytes derived from patient-specific hiPSC (hiPSC-CMs) great potentials for therapeutic applications. Several protocols have been established to derive hiPSC-CMs using growth factors, small molecules or matrix proteins; however, the maturity of differentiated hiPSC-CMs are not comparable with the cardiomyocytes in adult hearts, which constrains their therapeutic potentials for treating heart diseases. Numerous approaches have been employed attempting to promote the maturity of hiPSC-CMs including long-term culture, substrate modification, cell alignment improvement, chemical and mechanical stimulation. Here we show that the use of a small molecule for metabolic regulation during three-dimensional cardiac differentiation could promote the maturity of hiPSC-CMs. We treated the hiPSC-CMs with the small molecule starting from day12 of the differentiation for 16 days. Flow cytometry analysis revealed that the small molecule-treated hiPSC-CMs displayed significantly increased mitochondrial membrane potential compared with vehicle-treated hiPSC-CMs. Quantitative real-time PCR revealed a significant increase in mitochondrial DNA (mtDNA): nuclear DNA (nDNA) ratio in the small molecule-treated hiPSC-CMs. In addition, the small molecule significantly upregulated the expression of genes controlling oxidation phosphorylation and mitochondrial fusion, indicating an increased mitochondrial function. Consistent with these observations, the small molecule treatment significantly increased the expression of a subset of genes encoding cardiomyocyte structural proteins, Ca2+ handling proteins and Na+/K+ channels. Furthermore, a subset of genes involved in the metabolic regulation were also upregulated, including adrenergic receptors, glucose transport, fatty acid transport and activation. Our results demonstrate the small molecule is capable of promoting the molecular and structural development of hiPSC-CMs that lead to more maturation. We will further evaluate the effect of the small molecule on functions of hiPSC-CMs and examine the underlying mechanisms. We anticipate our research to provide insights on how the small molecular candidates contribute to the improvement of hiPSC-CMs maturity.

54. Cystic Fibrosis Sub-Microliter Exhaled Breath Condensate Metabolomics via Triboelectric Nanogenerator-Induced Nanospray Mass Spectrometry

Li, Yafeng; Areces, Marcos Bouza; Wu, Changsheng; Huang, Danning; Stecenko, Arlene A.; Wang, Zhong Lin; and Fernández, Facundo M.

Cystic Fibrosis (CF) is the most common life-shortening genetic disease, affecting 1 in 2,500-3,500 white newborns. CF is characterized by periodic pulmonary exacerbations that progressively decrease lung function, and by the development of complications later in life, such as CF-related diabetes, which increase mortality of CF patients. Exhaled breath condensate (EBC) is a biofluid that can be non-invasively collected and provides a wealth of information for studying CF disease progression. This biofluid is highly diluted by exhaled water vapor, and metabolomics approaches typically require at least 1 mL of sample and a 20-fold concentration by lyophilization to yield meaningful metabolic profiles by mass spectrometry (MS). A healthy adult normally requires 15-20 min to collect such quantity of EBC, but this type of collection can be much more challenging, or even impossible, for CF children or infants. To address this problem, we here present the first sub-microliter metabolomics platform based on Triboelectric Nanogenerator-induced (TENGi) nanospray MS. It not only requires sub-microliter sample volumes for
analysis, but it also has very low detection limits, yielding high quality spectra with only a few MS scans. As a proof-of-concept, EBC from CF patients with prediabetes was collected prior to, and following an oral glucose tolerance test, and studied successfully with as little as 0.8 µL per test using TENGi MS. Metabolite profile differences observed in EBC shed additional light on the mechanisms of CF-related diabetes onset. These results prompt us to believe that TENGi MS will likely enable CF metabolomics studies in infants or children that have so far remained elusive by standard MS approaches.

55. Stat3 Inhibitor WP1066 Inhibits Medulloblastoma Growth In Vivo
Liu, Jingbo; Malhotra, Anshu; Park, Jaekeun; and MacDonald, Tobey

Medulloblastoma is the most common pediatric malignancy of the central nervous system. Transgenic mouse models of medulloblastoma (MB) have enabled investigators to identify molecular targets for designing new therapies. In the present study we investigated the effect of WP1066, a small molecule inhibitor of Stat3, on MB growth in vivo using two different murine models of sonic hedgehog (Shh) type MB (Math-Cre-ER-PTC flox/flox and SmoA1). To initiate tumor formation in the Math-Cre-ER-PTC flox/flox mouse strain, pregnant females were induced with Tamoxifen by oral gavage at E17. The pups born were subsequently scanned by MRI at 5-6 weeks of age (n=10) to confirm tumor formation and measure volumetric size. SmoA1 mice (n=10) were also evaluated for tumor formation by MRI scanning at 10-12 weeks old. The tumor bearing mice within each strain were divided into two groups, which received either WP1066 or vehicle control, by I.P. injections every other day for 3 weeks. At the end of treatment, all mice had tumor volume measured by MRI, and were euthanized immediately thereafter. Immunohistochemistry of tumor tissue was performed for p-Stat3, total-Stat3, cleaved caspase 3 (CC3), and immune cell markers, Iba1, CD8 and CD56. Our results show that WP1066 treatment significantly reduces MB growth in vivo. Additionally, relative to control treated tumors, p-Stat3 levels were significantly decreased while CC3, Iba1 and CD8 levels were increased in the WP1066 treatment group. In conclusion, we show that the Stat3 inhibitor, WP 1066 is able to target MB in vivo and inhibits tumor growth. WP1066 treatment inhibits MB Stat3 phosphorylation, induces tumor cell apoptosis while enhancing the anti-tumor immune response by recruiting CD8 T cells and NK cells. The results provide critical evidence to support the translation of WP1066 for investigative clinical trials for refractory MB.

56. Developing a High-Throughput Method to Detect the Viability of Cardiomyocytes Derived from Human Induced Pluripotent Stem Cells
Liu Rui; Saraf, Anita; Du, Yuhong; Fu, Haian; and Xu, Chunhui

Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) have the potential to complement and replace animal cell equivalents and to be applied in many applications including drug development, disease modeling, developmental biology, personalized medicine, and regenerative medicine. Implementation of high-throughput cardiotoxicity screening will facilitate these applications. For example, cell viability is a basic index of cardiotoxicity and establishing a high-throughput method is critical to test cell viability of hiPSC-CMs in response to drug treatments. We found that cell viability of hiPSC-CMs could be measured effectively using the CellTiter-Blue® Cell Viability Assay in a high-throughput manner. The assay is based on the ability of live cells to convert a reduct dye (resazurin) into a fluorescent end product (resorufin). This assay has several advantages, including easy-handling, one step procedure, no toxicity to cells, and simple-recording of fluorescence readout. To determine if this assay is reliable and sensitive for detecting cell viability of hiPSC-CMs, we used hiPSC-CMs generated by growth
factor induction and microscale tissue engineering from two cell lines. The differentiation cultures contained >70% CMs based on the expression of cardiac transcription factor NKX2-5 as quantified by ArrayScan™XTI Live High Content Platform analysis. These cells were seeded at various densities and subjected to the CellTiter-Blue® Cell Viability Assay. After adding a single reagent directly to hiPSC-CMs cultured in a serum-free medium, cells were incubated for various durations and fluorescence signals were recorded using a plate-reading fluorometer. We found that there was a linear relationship between cell numbers and fluorescence signals ($R^2>0.988$) when cells were seeded from 0 to 8x10^4 cells/well in 96-well plates for the incubation time less than 6 hours. The signal detection threshold (detection limit) was 800-2,700 cells/well, and well-to-well variation was negligible with the coefficient of variation (CV) of 0% to 14%. These results suggest that CellTiter-Blue® Cell Viability Assay is a reliable and well-controlled high-throughput approach to detect cell viability of hiPSC-CMs.

**57. Attainment of the Sit Milestone is Related to Trajectories of Social Communication in Children with Autism Spectrum Disorder**

*Markert, Sarah; Klin, Ami; Klaiman, Cheryl; Jones, Warren; and Shultz, Sarah*

**BACKGROUND:** In typically-developing (TD) infants, the attainment of motor milestones transforms the ways in which infants are able to interact with their surroundings, affording them a wide range of new experiences that are fundamental to the emergence of social communication skills. In children with ASD, a small number of studies have begun to address how motor development relates to communication development, but have thus far focused only on the onset of walking. Sit milestone attainment relates to increases in social communication in TD infants, but remains unaddressed in infants with ASD.

**OBJECTIVE:** (1) Model longitudinal growth in social communication for children with ASD and TD age-matched peers. (2) Assess the extent to which attainment of the sit milestone impacts within-group trajectories of social communication and individual future social communication scores.

**METHOD:** 81 age-matched infants (38 ASD; 43 TD) were administered the Communication and Symbolic Behavior Scales (CSBS) at 9 and 12 months of age. The age at which each infant first sat without support was collected from a parent caregiver questionnaire (PCQ). Trajectories were modeled for social communication scores on the CSBS across the two time points using (a) chronological age and (b) an adjusted age based on sit onset age. "Early" and "late" sitters were identified relative to group median sit-onset age, and scores were plotted and compared. Within-group analyses were run to test for correlations between sit onset and social communication scores at 9 and 12 months.

**RESULTS:** Trajectories of CSBS scores adjusted for age-of-sitting show improved goodness-of-fit statistics ($r$-square, SSE, RMSE, adjusted $r$-square) compared to unadjusted, chronological-age trajectories. Early sitters exhibit higher CSBS scores at 9 months than late sitters: sit onset is significantly correlated with CSBS total scores and with Symbolic and Speech composites ($p$'s < .05), and approaches significance for Social composite scores ($p = .0503$).

**CONCLUSIONS:** This study reveals that adjusted age based on sit onset better models growth in social communication than chronological age alone. In addition, infants who sit earlier exhibit stronger social communication at 9 months, suggesting that sitting affords these infants experiences that may have positive cascading effects on communication development.
58. Characterization of Burkholderia Secondary Metabolites Produced Under Antibiotic-Induced Stress

McAvoy, Andrew; and Garg, Neha

The lungs of cystic fibrosis (CF) patients are often colonized by multiple bacterial species. Infections from opportunistic pathogens within the Gram-negative *Burkholderia cepacia* complex (Bcc) are particularly lethal in immunocompromised patients. Although the primary metabolome of most Bcc members is fairly well characterized, less is known about secondary metabolites that are only produced under specific conditions, such as antibiotic-induced stress or under host-specific conditions. In order to explore the effect of sublethal antibiotic concentrations on the metabolome of Bcc complex members, liquid-chromatography-tandem mass spectrometry (LC-MS/MS) was performed on extracts from different Burkholderia species with and without trimethoprim. Analysis of mass spectrums using a molecular networking-based approach revealed species-specific differences in the observed metabolomes. Understanding how opportunistic pathogens within the Bcc respond to antibiotics will lead to more informed treatments for Burkholderia infections in immunocompromised CF patients.

59. Characterization of Mesenchymal Stem Cells Used for Therapeutic Purposes

Medrano-Trochez, Camila; Chatterjee, Paramita; Ogle, Molly; Yeago, Carolyn; Botchwey, Ed; Roy, Krishnendu; and Gibson, Greg

BACKGROUND: Mesenchymal stem cells (MSCs) are multipotent cells that can differentiate into a variety of cell types and are also involved in the organismal immunoregulation. For this reason, there is increasing interest in the use of these cells for therapeutic intervention. We propose to use transcriptomics to characterize MSC and potentially identify biomarkers that correlate to and eventually predict clinical responses.

METHODS: RNA from single MSCs extracted from cord tissue or bone marrow was subject to scRNA-Seq using the BioRad drop digital platform followed by deconvolution of samples, cells, and transcript counts using the Illumina SureCell pipeline. We then developed a novel analytical strategy involving permutation of random cell-pools to overcome the zero-inflation (high drop-out rate) of mRNA abundance in single cells. EdgeR was used to perform differential expression analysis between MSC source samples.

RESULTS: The data (from bone marrow donors) clustered in 3 different groups: one of the subgroups is significantly different from the other two groups. This difference is due to unexpectedly large variation in read depth. It is still not clear if the difference in read depth is biological or technical. The other two groups separate cells involved in the cell cycle and active cells, expressing immunoregulation and differentiation genes.

CONCLUSIONS: scRNA-Seq provides a novel approach to contrast the gene expression of single cells of a tissue or cell type. This pilot experiment shows that adjustments for cell cycle will need to be accounted for when considering the profiles of MSC in the context of therapeutic intervention.
60. Examining the Association Between Parent-reported Developmental Concerns and the Development of Social Visual Engagement in Young Children

*Mendez, Adriana; Ponzo, Tristan; Klin, Ami; Klaiman, Cheryl; Shultz, Sarah; and Jones, Warren*

Parent-report plays a crucial role in the diagnostic landscape for autism spectrum disorder (ASD). Here, we investigate the association between performance-based, objective measures of a child’s behavior, collected via eye-tracking, and parent-reported concerns on the Ages & Stages Questionnaire - 3rd Edition (ASQ). Specifically, whether parent’s concerns are associated with a child’s social visual engagement when viewing scenes of social interaction. Social visual engagement is a means through which children define their developmental trajectories: by engaging with certain aspects of their social environment, children gather unique information that alters their learning. In ASD, variation in social visual engagement is strongly associated with levels of clinician-measured social-communicative competence.

Parent-reported developmental concerns and eye-tracking data were collected from N=146 consecutive referrals (Mage=28.23 months, SD=6.89) to a community-based clinic. Children were referred because of general or ASD-specific developmental concerns. All children received a gold-standard diagnostic evaluation, including the ADOS-2, developmental history, and developmental functioning measures. Parent-reported developmental concerns were collected through the ASQ. Eye-tracking data were collected while toddlers watched videos of children in daycare settings and were quantified by measuring percentage of visual fixation to faces and objects.

The data show significant associations between measures of social visual engagement and parents’ concerns about their children’s social-emotional and communication development, with increased attention to faces predicting fewer concerns ($r=0.451, p<0.001$ and $r=0.330, p<0.001$, respectively; see Figure 1) and increased attention to objects predicting greater concerns ($r=-0.452, p<0.001$ and $r=-0.266, p=0.003$, respectively). Fixation on faces was significantly associated with parents’ concerns about whether their child points, follows directions, plays pretend, plays with dolls, and talks like other children (all $p<0.05$). These associations appear specific to social-emotional and communication concerns, rather than general developmental concerns, as fixation on faces was unrelated to parental concern about their child’s ability to walk, jump, use stairs, turn doorknobs, or copy a line (all $p>0.05$).

Results indicate significant moderate convergent validity between eye-tracking-based measures of social visual engagement and parent-reported developmental concerns—consistent with prior research measuring the extent to which measures of social visual engagement are associated with clinician-administered assessments.

61. Respiratory Syncytial Virus: Evaluation of a Transdermal Microparticulate Vaccine using Laser Ablation

*Menon, Ipshia; D’Sa, Sucheta; Kang, Sang-Moo; D’Souza, Cherilyn; and D’Souza, Martin*

**INTRODUCTION:** Vaccine-enhanced respiratory disease has thwarted the attempts to develop a vaccine for Respiratory Syncytial Virus (RSV) using the inactivated form of the virus. As a result, there is a need for a safe and effective vaccine for RSV. Fusion protein is a one of the major proteins present on the surface of the virus, which can be integrated into a virus-like particle (VLP), yielding a highly immunogenic non-virulent F-VLP antigen. In this study we have used Precise Laser Epidermal System (P.L.E.A.S.E), a minimally invasive ablative fractional (Er: YAG) laser for creating micro-channels to deliver the antigen.
METHODS: In this study, the F-VLP antigen was incorporated into a biodegradable polymer matrix and spray dried to form microparticles. Micro-channels were created on the surface of the skin of Swiss Webster (CFW) mice using Er: YAG laser with an energy of 17.8 J/cm² and a pulse duration of 125µs. The F-VLP microparticles along with microparticles of adjuvant monophosphoryl lipid A (MPL) was dispersed in a hydrogel base and was applied onto the micro-channels. Post immunization, the mice were then challenged with live RSV A2 (1 x 10⁶ PFU per mouse). The mice were then sacrificed and their immune organs such as lymph nodes and spleen were collected to evaluate the in vivo immunogenicity.

RESULTS: The transdermally administered microparticles with adjuvant (F-VLP MP+Adj) group elicited a higher CD8+ and CD4+ cell count in lymph node and spleen cell populations when compared with the control group which was administered with transdermal F-VLP solution. The CD8+ expression in the spleenocytes increased by 7.2% and by 8% in the lymphocytes in the test group as compared to the control group. It was observed that the CD4+ cell count increased by 30.7% in the spleenocytes.

CONCLUSION: Microparticulate vaccine of F-VLP along with adjuvant MPL A helped in potentiating the immune response. Transdermal route of administration using an ablative laser augmented the efficacy of the microparticle vaccine. In the absence of a licensed RSV vaccine, transdermal microparticulate vaccines have immense potential and offer an alternative approach in the development of a vaccine against RSV.

62. Novel Insights into Red Blood Cell Metabolomics in Patients with Sickle Cell Disease at Risk for Early Mortality Through Biomarker Discovery
Morris, Claudia; Ma, Chunyu; Li, Shuzhao; Jones, Dean P.; Brown, Lou Ann; Kato, Gregory; Zhang, Yingze; Nouraie, Seyed; and Gladwin, Mark

BACKGROUND: In adults with sickle cell disease (SCD), an elevated tricuspid-regurgitant-jet-velocity (TRV) by Doppler-echocardiography is associated with increased morbidity/mortality. Metabolomics is an integrated biosystems approach to molecular fingerprinting that may be able to differentiate individual metabolic characteristics of patients at high risk (TRV>2.9m/s) vs. low risk (TRV<2.5m/s) of mortality based on TRV-elevation.

METHODS: A liquid chromatography-high resolution-mass-spectrometry method was used to determine metabolites in sickle-erythrocytes from 90 case-control selected participants from the walk-PHaSST screening cohort, matched for sickle genotype (Hb-SS), age, gender hydroxyurea use and alpha-thalassemia trait, divided equally into patients with a TRV>2.9m/s versus TRV<2.5m/s. Statistical/bioinformatics analyses used one-way analysis of variance (ANOVA) and partial least-squares discriminant analysis (PLS-DA) to identify groupwise discriminatory factors contributing to 95% separation of high vs. normal TRV. Significant features with one-way ANOVA p-value<0.05 were used to perform pathway analysis.

RESULTS: Patient characteristics are summarized in Figure1. Differential expression analysis showed that 145 accurate mass m/z features extracted from C18 column with positive ionization mode were significantly different between the high TRV and normal TRV subjects based on one-way ANOVA with p<0.005 (FDR<0.2) and PLS-DA with VIP>2 but no significant metabolism pathways were identified. With less stringent threshold p<0.05, one-way ANOVA revealed 1702 significant features which can discriminate the samples according to TRV status. Pathway enrichment analysis using Mummichog and the 1702 significant features with p-value<0.05 revealed 9 significant pathways.
CONCLUSIONS: Metabolic profiles in sickle-erythrocytes differentiate SCD patients with a normal TRV and low mortality risk from those at high risk for early mortality with a TRV > 2.9m/s through unsupervised pathway enrichment analysis. The pathways identified (in particular, porphyrin metabolic pathway/hemolysis, carnitine/mitochondrial function, vit-D3 and omega-3-fatty-acids/cell membrane function) warrant further study. This preliminary work identifies metabolic pathways for future targeted analysis, and may lead to novel mechanistic insight and therapies that target these pathways. Pediatric metabolomics studies in SCD are planned in the near future.

63. Gene Regulators and Cardiovascular Risk Evaluation in Childhood/Adolescent Obesity (GRACE STUDY): Relationship Between Blood Pressure, Insulin Resistance and Inflammation Among GRACE Study Participants (Preliminary Findings)

Mosimah, Charles; Murray, Pamela; Collin, John; Simpkins, James; Lilly, Christa L.; and Eggleston, Emma-Morton

BACKGROUND: MicroRNAs mediate environmental influences on genetic expression and cardiovascular risk factors (CVRF). Little is known regarding the impact of inflammation and insulin resistance on blood pressure in children. The GRACE study explores relationships between select microRNAs, family history and CVRF in obese children/adolescents compared to their normal weight peers (NWP).

METHODS: Participants (n=180) who met our eligibility criteria were approached: Forty-two percent (n=75/180) who expressed interest and met our eligibility criteria were recruited from WVU Adolescent, Pediatrics and Lipid Clinics. Normal weight was a BMI between the 5th and 85th %; obesity as BMI above the 95th percentile. Insulin resistance (IR) was calculated using HOMA-IR. Blood pressure was defined as age, height and gender adjusted blood pressure ≥ 95 percentile (HTN): elevated CRP (ECRP) levels as CRP levels ≥ 3.0 mg/dl. Participants and a parent completed a self-administered survey at enrollment, and completed fasting blood work: lipid profile, insulin, comprehensive metabolic panel, and microRNA assay on another day. Clinical measurements including blood pressure were manually measured by an investigator. Descriptive statistics, chi-square test and logistic regression was used to

RESULTS: Majority of the study population was white, 94.44% (n=68/72), female, 75.68% (n=56/74), obese, 64.79% (n=46/71), and were in the 15-19 age category, 68.2% (n=45/66). The proportion of participants with insulin resistance, blood pressure ≥ 95th percentile and ECRP were: 36% (n=27/75), 33.33% (n=22/66) and 38.5% (n=23/66) respectively. High blood pressure was more common among obese participants (47.62% vs 8.33%, x² = 10.6071, p= 0.0011) compared to normal weight participants. The proportion of participants who had elevated blood pressure was higher in the IR group compared to those without IR respectively (60.87% vs 18.60%, x²= 12.05, p= 0.0005). Similarly, proportion of participants who had HTN was higher in ECRP group compared to those with normal CRP levels (72.22% vs 17.95%, x²= 15.93, p= <.0001). The odds of having high blood pressure was higher in participants who were IR (OR:6.81, CI: 2.18-21.2), had ECRP (OR: 27.63, CI: 6.89-110.63). MicroRNA assays are underway.

CONCLUSION: There is a significant relationship between high blood pressure, insulin resistance and inflammation worth investigating; especially from a genetic stand point.
64. Identification of Strong Candidate Variants for Short Stature and Insulin Resistance

Mosley, Treuell; Wang, Chuan-En; Wilcox, William; and Zwick, Michael

We identified two Middle Eastern adolescent offspring (male and female) of unaffected consanguineous parents presenting with short stature and insulin resistance. We hypothesized these phenotypes result from an autosomal recessive disorder and predicted both probands should be homozygous for the same identical-by-descent allele. To identify candidate variants, we performed whole-genome sequencing and two analyses. The first was a genome-wide search for rare, high-CADD variants homozygous in both cases. Second, we located runs of homozygosity where both samples were homozygous for the same alleles. The analyses converged on nine candidate variants on chromosome 3. We pursued three top candidate variants in genes DUSP7, POC1A and USP19. The DUSP7 variant (g.52020873:T>C; hg38) substitutes a cysteine residue for a tyrosine (p.Y401C). DUSP7 is a dual-specificity phosphatase that interacts with the MAP kinase (MAPK) pathway, which is associated with dwarfism and the insulin signaling pathway. The variant in POC1A (g.52122374:C>G) is predicted to mutate a splice donor site between the ninth and tenth exons. Mutations in POC1A are linked to short stature, onychodysplasia, facial dysmorphism and hypotrichosis (SOFT) syndrome. Specifically, mutations affecting the tenth exon in mature POC1A RNAs are linked to short stature and insulin resistance. The final top variant in USP19 (g.49112616:C>T) has the highest CADD score (28.8) and is an arginine to histidine missense mutation (p.R840H) in the ubiquitin specific protease domain of the encoded protein. The USP19 protein, UBP19, has been shown to modulate insulin signaling. We hypothesized Y401C abolished the phosphatase activity of DUSP7 towards its substrate, ERK2, but functionally determined it does not affect the phosphatase or binding activity of DUSP7 towards ERK2, and therefore is likely not the causal mutation. We are currently investigating the POC1A and USP19 variants for causal links to the phenotype. We hypothesize the POC1A variant affects splicing of the tenth exon, while the USP19 variant affects the ubiquitin protease activity of UBP19. Investigation of these putative causal variants will provide insight into the dynamics of developing chondrocytes and the insulin signaling pathway, while broadly elucidating the mechanisms influencing metabolic insulin signaling, bone development, and the etiology of insulin resistance.

65. Alterations in Genetic Regulation of Rectal Gene Expression from Diagnosis to Recovery in Pediatric Ulcerative Colitis

Nagpal, Sini; Mo, Angela; Hyams, Jeffrey; Marigorta, Urko M.; The PROTECT Consortium; Walters, Thomas; Kugathasan, Subramanium; Denson, Lee; and Gibson, Greg

Elucidation of the genetic mechanisms underlying the progression of pediatric Ulcerative colitis (UC) is a high priority for guiding therapeutic intervention. Characterization of molecular signatures of disease progression should improve prognostic accuracy and increase precision of therapy. We utilized genetic and transcriptomic data obtained prior to initiation of therapy and at 52-week follow-up from PROTECT study (Predicting Response to Standardized Pediatric Colitis Therapy), to identify genetic regulators of patterns of disease progression. We first show that at diagnosis, gene expression is indicative of greater pathology than after a year of recovery, since the first principal component is highly correlated with the Pediatric UC Activity Index (PUCAI). Intervention with 5-ASA or Corticosteroids leads to improvement for 80% of the subjects, and remission within 12 weeks for one third, and healthy gene expression is recovered with both treatments. We next performed expression quantitative trait locus (eQTL) studies contrasting baseline and week-52 groups to identify genes that are differentially regulated upon recovery of more normal rectal physiology. Most of the 1,467 peak eQTLs identified at baseline have similar effects.
at follow up, and vice versa for the 445 identified at follow up. However, there were instances of eQTL with a specific effect on expression exclusively in the baseline or week 52 groups. We explored some of the potential functions of the genes influenced by these SNPs that have also been associated with UC. Furthermore, since genome-wide predicted gene expression association studies have enhanced the discovery of genetic risk loci, we performed gene-based association tests based on predicted gene expression (also known as Transcriptome-wide association study or TWAS) for approximately 12,000 genes at baseline and at follow up. We show how TWAS can be used to prioritize loci that associate with UC based on predicted patterns of gene expression.

66. Intestinal Organoids to Model Ex Vivo Patient Response in Pediatric Inflammatory Bowel Disease
Niklinska-Schirtz, B. Joanna; Matthews, Jason; Prince, Jarod; Dodd, Anne; and Kugathasan, Subramanium

BACKGROUND: Inflammatory Bowel Disease (IBD) is a chronic autoimmune condition which causes progressive tissue damage requiring frequent surgical interventions. Even the best available anti-Tumor Necrosis Factor (TNF) therapy, which targets immune cells and macrophages, is only effective in 45% of patients, suggesting alternative disease mechanisms. Thus, we sought to examine the contribution of the epithelium in IBD. Patient-derived organoids (PDOs) have enormous potential to elucidate disease mechanism and they can be used to screen future therapeutics. PDOs are currently being studied in many cancers including colorectal, gastroesophageal, and prostate cancer as well as cystic fibrosis. PDOs derived from patient biopsies can be expanded in culture to produce epithelial crypts, dome like ‘mini-guts,’ as well addition of patient specific immune cells into a co-culture system.

AIM: To establish a biobank of patient-derived intestinal organoids to mechanistically define the role of the epithelium in IBD in response to inflammatory signaling molecules.

METHODS: Colonoscopy biopsies from the terminal ileum and rectum at diagnosis, as well as on-going routinely treated patients, are being cultured as PDOs. These samples are then subjected to a variety of biochemical analysis in conjunction with histopathologic and immunofluorescence microscopy. Using RNA sequencing on the PDOs derived from healthy vs disease tissue, we will define epithelial specific transcriptional changes in response to inflammatory cytokines.

RESULTS: To date 40 PDOs (18 ileum, 22 rectum) have been generated and banked. Staining with ZO-1, E-cadherin, beta-catenin, DNA, and MUC2 showed the intact mini-gut structure and the presence of tight junctions, adheren junctions, Paneth, and goblet cells.

CONCLUSION: We demonstrate feasibility of PDOs in our laboratory on a routine basis. We anticipate variability in epithelial components between disease and non-diseased organoids, as well as differences in growth and behavior between healed mucosa vs endoscopically active disease. Specifically, with this information, these ‘mini-guts’ will be able to pioneer a model to predict disease activity, as well as drug response and efficacy, for personalized medicine in the near future.

67. The Impact of Caregiver Stress on a Parent-Mediated Feeding Intervention
Nuhu, Nadratu; Burrell, T. Lindsey; McCracken, Courtney; Gillespie, Scott; Wawrzonek, Addam; Cohenour, Jessica; Gonzalez, Jacquelin; and Sharp, William
INTRODUCTION: Feeding problems are common in children with ASD; however, access to care is limited due to few trained therapists and treatment facilities. Parent training to address feeding problems may ease access and expand treatment options. Parental stress is a crucial factor to consider when utilizing a parent mediated intervention. However, few studies evaluate the effects of parental stress on treatment outcome. This purpose of the current study was to examine caregiver stress during a parent-mediated feeding intervention between participants in the treatment and control condition.

METHODS: Participants included children (ages of 3 to 8) with moderate food selectivity and ASD. Participants were randomized to the Autism MEAL Plan (treatment) or Parent Education (control) condition. The treatment and control conditions included 10-weekly sessions. Outcome measures included baseline clinical scores from the Social Communication Questionnaire, Vineland, Aberrant Behavioral Checklist, the Brief Autism Mealtime Behavior Inventory (BAMBI), and the Parental Stress Index (PSI). Data were also collected on the grams consumed during structured meal observations at baseline and post-treatment.

RESULTS: Thirty-eight children and their caregivers participated in the study. There were no significant differences in baseline clinical scores between caregivers of individuals in the treatment condition when compared to those in the control condition. However, to evaluate the effects of stress within participants in the treatment condition, we assessed stress between treatment responders and non-responders. Caregivers of participants, who were considered responders to treatment, demonstrated no differences in child baseline scores compared to participants who did not respond to treatment. However, parents of children who were responders to treatment demonstrated higher parental stress than those children who did not respond to treatment.

DISCUSSION: Results suggest that the caregivers from the treatment condition that observed improvements in their child’s feeding behaviors reported increased levels of stress. To further explore the variables influencing stress within the responder group treatment data such as caregiver integrity, parental engagement, and homework completion will be further evaluated.

68. Neurological Effects of Concussions in Adolescents: A Literature Review
Oberfeld, Austin; Ratcliffe, Lauren; Gandhi, Mayank; Sutcliff, Jane; and Robinson, Brittany

The Center for Disease Control (CDC) reported 38 million children and adolescents (age ≤18) compete in sports each year with 1.6 to 3.8 million children a year being diagnosed with sports related concussions. Concussion symptoms include memory deficits, confusion, fatigue, syncope, diplopia/blurred vision, headache, nausea/vomiting, photophobia, phonophobia, tinnitus, delayed processing speed, balance problems, and slowed reaction time. The onset of symptoms varies from just after the moment of impact to lasting for hours, days, or weeks after impact, generally lasting up to six weeks. Literature in this area has shown increases in myelin synthesis, chemical imbalance, decreased blood flow, and problems with neural connectivity, resulting in microstructural integrity, to be associated with sports related concussion (Borich et al., 2015; Halstead et al., 2018; Moore et al., 2018). These findings indicate that adolescents are able to return to their baseline level of functioning within six months of impact. The purpose of this review is to examine the current literature on the effects of concussions on the adolescent brain. This is an important time in neural development and a mild traumatic brain injury can effect neural development and neural chemistry. Thus, an analysis of this process can aid in the understanding of recovery and long-term implications of contact sports.
69. A Nonhuman Primate Model of Pediatric HIV Infection to Evaluate Functional Cure Strategies

Obregon-Perko, Veronica; Bricker, Katherine; Uddin, Ferzan; Fouda, Genevieve; Bar, Katharine; Shaw, George; Silvestri, Guido; Permar, Sallie; and Chahroudi, Ann

HIV infection in the setting of breastfeeding transmission commits infants to lifelong ART, as interruption is typically followed by viral rebound. With prolonged drug exposure in HIV-infected children now associated with metabolic complications and the risk of triple drug class virologic failure, there is a need to develop alternatives to ART-based treatments that can achieve viral remission. Advancements in such cure strategies have been hindered by the lack of a relevant animal model for understanding viral rebound and reservoir size in infants. We sought to develop and characterize a nonhuman primate (NHP) model of oral pediatric HIV infection and long-term ART using a SHIV expressing clade C HIV Env, a subtype highly relevant to the current epidemic and one that will allow investigation of HIV envelope-targeted therapies. Sixteen 4-week-old Mamu B08-/B17-/A01- or + rhesus macaques were administered SHIV CH505 in two high-dose oral inoculations and placed on ART at 8 wpi. Median viral loads at peak and immediately prior to ART were about 500,000 and 100,000 copies/mL, respectively. Although the majority of macaques were infected in 1 to 2 exposures, two required 3 to 4 exposures. Refraction to infection in these two macaques was reflected in their viral loads, which were roughly 1 log lower than those of the other animals. We identified low frequency of peripheral CCR5+CD4+ T cells as one factor potentially underlying this observed resistance. Males and females had comparable replication kinetics but there was a trend for lower pre-ART viral loads in Mamu A01+ animals. Infants showed little to no drop in peripheral CD4 T cell frequency during acute infection. ART was well tolerated and decreased plasma viral loads to undetectable levels after 1 to 10 weeks on the regimen. This was accompanied by a 2-log reduction in cell-associated viral DNA in peripheral CD4 T cells. In summary, we have developed a pre-clinical NHP model that displays key features of pediatric HIV infection, including limited CD4 T cell depletion and sustained viremia before ART. Ongoing work involves continued CA-DNA measurements in various tissues as well as whole-body imaging to visualize anatomical sites of infection.

70. Impact of Preceding and Co-Existing Autoimmune Cytopenias on Severity of Childhood-Onset Systemic Lupus Erythematosus: A Single-Center Retrospective Cohort Study

Ogbu, Ekemini; Chandrakasan, Shanmuganathan; Rouster-Stevens, Kelly; Greenbaum, Larry; Marion, Chelsea; Singer, Karl; Sanz, Iñaki; and Prahalad, Sampath

BACKGROUND: Autoimmune cytopenias may precede or occur with childhood-onset systemic lupus erythematosus (cSLE). Adult studies suggest that lupus patients with concurrent autoimmune cytopenias have relatively lower prevalence of lupus nephritis (LN) and are a unique sub-population. Therefore, the objectives of our study were to assess if in cSLE, autoimmune cytopenias decrease the 2-year risk and severity of LN. To assess associated serological differences and the effect of prior immune therapy for autoimmune cytopenia on 2-year risk of LN. To perform descriptive analyses of pediatric patients without LN at cSLE diagnosis.

METHODS: Ours was a retrospective cohort study of incident cSLE cases over a 16 year period. We included patients aged less than 17 years who met the classification criteria for systemic lupus erythematosus. We excluded patients diagnosed outside our institution and those with LN at cSLE diagnosis. Our follow-up period was 2 years. We defined autoimmune cytopenia as either autoimmune hemolytic anemia, Coombs positive anemia without hemolysis, immune thrombocytopenia or Evan’s syndrome.
RESULTS: Our study included 130 patients. Of these, 43 (33.08%) had autoimmune cytopenia before or at cSLE diagnosis. Those with autoimmune cytopenia had significantly more neuropsychiatric symptoms and higher mean ESR. However, they had less arthritis, malar rash and myositis. 2-year incidence of LN was 12.31% in our cohort. Patients with autoimmune cytopenia had lower 2-year risk of LN compared to other cSLE patients (6.98% vs 14.94%). Of the 16 patients that developed LN, those with autoimmune cytopenia had mostly class V (2 of 3 patients) versus mostly class III and IV in those without autoimmune cytopenia (6 of 12 patients). None of the 13 patients pre-treated for autoimmune cytopenia prior to cSLE diagnosis developed LN. Low C3 complement at cSLE diagnosis was independently associated with 4-fold increased odds of LN.

CONCLUSION: Patients with autoimmune cytopenia before or at cSLE diagnoses have significant and clinically relevant differences in their presentation from other cSLE patients. Our findings call for further studies on the immunologic and genetic basis of these differences.

71. Psychotropic Prescribing Patterns in a Specialty Service for Youth with Autism Spectrum Disorder and Serious Behavioral Problems

Ogunyankin, Forest; Scheithauer, Mindy; Lark, Catherine; and Scahill, Lawrence

OBJECTIVE: To document prescribing patterns in a sample of youth with autism spectrum disorder, or other developmental disabilities treated in a specialty day treatment program between 2012 and 2017.

Methods: One-hundred-forty-six consecutive first-time admissions to the Severe Behavior Day Treatment Program were identified via electronic medical records between January 1, 2012 and December 31, 2017. The inclusion criteria for further review were: prior diagnosis of autism spectrum disorder or other developmental disorder; chief complaint(s) of aggression, tantrums, property destruction and/or self-injurious behavior; admission to day treatment for at least two weeks.

RESULTS: The sample included 112 males, 31 females; mean age 11.1 ± 4.0 years; range 3 to 20. Parents reported aggression as the chief complaint for 86.0% of youth. At admission, 83.2% of Day Treatment patients were taking at least one psychotropic medication; 37.1% were taking three or more medications. Between 2012 and 2014 the most commonly prescribed class of medication was atypical antipsychotics; alpha-2-agonists were the most commonly prescribed medication class from 2015 to 2017. Clonidine was the most prescribed medication in young children (12 of 33 in 3-8 year-olds), and risperidone was the most frequently prescribed in adolescents (20 of 78 in 9-15 year-olds) and young adults (6 of 26 in 16-20 year-olds). Weight gain was reported in 8 of 56 of patients on risperidone or aripiprazole.

DISCUSSION: From 2012 through 2017, the use of atypical antipsychotics and stimulants declined, while that of alpha-2-agonists, antidepressants, anticonvulsants, and anxiolytics increased. The explanation of these trends is not clear. Additional investigation on the integration of behavioral intervention and psychopharmacological treatment is warranted.


Olmstead, Jack A; Klin, Ami; Shultz, Sarah; and Jones, Warren

Eye-tracking is a popular research method for neuroscience and biomedical research in general, and for studying social development in particular (Jones & Klin, 2013). In theory, the technology is applicable to
participants of all ages and all levels of cognitive and adaptive ability. In actuality, however, traditional calibration techniques required by many model-based eye-tracking technologies (i.e., those that use models of infrared corneal reflection and pupillary geometry to determine gaze, such as EyeLink, SMI, and ISCAN) are difficult to complete for neonates and for individuals with behavioral or cognitive challenges. This limitation can prevent data collection from important stakeholders in developmental research.

By using a set of accurately calibrated, high-quality eye-tracking sessions (N = 18) as ground truth estimates during supervised machine learning, we empirically constructed a calibration transformation from 2-D eye-image space into 2-D screen-coordinate space. We then used an independent set of accurately calibrated, high-quality eye-tracking sessions (N = 111) to test the calibration transformation on novel data collected under the same lab conditions.

The median absolute point of gaze (POG) error (actual POG - predicted POG) subtended 2.64 degrees of visual angle. We then used the empirical error distribution to demonstrate potential future area-of-interest (AOI) analyses: In videos that are segmented into AOIs at each frame, we can probabilistically determine what AOI is being fixated.

Data collected through model-based eye trackers can be accurately calibrated post-session using machine learning. Once an error distribution has been empirically determined, it may be used to probabilistically determine fixated elements in naturalistic social scenes shown to participants. The strength of this eye-tracking approach is that it can be developed and deployed to collect data from participants for whom traditional eye-tracking experiments may not be possible—i.e., from neonates or from individuals with behavioral challenges or with substantial comorbid intellectual disability. Probabilistic eye-tracking analyses have the promise of extending quantitative research methods into populations of both extreme interest and vulnerability, allowing insight into the earliest periods of development and the results of its disruption.

73. Strategies to Analyze High Dimensional Untargeted Metabolomics Data

Oza, Vishal H.; and Reed, Laura K.

Untargeted metabolomics studies are currently widespread in characterizing the metabolic phenotype, defined as the ensemble state of all metabolites in an organism at any point in time. Most of these studies focus on identifying important metabolic biomarkers that change between two different disease conditions or environments. These studies also have highlighted the fact that the known metabolic networks are incomplete. However, untargeted metabolomics studies can be a great tool of getting a snapshot of metabolome of an organism at any given point in time and hence can be used to get a more complete picture of the metabolome. Here, we demonstrate the use of Probabilistic graphical models, particularly Bayesian networks to identify the network structure from the untargeted metabolomics data in Drosophila melanogaster.

Here, we demonstrate the use of Probabilistic graphical models, particularly Bayesian networks to identify the network structure from the untargeted metabolomics data in Drosophila melanogaster. A total of 856 Drosophila melanogaster larvae were subjected to untargeted metabolomics analysis using Liquid Chromatography-Mass Spectrometry and Gas Chromatography-Mass Spectrometry at The West Coast Metabolomics Center. The untargeted metabolomics approach isolated and identified relative concentrations of 422 different metabolites, out of which 169 are known metabolites and 253 are
unknown metabolites. The dataset obtained was used to construct Bayesian networks using both score
and constraint based structure learning algorithms. The analysis was performed using the bnlearn and
sparsebn package in R. The networks obtained were then compared to known global metabolic networks
in various organisms present in KEGG. We found that the generated Bayesian networks showed a similar
degree distribution to the known KEGG networks, had similar subgraph structure composition, and when
fitted to power-law distribution Bayesian network models showed comparatively smaller alpha values.

Thus, we demonstrate that Bayesian network analysis can be successfully utilized for untargeted
metabolomics data to generate data-driven network models that have similar underlying characteristics as
known metabolic networks.

74. Genome-Wide Non-Coding RNA Analysis in Both Ileum and Rectum Biopsies from Pediatric
Crohn's Disease
Pellet, Ranjit; Venkateswaran, Suresh; Somineni, Hari; Gibson, Greg; Okou, David; Cutler, David; and
Kugathasan, Subramanium

BACKGROUND: Utilizing our treatment-naive pediatric Crohn's disease (CD) cohort, we previously defined
the inflammatory and metabolic genomic signatures within ileal biopsies. Recently, long-noncoding RNAs
(lncRNA) have been observed to be part of multiple facets in regulating gene transcription, translation,
and expression. Our aim was to identify Crohn’s Disease (CD) specific lncRNAs from the RISK study, a large
pediatric CD cohort.

METHODS: The primary dataset consisted of 345 ileum biopsies, including 274 CD patients and 71
controls. The secondary dataset was comprised of 431 rectum biopsies, of which 81 were controls and 350
CD patients. Whole RNA-Sequencing was conducted using Illumina deep RNA-Sequencing. Reads were
aligned with hg38 reference panel by STAR package. EdgeR was used to analyze Differential Expression
(DE). The results were verified using DESeq2 tools in R. The DE lncRNA were defined by log2FoldChange
(FC) > or < 1.25 and adjusted p-value (FDR) < 1e-6.

RESULTS: Totally, 10,111 out of 17603 ncRNAs were retained after the quality control i.e. any ncRNA reads
with >10% missing data was removed. In total, 1834 up- and 1140 down- regulated IncRNAs were
observed within ileal biopsies between CD patients compared with healthy controls, FDR p < 0.05. After
stratifying the results based on log2FC 1.25 and FDR<1e-6; totally, 19 up- and 13 down- regulated
IncRNAs were retained for extensive analysis. In contrast with other ncRNA studies, our results are
interesting that CD specific ncRNAs are not tissue-specific. Notably, RN7SL2 was explicitly down regulated
in CD patients than healthy controls. The results were consistent when the rectal biopsies were examined.
RN7SL2 is a major component of the signal recognition particle (SRP). It functions as a 7SL RNA molecule
to the cytoplasmic ribonucleoprotein complex. The SRP serves to mediate cotranslational transport into
and out of the endoplasmic reticulum lumen.

CONCLUSION: An extreme down regulation of the gene RN7SL2 was observed in multiple manifestation
sites of CD patients such as ileum and rectum. It leads to the postulation that its dysregulation may be
required for gene regulation. Further functional studies may provide mechanistic insights to improve the
diagnosis, prognosis, and treatment.
75. Post-Translation Acylations as New Pathway Effectors of the Mitochondrial Energy Stress Response

Peoples, Jessica; Ghazal, Nasab; and Kwong, Jennifer Q.

Mitochondria are dynamic hubs of cellular metabolism. In addition to their role in energy production, mitochondria can act as signaling platforms using metabolites as messengers. One group of messenger metabolites are reactive acyl-CoA’s, such as acetyl-CoA, and malonyl-CoA which are intermediary metabolites derived from mitochondrial metabolism which have the potential to modify and regulate proteins through post-translational acylations. Here we propose that reactive acyl-CoAs play an important role in the mitochondrial response to energetic stress. We have modeled mitochondrial dysfunction in vivo in the heart, a tissue of high-energy demands, by generating mice with the inducible cardiomyocyte-specific deletion of the mitochondrial phosphate carrier (Slc25a3), a mitochondrial transporter critical for energy production. Induction of Slc25a3 deletion in the adult heart results in a striking elevation of heart weight to body weight ratios, a common feature of cardiac hypertrophy, by 10 weeks following induction of gene deletion. Importantly, cardiac Slc25a3 deletion results in a specific signature of acylations: increased acetylation and malonylation. Using liquid chromatography-mass spectrometry metabolomics, we observed that PiC-deleted hearts have significantly increased malonyl-CoA, citrate, and alpha-ketoglutarate levels suggesting that increased protein malonylation originate from alterations in pathways that converge on malonyl-CoA synthesis. Interestingly, acetyl-CoA levels were unchanged with Slc25a3 deletion. As acylations may occur either enzymatically through the action of acyl-transferases or non-enzymatically under permissive conditions (such as high substrate availability), these results suggest differential regulation of the two acylations, and support nonenzymatic malonylation. Finally, proteomic analyses to identify Slc25a3 deletion-induced acetylated and malonylated proteins revealed upregulation of acylations in number of mitochondrial proteins, including cyclophilin D, isocitrate dehydrogenase 2, and several isoforms of acyl CoA dehydrogenase. Using our studies combining cardiac physiology with the systems biology-based approaches of metabolomics and proteomics we intend to uncover how mitochondria regulate the acylome and the molecular functions of protein targeted by acylations to further understand mitochondrial signaling following cardiac metabolic dysfunction.

76. Optical Control of Tumor Immunity via Engineered Cytokines

Perdue, Lacey A.; Do, Priscilla; Kelvin, James M.; Kim, Hye Ryong; and Dreaden, Erik C.

Recombinant cytokines were the first cancer immunotherapies approved by the FDA and have demonstrated therapeutic benefit in subset of cancer patients. Recently however, their clinical use to treat cancer has declined due to off-target toxic effects and poor circulation that necessitates frequent, high dosing and complex treatment management. To overcome these limitations, we recently engineered a new class of cytokines that enable tissue-restricted activation in response to external triggers such as light. Through chemical modification with photosensitive and biocompatible polymers, these so-called photokines can modulate the activity of T cell stimulating cytokines such as IL-2 (i.e. Proleukin) and IL-15 by approximately three orders of magnitude in response to simple LED light exposure. In vitro, photokines are rapidly triggered (ca. 10 min), highly color selective, and their activity can be spatially constrained with micrometer-scale resolution. By optically controlling the location, magnitude, and duration of cytokine signaling within the body and in chip-based organoids, we aim to provide new insights into the mechanics of tumor immune elimination or evasion and to simultaneously use these engineered proteins as immunotherapies that can drug tumors in a more tissue-exclusive and patient-personalized fashion.
77. Graft Choice for Adolescent Athletes Returning to High-Risk Sports: A Matched Cohort Analysis of Patellar Tendon and Hamstring Autografts
Perkins, Crystal A.; Busch, Michael T.; Christino, Melissa A.; and Willimon, S. Clifton

BACKGROUND: Graft selection for skeletally mature adolescents undergoing anterior cruciate ligament (ACL) reconstruction is guided by surgeon and patient preference. The purpose of this study is to compare the rates of graft failure between patellar tendon (BTB) and hamstring (HS) ACL reconstruction cohorts matched by age, sex, and sport.

METHODS: A single-institution retrospective review was performed of consecutive patients 13-18 years of age treated with ACL reconstructions using either BTB or HS autograft performed by a single surgeon with minimum 12-month follow-up. Skeletally mature or nearly mature patients in “high-risk” ACL injury sports (basketball, football, soccer, lacrosse, and gymnastics) were initially treated with hamstring autografts but the graft preference transitioned to BTB autografts as the preferred graft choice during the study period. This transition in graft preference allows for a comparison of outcomes based on graft types. The two cohorts of patients were matched by age, gender, and sport. The primary outcome measure was graft rupture.

RESULTS: 138 patients with an average age of 15.9 years (range 13-18 years) met inclusion criteria. There were 66 BTB and 72 HS reconstructions. There were 59 females and 79 males. There was no difference in age, sex, BMI, or laterality between groups. There were no differences between the BTB and HS cohorts in terms of meniscus tears (62% v 72%, p=0.15), meniscus repair (21% v 32%, p=0.13), or partial meniscectomy (33% v 33%, p=1.0).

Mean duration of follow-up was 31 months (range 12-73 months). There was no difference in follow-up between cohorts (BTB 28 months and HS 33 months, p = 0.07). There were 16 graft ruptures (11.6%). There was no difference in the rate of graft rupture between cohorts (BTB 9.1% vs HS 13.9%, p = 0.38). Mean time to graft rupture was 21 months (range 8-35 months) and Kaplan-Meier survival curves demonstrated no difference between cohorts.

CONCLUSIONS: ACL reconstruction in adolescents returning to high-risk sports can be performed utilizing BTB or HS autografts with similar rates of graft rupture.

78. Outcomes of Discoid Meniscus Repairs in Children and Adolescents
Perkins, Crystal A.; Busch, Michael T.; Christino, Melissa A.; and Willimon, S. Clifton

BACKGROUND: Discoid meniscus tear patterns typically involve horizontal cleavage tears of the central discoid component with or without anterior or posterior meniscocapsular tears. Many meniscal transplants are performed secondary to unsalvaged or unsalvageable discoid meniscus tears. The purpose of this study is to describe the outcomes of meniscus repair and saucerization in pediatric patients with symptomatic discoid menisci.

METHODS: A single-institution retrospective review was performed of consecutive patients less than 18 years with surgical repair of a discoid meniscus tear with minimum 12 month follow-up. The primary outcome was revision meniscus surgery.
RESULTS: Forty patients were identified to meet inclusion criteria. There were 22 males and 18 females with a mean age of 12.4 years (range 5-17 years). The right knee was affected in 63% of patients. The lateral meniscus was involved in all patients. Tear patterns included anterior meniscocapsular (17 patients, 43%), posterior meniscocapsular (14 patients, 35%), radial (5 patients, 13%), and bucket-handle (5 patients, 13%).

Arthroscopic meniscus repair and saucerization was performed in all patients. Marrow stimulation, as a biological approach to improve repair healing, was performed in 14 patients (33%). The repair types and mean number of sutures for each type is: outside-in (13 patients, 4.1 sutures), inside-out (10 patients, 6.5 sutures), all-inside (8 patients, 4.3 sutures), hybrid (9 patients, 4.8 sutures).

Mean follow-up was 31 months (range 12-70 months). Four patients (10%) underwent revision meniscus surgery following the primary repair, including 2 all-inside repairs and 2 partial meniscectomies. There were no statistically significant differences between patients who did or did not require a secondary surgery with respect to sex, age, tear location, tear pattern, repair type, or number of sutures.

CONCLUSIONS: Saucerization and repair of discoid lateral meniscus tears in the pediatric population have good outcomes with low rates of reoperation. Appropriate saucerization, followed by an arthroscopic assessment of stability and tear patterns is critical to successful treatment of symptomatic discoid menisci. If tissue quality permits, meniscal preservation should be considered in all patients to avoid the consequences of subtotal meniscectomy.

79. The Rate of Meniscus Tears in Association with Anterior Cruciate Ligament Injuries Increases with Age
Perkins, Crystal A.; Christino, Melissa A.; Willimon, S. Clifton; and Busch, Michael T.

BACKGROUND: Anterior cruciate ligament (ACL) tears are frequently associated with meniscal injury. The purpose of this study was to evaluate the relationship between age and the presence of a meniscal tear at the time of ACL reconstruction in children and adolescents.

METHODS: A single-institution retrospective review was performed of patients less than 20 years of age who underwent ACL reconstruction over a 3-year period at a single institution. Meniscus tears were defined as tears documented during diagnostic arthroscopy.

RESULTS: 461 patients, 226 males (49%) and 235 females (51%), with a mean age of 15 years (range 7-20 years) were included. 270 patients (80%) had intra-operative evidence of a meniscal tear, including 215 lateral meniscus tears (56%) and 119 medial meniscus tears (44%). 64 patients (14%) had both medial and lateral meniscus tears.

Age at surgery was found to be a statistically significant independent predictor of the presence of a meniscus tear, odds ratio=1.14, 95% CI (1.05–1.25), p=0.003. For every 1-year increase in age, there is a 14% increase in the odds of having a meniscus tear.

Among patients 13 years of age and younger (n=89), 47% had a meniscus tear. In contrast, 62% of patients 14-19 years of age (n=301) had a meniscus tear, which was significantly greater (p=0.01). Age was also a statistically significant independent predictor following subgroup analysis of medial and lateral
meniscus tears. For every one year increase in age at surgery, there was a 21% increase in the odds of having a medial meniscus tear (odds ratio=1.21, 95% CI: (1.08-1.35), p<0.001) and a 13% increase in the odds of having a lateral meniscus tear (odds ratio=1.13, 95% CI: (1.04-1.24), p=0.01.

CONCLUSIONS: Among children and adolescents with anterior cruciate ligament tears, for every 1-year increase in age, there is a 14% increase in the odds of having a meniscus tear. Adolescents over the age of 13 years had a significantly greater rate of meniscus tears than did those 13 years of age and younger.

80. Unilateral Patchy Hair Growth is Frequent in Children with Proteus Syndrome

Pithadia, Deeti; Roman, John; Biesecker, Leslie; and Darling, Thomas

Proteus syndrome is caused by a somatic mosaic activating variant of AKT1, a gene that plays vital roles in cell cycle progression, cell survival, and apoptosis. The disorder presents in early childhood and is characterized clinically by progressive and asymmetric overgrowth of skin, bones, and various internal organs. Dermatologic manifestations include cerebriform connective tissue nevi, epidermal nevi, lipomas, and vascular malformations. There have been isolated reports of hair-related aberrations in Proteus syndrome, and we sought to survey the frequency, distribution, and appearances of these abnormalities. A retrospective chart review was performed on a cohort of 41 children at the National Institutes of Health with genetically-confirmed Proteus syndrome; 4 were excluded due to inadequate photos. The remaining 37 patients included 25 males and 12 females with mean age of 7.9 years. Overall, 81% (30/37) of patients displayed at least one hair-related abnormality. Twelve of the 37 (32%) showed unilateral hypertrichosis in the distribution of Blaschko’s lines. The majority were located on the back and posterior arm and contained terminal hair growing in a uniform arrangement, and 7 of the 12 (35%) were overlaying or directly adjacent to a linear epidermal nevus. Unilateral, localized, non-Blaschkoid patches of increased terminal hair growth were observed in 8 (22%) patients; 2 were present on the dorsal portion of the hand overlying a connective tissue nevus. Eight (22%) patients displayed terminal hair overgrowth isolated to one lower extremity. Other abnormalities, observed in 14 patients, included segmental acne, asymmetric axillary hair development, discrepancies in speed of hair growth with respect to midline, and irregularities in scalp hair density. These results demonstrate that asymmetric hair growth may be a previously underreported mosaic phenotype of Proteus syndrome, and recognition of its presence may aid in earlier diagnosis. Given that Akt activation has been established to drive hair follicles toward the growth phase, we postulate that increased Akt activity within hair-bearing skin of Proteus syndrome patients may influence hair growth within these regions.

81. Metabolic Dysfunction as a Contributor to 3q29 Deletion Syndrome Phenotypes

Pollak, Rebecca; Rutkowski, Timothy; Grewenow, Stephanie; Malone, Tamika; Purcell, Ryan; Pachura, Kimberly; Wynn, Grace; Bassell, Gary; Caspary, Tamara; Dawson, Paul; Jones, Dean P.; Warren, Stephen; Weinshenker, David; Zwick, Michael; The Emory 3q29 Project; and Muller, Jennifer

3q29 deletion syndrome (3q29Del) is caused by a rare (~1:30,000) 1.6 Mb heterozygous deletion on chromosome 3 and is associated with a wide range of pediatric phenotypes, including mild to moderate intellectual disability, increased risk for autism and ADHD, and reduced birth weight and growth deficits. Individuals with 3q29Del have significantly reduced birth weight compared to matched controls (15.04oz difference, p=1.5E-6); this phenotype is recapitulated in the mouse model of 3q29Del created at Emory University (1.81g difference, 8% of body weight, p=8.8E-12). Based on the consistency of this phenotype
and the presence of four mitochondria- or metabolism-associated genes within the 3q29 interval (Pcyt1a, Tfrc, Bdh1, and Senp5), we hypothesize that there may be metabolic dysfunction in individuals with 3q29Del. Untargeted metabolomics is an ideal platform to interrogate this hypothesis. Using untargeted metabolomics in liver tissue from our mouse model, we found that palmitoylcarnitine was significantly increased in 3q29 animals after adjusting for sex (p<0.05, FC=0.314). In stratified analyses, phosphatidate was significantly increased in female mutants versus wild-type (WT) littermates (p=0.02, FC=1.997). Metabolome-wide association studies identified the carnitine shuttle and fatty acid metabolism pathways as significantly associated with these target metabolites (p<0.05), suggesting that mitochondrial function and fat metabolism may be impacted by the 3q29 deletion. We used metabolic chambers to measure gross features of metabolism in our mouse model, including food and water consumption, activity, and energy expenditure. On standard chow, the 3q29Del mice were not significantly different from their WT littermates for any of the parameters measured. After correcting for weight, 3q29Del mice did not eat significantly less than their WT littermates, did not move more than their WT littermates, and energy expenditure, as measured using indirect calorimetry, was not different. These data confirm that the 3q29Del-associated weight deficit and metabolic features are not due to decreased feeding or increased energy expenditure, and instead likely result from altered metabolism. Studies are underway to assess specific deficits in mitochondrial function and fatty acid metabolism in the mouse model of 3q29Del; together with our metabolomics results, these data may highlight metabolic pathways relevant to intellectual disability, autism, and other pediatric phenotypes.

82. Developing High-Throughput Screening Methods for Testing Drug-Induced Cardiotoxicity
Rampoldi, Antonio; Maxwell, Joshua; Liu, Rui; Fu, Haian; Du, Yuhong; and Xu, Chunhui

Current drug development relies on animal models which have limitations to predict cardiotoxicity in humans as a side-effect response to drugs, mainly due to physiological differences between human and animal cardiomyocytes. Cardiotoxicity is a severe side effect of some drugs, including those for chemotherapeutics, and is a risk factor for long-term morbidity of patients. The drugs can induce an electrophysiological dysfunction and structural damage to cardiomyocytes, resulting in loss of contractility and arrhythmia. There is the need to develop a new physiologically relevant model that can be used to reliably investigate drug-induced cardiotoxicity in high-throughput systems. Cardiomyocytes derived from human induced pluripotent stem cells (hiPSC-CMs) can serve as a novel human cell-based model for the characterization of cardiac defects. To study cell contractility, we performed live cell imaging to analyze changes in intracellular Ca2+ transients, since dysregulation of intracellular Ca2+ handling plays an important role in the pathogenesis of cardiac arrhythmias. Using a Ca2+ sensitive fluorescent dye and an automated high-throughput microscope, we detected two categories of Ca2+ release events, regular and irregular (arrhythmic) Ca2+ transients in hiPSC-CMs in a highly efficient and scalable manner. Ca2+ transients were categorized as regular if the Ca2+ transients had mostly consistent amplitudes and beat periods (rapid upstroke and decay kinetics), and instances of Ca2+ release in-between transients. Ca2+ transients were categorized as irregular if they exhibited oscillations of diastolic cytosolic Ca2+, indication of an arrhythmic events. The arrhythmic events were further classified in 4 subtypes based on the severity of Ca2+ oscillations: Type A (single notch), B (multiple notches), C (ectopic beat) and T (tachyarrhythmic). The number of cells exhibiting regular or irregular Ca2+ transients was counted, and percentages of the cells in each category were calculated for each condition tested. Our combination of hiPSC-CM arrhythmia modeling and high-throughput intracellular Ca2+ screening has the potential to predict drug-induced proarrhythmic effects in a highly efficient manner and improve the development of therapeutics for potentially life-threatening conditions.
83. Quantifying Stem Cell-Derived Cardiomyocytes Traction Forces as a Function of Differentiation Using Molecular Tension Probes
Rashid, Sk Aysha; Forghani, Parvin; Xu, Chunhui; and Salaita, Khalid

Heart disease often arises from abnormal contractile profiles of the unit muscle cells (cardiomyocytes), which comprise a major component of the human heart. There is a direct correlation between heart diseases and loss of functional cardiomyocytes. Accordingly, human-induced pluripotent stem cell derived cardiomyocytes (hiPSC-CMCs) are being investigated as a promising cell source for replacement therapeutics due to their ease of availability and high proliferation rates compared to primary cardiac cells. hiPSCs can potentially serve as a source of patient-specific cardiomyocytes, which is desirable, unlike human embryonic stem cells (hESC), as their use is noncontroversial. The process of inducing fibroblasts into PSCs is heterogenous and low efficiency. Therefore, methods that can characterize the molecular properties of hiPSC-CMCs are severely needed. While most methods rely on profiling the chemical properties (protein/gene expression), there is a need of developing methods for characterizing their mechanical properties, given the importance of mechanics to cardiomyocytes. Current technologies such as traction force microscopy, atomic force microscopy and optical edge detection are useful, however, these approaches are either serial in nature, or their spatial (≈µm²), and force (≈nN) resolutions are limited and therefore unable to map the pN forces applied by integrin adhesion receptors in CMCs. In this project, we address this need by demonstrating the use of molecular tension fluorescence microscopy (MTFM) to directly map integrin tension of CMCs and its relationship with differentiation. MTFM probes used in this study are comprised of an extendable DNA hairpin linker or DNA duplex, flanked by a fluorophore and quencher pair. Forces applied by CMCs lead to mechanical melting of DNA and an increase in fluorescence. Skin-derived hiPSCs were differentiated toward cardiomyocytes in a feeder-free environment using activin. Post-differentiated cardiomyocytes in 2D and 3D format at early (7-14 days) and late stages (> 24 days) were dissociated. Next, we measured the fluorescence signal generated by MTFM probes upon plating these cells. Varying ages of hiPSC were tested allowing us to quantify traction forces as a function of differentiation. These results indicate that the mechanical properties of cardiomyocytes can be used for functional screening of the unit muscle cells.

Ponzo, Tristan; Mendez, Adriana; Klin, Ami; Klaiman, Cheryl; Shultz, Sarah; and Jones, Warren

Parents have unique insights into their children’s development. Although clinician best estimate remains the gold standard for diagnosis of autism spectrum disorder (ASD), parents contribute critical insights that impact initial screening, symptom profile reports, and medical histories. Despite these strengths, parental impressions are inherently subjective. Here, we measure the extent to which parent-report measures can be predicted by performance-based measures of social visual engagement collected via eye-tracking. These measures offer an assessment of the real-world validity of lab-based eye-tracking measures as well as the potential for objective metrics of child behavior.

Participants were N=146 consecutive referrals (ages 1.1–4.75 years) to a community diagnostic clinic. Parents completed the Ages & Stages Questionnaires: Social-Emotional, Second Edition (ASQ:SE-2) to identify early signs of social-emotional difficulties. Children viewed video scenes of age-matched peers engaging in naturalistic social interactions while eye-tracking data were collected. After calculating
fixation time to faces, we used hierarchical logistic regression to predict parent responses on the ASQ:SE-2 from children’s eye-tracking measures.

Results show that eye-tracking-based measures significantly predict ASQ:SE-2 parent responses. Items more relevant to social visual engagement (e.g., Does your child look at you when you talk to him?) are more strongly associated (r=0.406, p<0.001), while items less relevant (e.g., Does your child hurt himself on purpose?) were unrelated (r<0.01, p>0.05). Hierarchical logistic regression yielded parameter estimates modeling (a) the odds of a child rarely or never looking at a parent’s face when talking (versus looking sometimes or most times) and (b) the odds of a child looking at least sometimes (versus most times). In both cases, eye-tracking-based measures significantly predict the odds of a child’s real-world behavior as reported by parents (t>2.6, p<0.01): for every 1% decrease in eye-tracking-based measures of face-looking, the model indicates a 15% increase in the odds that a child rarely or never looks at a parent’s face; likewise, every 1% decrease in face-looking indicates a 6% increase in the odds that a child looks only sometimes. These results highlight the real-world relevance of lab-based eye-tracking measures and offer potential for capitalizing on measures that converge with the unique insights gleaned from parents.

85. Clinical Symptoms and Abnormal MRI Findings in Pediatric Patients Diagnosed with Anti-NMDAR Encephalitis
Ratcliffe, Lauren; Blackwell, Laura; and Howarth, Robyn

BACKGROUND: Anti-N-methyl-D-aspartate receptor (NMDAR) encephalitis is an autoimmune neurological disorder first described by Dalmau and colleagues in 2007. It is characterized by acute onset of behavior changes and/or psychiatric symptoms followed by the emergence of seizures, memory loss, unresponsiveness, catatonia, dyskinesias, and autonomic instability. Diagnostic work-up typically involves labwork (presence of antibodies in the serum and/or cerebrospinal fluid [CSF]), pelvic ultrasound, electroencephalography (EEG), and magnetic resonance imaging (MRI). Previous research has noted variability in MRI abnormalities among adults, thus providing limited diagnostic and prognostic value. Little research exists on whether clinical symptoms of anti-NMDAR encephalitis are associated with MRI abnormalities in pediatric patients.

METHODS: This is a two-part study. Study one examined clinical symptoms and MRI findings within a sample of pediatric patients (N=31) with anti-NMDAR encephalitis (67% female, 61% African American; age M=10.1 years). Study two further investigated the clinical features and serial MRI findings in a subset of these patients (n=16) with MRI abnormalities.

RESULTS: Data was collected through retrospective chart review. Study One: An independent chi-square test for independence (with Yates’ Continuity Correction) indicated no significant association between MRI abnormalities and clinical symptoms (initial or cumulative). Nevertheless, an additional independent chi-square test approached significance for an association between ethnicity and MRI abnormalities, x2 (2, n=31)=5.819, p=0.055, phi=-0.71. Study Two: The subset of patients with MRI abnormalities (n=16; 69% female, 69% African American, 25% Latino; age M=9.9 years) were predominantly non-Caucasian. Results also revealed inconsistencies in regard to timepoints of MRI abnormalities relative to disease course, symptom onset to first abnormal MRI, and clinical symptoms experienced (initial or cumulative). Moreover, the number of clinical symptoms increased as the disease progressed in this group.
CONCLUSIONS: Clinical symptoms were not indicative of MRI abnormalities in a pediatric sample. Interestingly, non-Caucasian patients were more likely to have MRI abnormalities compared to Caucasian patients. Variability in MRI findings may not be useful for an anti-NMDAR encephalitis diagnosis but rather in exclusion of differential diagnoses. Future studies are warranted to assess implications of MRI abnormalities on cognitive status for pediatric patients diagnosed with anti-NMDAR encephalitis.

86. Coordinated Metabolic Reprogramming in Dendritic Cells Induced by Influenza Infection
Rezinciuc, Svetlana; Bezavada, Lavanya; Wang, Ruoning; Lopez-Ferrer, Daniel; Thomas, Paul G.; and Smallwood, Heather S.

Infection with influenza virus triggers an innate immune response aimed at initiating the humoral reaction to halt viral replication and spread. The underlying molecular mechanisms of these processes of change in innate immune cell homeostasis have not been determined and represent an area of considerable interest. Dendritic cells actively participate in the innate immunity process due to their ability to activate naive T cells. We questioned whether hampered dendritic cell metabolism controls T cell metabolism.

We have identified that, upon viral infection, dendritic cell metabolism is altered to control the uptake and catabolism of nutrients, fuelling the innate response and viral biomass production. The metabolic response of dendritic cells to viral infection differed from activation via TLR stimulation or infection with an inactive virus.

Using quantitative mass spectrometry along with metabolic flux measurements and functional assays, we found that the metabolic pathways of glycolysis and glutaminolysis were increased and oxidative metabolism was decreased, preferentially fueling the effector function of the immune cells. Also, our data suggests that, by altering the metabolism of the influenza infected dendritic cells, their function in activating T cells wasn’t affected.

Therefore, dendritic cell metabolic flexibility is a signaling mechanism that suggests metabolic switching may play a vital role in initiating the innate immune response.

87. Cell Death Induction by Engineered Reovirus in Triple-Negative Breast Cancer Cells
Rodriguez Stewart, Roxana M.; Berger, Angela K.; Guberman, Jaime; and Mainou, Bernardo A.

Triple-negative breast cancer (TNBC) constitutes approximately 15% of all breast cancer and is associated with worse prognosis when compared to other subtypes of breast cancer. There is a need for targeted therapeutics to treat this type of breast cancer as current therapies are largely limited to cytotoxic chemotherapy and radiation. Mammalian orthoreovirus (reovirus) is a nonenveloped, segmented, dsRNA virus in the Reoviridae family. Reovirus infects most humans during childhood but infection seldom causes disease. Reovirus has been shown to preferentially kill transformed cells and a serotype 3 reovirus is currently in Phase I and II clinical trials to assess its oncolytic efficacy against a variety of cancers. To engineer reovirus with enhanced infective and cytopathic properties against TNBC cells, we coinfected TNBC MDA-MB-231 cells with parental reoviruses T1L, T2J, and T3D. Following sequential serial passage, we isolated reassortant reovirus r2Reovirus. r2Reovirus has genomic segments predominantly from T1L, one gene segment from T3D, and synonymous and nonsynonymous point mutations. Infection of MDA-MB-231 cells with r2Reovirus is more efficient and induces cell death with faster kinetics than parental
reoviruses. Reovirus-mediated killing of MDA-MB-231 cells requires viral replication and is caspase-dependent but apoptosis-independent. Our data show poly (ADP-ribose) polymerase (PARP) cleavage during reovirus infection occurs in a caspase 3-independent manner, indicating PARP is cleaved by other proteases involved in alternative forms of cell death. These findings suggest reovirus induces MDA-MB-231 cell death in a manner that has not been previously observed for reoviruses. Understanding host mechanisms that reovirus uses to induce cell death will help generate an improved viral oncolytic therapeutic that specifically targets cancer cells and enhances the prognosis of patients affected with triple-negative breast cancer.

88. Using Human Induced Pluripotent Stem Cell Cardiomyocytes to Model Inflammation
Saraf, Anita; Rampoldi, Antonio; Jha, Rajneesh, Liu, Rui; Maxwell, Joshua; and Xu, Chunhui

Pro-inflammatory markers, such as TNF-α, are upregulated in multiple congenital cardiac diseases such as those modified for single ventricular Fontan physiology. Animal models for congenital heart disease are limited, hence understanding the pathophysiology of inflammatory factors on cardiomyocytes is challenging. Furthermore, evaluating long term influence of inflammation in vitro is limited by the short in vitro life span of primary cardiomyocytes. We investigated the influence of long-term exposure of TNF-α at various doses on cardiomyocytes derived from human induced pluripotent stem cells (hiPSCs). TNF-α at 1, 10, 20 and 100 ng/mL and 4 days exposure, did not affect differentiation of hiPSC-induced cardiomyocytes. Viability of hiPSC-induced cardiomyocytes decreased at higher concentrations of TNF-α (20, 100 ng/mL). Production of mitochondrial reactive oxygen species increased at 10, 20 and 100 ng/mL of TNF-α. hiPSC cardiomyocyte contraction and relaxation velocity and beat rate decreased significantly at all tested concentrations of TNF-α. In conclusion, hiPSC cardiomyocytes can be effectively used to model the influence of inflammatory factors such as TNF-α in vitro and can be used as a translational system replicating cardiomyocyte behavior.

89. Mathematical Techniques for Improving Clinical Care of Cerebral Palsy Patients
Schrum, Mariah; and Gombolay, Matthew

Cerebral palsy (CP) patients exhibit diverse, pathological gait patterns as a result of a variety of neuromuscular defects. Gait analysis provides insight into the physical limitation of a patient’s movements and is often used to inform therapeutic treatments. If these treatments are not successful, therapists iteratively apply alternative therapies to determine the best approach for an individual patient. Prior work has suggested that individual CP patients may be attempting to achieve certain optimality goals such as minimizing physiological costs or maximizing symmetry. Knowledge about the optimality principles with which CP patients are complying may serve as a better indicator for therapists when selecting a treatment plan.

We investigate applying inverse reinforcement learning (IRL) to discover these latent factors of CP gait to help clinicians gain a better understanding of an individual patient’s pathology. IRL is a method of finding a reward function and associated gait strategy that explain the observed patient EMG and motion capture data. This reward function will elucidate the self-optimization principles of the CP patient in an interpretable way and provide important insight for therapists when determining a treatment plan. Therapists will benefit from understanding how the kinematic limitations of the patient alter their goal formulations and how to tailor therapies to aid patients in achieving these goals.
Our clinical collaborators at Georgia State University have collected lower body motion capture and EMG data of CP patients. To enable our algorithms to infer patients’ goals given their kinematic limitations, we utilize the Stanford OpenSim dynamic musculoskeletal physics simulator. We apply biomechanical constraints to the simulator that match those of a CP patient and train the algorithms to reverse engineer the strategies of the patients. Preliminary results show we can learn a policy for walking with both a typical human and one with limited knee range of motion. In ongoing work, we extend our approach to providing clinicians with suggestions for how a patient’s gait may be augmented to better enable the patients to achieve their goals. As such, this novel pipeline enables both diagnosis and prescription of therapy mechanisms to support therapists.

90. BMI-1 as a Therapeutic Target in Rhabdomyosarcoma

Shields, Cara; Cuya, Selma M.; Chappell, Sarah; Rathi, Komal; Patel, Shiv; Potlapalli, Sindhu; and Schnepf, Robert W.

Rhabdomyosarcoma (RMS) is an aggressive soft tissue sarcoma which affects mainly children. There are two subtypes: alveolar rhabdomyosarcoma (ARMS) and embryonal rhabdomyosarcoma (ERMS). ARMS harbors currently undruggable PAX-FOXO1 fusion proteins and has a worse overall outcome, therefore underscoring the urgent need to identify novel targets for this cancer. We discovered that BMI-1, a protein member of the PRC1 complex is overexpressed in ARMS cells. BMI-1 is a known oncogene in other cancers, but its potential oncogenic role in ARMS has not yet been interrogated and so we aim to study it within this context.

We examined RNA-Seq tumor datasets and determined that BMI1 is robustly expressed in ARMS tumors. Additionally, we confirmed that BMI-1 is also overexpressed in ARMS cell lines at the levels of RNA and protein. Next, we depleted BMI-1 using multiple shRNAs/siRNAs and found that this led to striking (~70%) decreases in cell growth. We also observed increased levels of apoptosis within knockdown cells. We then turned to pharmacological inhibitors PTC-209 and the newer PTC-028. Both compounds inhibited BMI-1 function and greatly reduced cell proliferation in ARMS cell lines within the nanomolar range; however, as expected, PTC-028 showed a more pronounced effect compared to PTC-209. To further study the effects of PTC-028 on apoptosis and the cell cycle, we utilized Annexin V/PI staining and BrdU/7-AAD staining, analyzed by flow cytometry. We determined that PTC-028 treatment increased overall levels of apoptosis and decreased the number of cells in S phase, indicating a halt in cell cycle progression.

BMI-1 supports proliferation and survival in cell line models of ARMS. Both genetic and pharmacologic inhibition of BMI-1 led to striking decreases in cell proliferation and an increase in apoptosis, along with effects on the cell cycle. Currently, we are further investigating the molecular impact of BMI-1 inhibition, with plans to investigate its effectiveness within an in vivo ARMS model. Targeting BMI-1 pharmacologically could provide a novel therapeutic option for pediatric patients with ARMS and may apply more broadly to other sarcomas.

91. Mobilization of Mechanically Ventilated Pediatric Patients Using a Liberation Bundle

Shilt, Nataly; Jain, Shabnam; Delmeida, Mary; and Graessle, Shelby
Mobilization of mechanically ventilated (MV) intensive care unit (ICU) patients has been associated with improved functional outcomes. While mobility in MV children is feasible, barriers such as perception of patient safety, staff resources, and lack of education make consistent mobilization challenging. This single-center quality improvement initiative was designed to increase mobilization of MV patients through the implementation of an ICU Liberation bundle.

We included pediatric patients requiring MV for >48 hours. Review of mobility practices at our institution in 2017 provided the pre-implementation data. The post implementation phase spanned over 6 months, September 2018 through February 2019. The ICU liberation bundle included five facets: sedation and analgesia, sleep hygiene, delirium assessment, family engagement and mobility using an automated physical (PT) and occupational (OT) therapy order on admission. The main outcome measures were percent MV patients who were mobilized and time in days from MV to mobility. Process measures included time in days from MV to PT/OT consult order and from eligibility (readiness to mobilize) to mobility.

In 2017, 16% (51/316) MV patients were mobilized. The aim was to increase the percentage of mobilization to 40%. By February 2019, 46% (44/95) MV patients were mobilized. The median time from MV to PT/OT consult order was 3 days (IQR 1-3) unchanged from the pre-implementation phase, median of 3 days (IQR 1-7). The time to mobility increased by one day in the post implementation phase, 8 days (IQR 4-12.75) compared to 7 days (IQR 3-12). Patients were mobilized as soon as they were eligible. The median time from MV to eligibility was 8 days (IQR 4.25-12.5) and time from eligibility to mobility was zero days (IQR 0-1). There were no unplanned extubation events and only 2% of mobility sessions had a desaturation event in the post intervention period.

Implementation of an ICU liberation bundle in a mixed medical-surgical pediatric ICU was associated with an increase in mobilization of mechanically ventilated children. Mobility of MV pediatric patients is feasible but requires a multidisciplinary approach. Future quality improvement project would target decreasing time to mobilization.

92. Outpatient Ambulatory Departments’ Impact on Pediatric Patient Satisfaction
Silver, Susan; Dyjak, Patrick; Basaria, Kashif; Ingle, Alexis; Albu, Vanessa; Agosto, Katelyn; Shah, Yash; Basdeo, Devina; Mejias, Arnaldo; and Ette, Donna

BACKGROUND: It can be seen in numerous health care facilities that the quality of care provided is highly impacted by a variety of factors at a department level. Research suggests that adequate departmental performance leads to increased patient satisfaction and improved quality of the care provided. Medical care alone does not dictate patients’ perception of satisfaction but also relies on external elements, including cleanliness and positive interpersonal relationships.

PURPOSE STATEMENT: The purpose of this study is to better understand how the performance of hospital departments impact the overall satisfaction of pediatric patients in an outpatient ambulatory setting.

METHODS: Patients were surveyed about their experiences within four departments in an outpatient pediatric ambulatory clinic: registration, facilities, test/treatment services, and specialty services. Patients indicated their experiences with seven criteria in the four departments. A quantitative, causal-comparative approach was used to identify process opportunities that may significantly increase patient satisfaction.
while simultaneously increasing quality outcomes for patients. Initially, a MANOVA was conducted in order to compare the hospital units on the seven criteria across hospital units.

FINDINGS: The MANOVA findings identified two statistically significant criteria: 1. over 90% of patients were satisfied with the immaculate condition of the hospital. 2. 95% of patients reported having a positive rapport with their health care providers and further, were very satisfied with the education and explanations that they received (p<0.003 and p<0.002 respectively).

CONCLUSIONS: The findings of this analysis suggest that patients have a positive perception in regards to the cleanliness of the hospital environment. The level of satisfaction with the education patients received, while not low, may be improved. Nonetheless, findings from this study certainly show positive outcomes in the areas assessed.

93. Advanced Registered Nurse Practitioner’s (ARNP) Impact on Pediatric Outpatient Ambulatory Clinic Patient Satisfaction

Silver, Susan; Whiting, Victoria; Johnson, Sofie; Monasterio, Julia; Alqasemi, Rama; Ernest, Timothy; Sidhom, Natalia; Daya, Joshua; Basdeo, Devina; Mejias, Arnaldo; and Ettel, Donna

BACKGROUND: Evidence from the literature has provided insight about issues concerning patient satisfaction affect perceived care by the patients. Nurse practitioners play an important role in health care by developing patient-centered care models, and improving both patient outcomes and satisfaction simultaneously. There may be an association between patients’ perception of their quality of care and the ARNP’s clinical performance. There is a need for greater understanding of the relationship between ARNP’s and patient satisfaction.

OBJECTIVE: The study aims to examine the impact of ARNP’s on the pediatric patient perception of satisfaction in the outpatient ambulatory clinic; and to make recommendations for future practice.

METHODS: A retrospective review of 712 patient surveys were analyzed using a quantitative, causal-comparative approach using a multivariate analyses of variance (MANOVA). Effect size was calculated using Mahalanobis Distance. The independent variable is the ARNP to whom the patient was assigned. The dependent variables are the wait time in the examination room before being examined by a healthcare provider, the wait time before the healthcare provider explained the findings upon completion of procedures, the respectfulness of the physician, the respectfulness of the ARNP, the perception of facility cleanliness, facility accessibility, and proper patient education and rapport.

FINDINGS: Results of the MANOVA identified statistical significance of the following three criteria: (1) 63% of patients experienced a wait time of less than ten minutes in the examination room before being examined by a healthcare provider (p<0.0003); (2) 74% of patients experienced a wait time of less than ten minutes before the healthcare provider explained the findings upon completion of procedures (p<0.002), and (3) 95% of patients reported physician respectfulness (p<0.002). Data on ARNP respectfulness, facility cleanliness, accessibility, and patient education and rapport did not provide statistically significant results.

CONCLUSION: Overall, the patients and their parents were very satisfied with their physician encounter in regards to physician respectfulness. Wait time continues to be a driving factor in patient satisfaction. Efforts to improve timeliness may unlock the doors to maximizing patient satisfaction.
94. Positive Physician Interaction Promotes Pediatric Patient Satisfaction
Silver, Susan; Thomas, Laurel; Sepehri, Farrah; Lyn, Stephanie; Lee, Alexander; Leone, Brianna; Jagannath, Vybhav; Basdeo, Devina; Mejias, Arnaaldo; and Ettel, Donna

BACKGROUND: The literature suggests that patient satisfaction is a driving factor in healthcare delivery. The objective of this study was to examine how physicians impact patient satisfaction scores at a multisystem non-profit children's hospital.

DESIGN/METHODS: A quantitative, causal-comparative approach was used to identify process opportunities that may significantly increase patient satisfaction while simultaneously increasing quality outcomes for patients. The sample consisted of on-site patient satisfaction surveys (n=712) from 2018 that were provided by the hospital. The independent variable was the physician the patient was assigned to. The dependent variables included time in examination room before seeing the physician, time in examination room waiting for physician to present findings, physician respectfulness, ARNP respectfulness, cleanliness, accessibility, and patient education and rapport. A MANOVA was conducted in order to compare physician performance across the seven criteria.

FINDINGS: The MANOVA findings identified five statistically significant differences of the seven total criteria: (1) 70% of patients experienced a wait time before being examined by physician that was less than ten minutes (p<0.025); (2) 74% of patients experienced a wait time that was less than ten minutes before the physician presented findings (p<0.0001); (3) 95% of patients indicated physician respectfulness (p<0.05); (4) 98% of patients indicated that the hospital was accessible (p<0.05)) and (5) 90% of patients experienced satisfactory education and rapport (p<0.05). ARNP respectfulness and cleanliness data did not provide statistically significant results.

CONCLUSIONS: Patients and their parents were very satisfied with their physician encounter in regards to education and rapport development. Wait times will continue to be a driving factor of patient satisfaction. The length of the wait may be a factor in patient and parent anxiety. Despite the national effort placing emphasis on improving the delivery of high quality care and service, it is critical to note that hospital administrators who consider the significance of dealing with access issues such as reducing wait times or easing the burden of patient scheduling may unlock the doors to maximizing patient satisfaction.

95. A Single Regional Perinatal Center’s Experience with the 7th Edition NRP Recommendations for Meconium Stained Amniotic Fluid
Soorneela, Shrawani; Wachtel, Eleva V.; Goodly, Destini; Paredes, H.; and Desai, Prateen V.

BACKGROUND: Meconium aspiration syndrome (MAS) is a leading cause of morbidity and mortality in full term infants. Of the infants born through meconium stained amniotic fluid (MSAF), 3-12% develop MAS. In 2015, the neonatal resuscitation program (NRP) revised its recommendations to no longer performing routine intubation and suctioning below the vocal cords for non-vigorous infants born through MSAF.

METHODS: We performed a retrospective chart review of all live born, non-vigorous, full term (≥37 weeks gestation) infants born through MSAF at New York University Langone Health Center (NYULHC) over a two year period from January 2016 to December 2017. The first year assessed infants resuscitated using 6th edition recommendations and the second year, infants resuscitated with 7th edition recommendations. We also conducted an anonymous cross sectional survey of delivery room personnel.
assessing their knowledge of, attitude towards, and self-reported compliance with the new NRP recommendations.

RESULTS: A total of 274 patients met criteria for study inclusion. Fewer patients (32%) were admitted to the NICU in 2017 following the change in NRP guidelines compared to 46% in 2016, p=0.016. The incidence of MAS was also reduced following the change, with 17% of infants born through MSAF developing MAS in 2017 compared to 29% in 2016, p=0.02. There was no significant difference in the severity of MAS between the two eras. Most (94%) of our survey respondents reported at least some familiarity with the recommendation updates and most (80%) correctly answered questions regarding the management updates. The vast majority of respondents (96%) reported compliance with the updated recommendations.

CONCLUSIONS: After implementation of the 7th edition NRP recommendations, the incidence of MAS and NICU admission rates for non-vigorous infants born through MSAF at our institution decreased though severity of disease in those who developed MAS remained unchanged. We also found that the majority of our delivery room personnel were familiar with and knowledgeable about the updated recommendations and complied with change in practice.

96. Application of Metabolomics for Evaluation of the Mechanism of Action of an Antibacterial Oligomeric Polyphenol from Cladophora Socialis Against Methicillin-Resistant Staphylococcus Aureus

Sweeney-Jones, Anne Marie; Fernández, Facundo M.; and Kubanek, Julia

Evaluating the mechanism of action (MOA) of small molecule drugs is an important step in bringing a drug to market as it provides insights about the interactions responsible for the molecule’s pharmacological effect. Identifying the biological targets of a drug can lead to optimization of the drug molecule to improve binding properties, detection of possible resistance pathways that might develop, and determination of binding events that might lead to side effects. One method that has found increasing utility in evaluating a drug's MOA is metabolomics, which allows for systematic, unbiased analysis of metabolic outcomes from drug exposure. Metabolomics provides a wealth of information about the effects of drugs on a pathogen by identifying perturbations that are present downstream of biomolecular processes.

Numerous studies have evaluated the metabolome of methicillin-resistant Staphylococcus aureus (MRSA), including experiments to understand metabolic pathways impacted by drugs of known MOA's. These methods can be applied to study effects of novel antibiotics, such as a series of oligomeric polyphenols recently identified from a Fijian green alga, Cladophora socialis, with potent and selective activity against MRSA. These compounds, cladophorols A–I, were discovered using a combination of nuclear magnetic resonance spectroscopy, mass spectrometric analysis, and computational modeling. MRSA will be incubated with a sublethal dose of cladophorol D, which has an MIC90 of 9±2 µg/ml against MRSA and no potency against other tested pathogens, to evaluate metabolite changes. Metabolomes will be compared for untreated MRSA and MRSA exposed to drugs that target different pathways, including penicillin, ciprofloxacin, streptomycin, and monensin, as well as cladophorol D. Based on the resulting data, predictions will be made about plausible MOA's by which the cladophorols inhibit MRSA growth.
97. Single Cell Transcriptional Profiling of TNF-Alpha Stimulated Peripheral Blood Mononuclear Cells from Patients with Untreated Juvenile Idiopathic Arthritis

_Treadway, Nicole; Duan, Mexiue; Kosters, Astrid; Gergely, Talia; Arafat, Dalia; Prince, Chengyu; Thakral, Amit; Taneja, Angela; Chandrakasan, Shanmuganathan; Ghosn, Eliver; Gibson, Greg; and Prahalad, Sampath_

BACKGROUND: Juvenile Idiopathic Arthritis (JIA) is an autoimmune mediated inflammation of synovium affecting 1 in 1000 children. Currently, at onset, physicians cannot predict the clinical course of disease with regard to severity or symptom duration and subtypes of disease do not respond homogeneously to treatment options. Recent utilization of methods including flow cytometry and RNA transcript analysis have allowed researchers to examine immunologic cellular populations and dissect inducible immune pathways, offering an improved biological basis to diagnose and stratify patients with autoimmune diseases and predict patient outcomes.

METHODS: This pilot study utilized Chromium 10x single cell RNA sequencing (scRNAseq) to examine cellular populations and transcript expression in PBMCs from children with newly diagnosed oligoarticular or polyarticular JIA. Whole blood samples from 4 newly diagnosed patients and 2 healthy controls were stimulated for 24 hours _in vitro_ with TNF-alpha to enable analysis in the context of simulated active disease.

RESULTS: We obtained approximately 2,000 single PBMC from each of 12 samples (unstimulated and TNF-alpha treated), at an average read depth of 50,000. Clustering with Seurat and a variety of other emerging R scripts for single cell processing identified between 9 and 14 cell types per sample, including multiple T-cell subtypes, NK cells, two major B cell sub-types, monocytes, macrophages and other minor components. Cell-type abundance varies significantly among individuals, and is also affected by TNF-treatment. We also observe differential expression within cell types among samples, which is associated with treatment in some cases, and differentiates individuals in others. No clear trends emerged when contrasting healthy controls and JIA patients in our preliminary analyses of this small sample.

DISCUSSION/CONCLUSION: We are working toward robust procedures for monitoring the complete network of differential gene expression at the level of contributing peripheral blood cell types. This pilot study sets the stage for large-scale personalized immune-transcriptomics that we hope may distinguish patients with different classes of immune-mediated disease.

98. Facilitators and Barriers to the Training and Maintenance of Young Persons’ Advisory Groups (YPAGs)

_Tsang, Vivian W. L.; Chew, Siaw Yee; and Junker, Anne K._

INTRODUCTION: Increasing demands from public and private healthcare coupled with national initiatives in patient-oriented research encourages patients to be more directly involved in the research process. The push towards child and youth participation in research resulted in the formation of paediatric patient advisory groups with broad partnerships and consultations requests worldwide. However, there is a lack of evidence that examines the challenges in formation and training of young persons’ advisory groups (YPAGs) and management process involved thereafter.

PURPOSE: This study’s purpose is to document YPAG formation and training protocols around the world, highlight common strengths, and evaluate pitfalls and challenges. The results from this study will
subsequently inform the development of standardized training protocols for children and youth to be piloted globally.

METHODS: 17 select YPAG team leaders from 7 countries were surveyed to determine their current training techniques. 17 youth and 15 team leaders were then interviewed to gather further qualitative data on facilitators and barriers that aid or prevent successful initiation and maintenance of these groups. Qualitative interview data was coded and analyzed using NVivo.

RESULTS: The most common training topics include consent and assent (64.71%), clinical trials (64.71%), and patient safety (70.59%). Most YPAGs receive no formal training (58.82%) while training sessions in the remaining 7 groups vary in both duration and frequency. Collectively, meetings ranged from 15 minutes to 6 hours long, with the majority of team meetings being 2-3 hours long (58.82%). The most common training facilitators are a positive relationship with a local hospital (82.35%) and access to a dedicated team coordinator (64.71%). The most common barrier identified by 70.59% of team leaders is the lack of access to appropriate educational materials.

CONCLUSION: Bringing children and youth to the forefront of paediatric trials and clinical research facilitates appropriate patient representation in subsequent research decision-making. There is an urgency to create standardized protocols for the training of children and youth, especially in preparation for national and international research consultations. Recommendations are suggested based on the goals, training, support, and contractual arrangements necessary for successful YPAG initiation and maintenance.

99. Blood mQTL Effects are Consistent During the Course of the Disease Regardless of Remission or Relapse Status in Pediatric Crohn’s Disease Patients
Venkateswaran, Suresh; Somineni, Hari; Kilaru, Varun; Cutler, David; Smith, Alicia; Conneely, Karen; and Kugathasan, Subramanium

BACKGROUND: Our recent findings suggested that Crohn’s Disease associated DNA methylation (DNAm) signatures in blood are a transient consequence of inflammation. Here, we examined the longitudinal relationship between genetic variants and DNAm levels in blood of pediatric CD patients at diagnosis and later time point.

METHODS: In total, 402 samples obtained from 164 newly diagnosed pediatric CD patients at two time points – at diagnosis and follow-up. Genome-wide DNAm profiling and Genome-wide genotyping were done using Illumina HumanMethylationEPIC ‘850K’ and Illumina Multi-Ethnic Genotyping arrays. Missing genotypes were imputed with 1000 Genomes phase3 using IMPUTE2. MatrixeQTL, an R package was used to test the association between variant and CpG sites in both cis (±500 kb) and trans (>500 kb on the same chr or different chr). Age, gender, disease status (case-control), cell-type proportions and the first 3 genotype-based principal components were used as covariates.

RESULTS: A total of 3,109,863 high quality imputed SNPs were tested against 609,192 CpGs in 164 cases at baseline; and follow-up, separately. We detected 236,936; and 194,282 mQTLs in the analyses, respectively (Bonferroni p<8.2 x 10-14; Table 1). ~95% of mQTLs from each dataset were cis-acting. Of the 142 known CD-associated SNPs from large, meta-analyses of genome-wide association studies, 28 (23.3%) were found to exert mQTL effects. Of the 1189 CpG sites previously reported as differentially methylated between controls and cases at baseline, 22 (1.6%) demonstrated significant associations with
SNPs. On the other hand, despite a significant change in CRP levels between baseline vs. follow-up patient samples (P = 8.4 x 10^-9), we noted an extremely strong correlation in mQTL effects (R^2=0.99; P<2.2 x 10^-16).

CONCLUSION: Our data suggest that the blood mQTL effects remain consistent during the course of the disease in pediatric CD patients, irrespective of the improvement in inflammatory status, suggesting that these mQTL effects are not modified by inflammation. Whether or not the associations of mQTL are causally related to IBD is uncertain. Our findings support the utility of DNAm and mQTL to improve the functional interpretation of CD-associated genetic variation that is predominantly localized to non-coding regions.

100. Pediatric Feeding Concerns Within 3q29 Deletion Syndrome Phenotypes
Wawrzzonek, Addam; Burrell, T. Lindsey; Pollak, Rebecca; Nuhu, Nadratu; DeBarros, Berhane; Sharp, William; and Mulle, Jennifer

INTRODUCTION: 3q29 deletion syndrome (3q29DS) is caused by a rare 1.6 Mb heterozygous deletion on chromosome 3 and is associated with a wide range of phenotypes, from neurodevelopmental and neuropsychiatric disorders to reduced birth weight and growth deficits. Common comorbid diagnoses of 3q29DS are gastrointestinal (GI) complications, autism, intellectual disabilities and global developmental delays. Little is known about the phenotype of individuals with this disorder. However, the known increased risk for co-occurring disorders may also place them at risk for feeding problems food refusal or food selectivity. Feeding problems can further contribute to a number of nutritional and medical sequelae that would further complicate their medical complexity. Therefore, the purpose of the current study is to evaluate the feeding problems that present in individuals with 3q29 deletion syndrome.

METHODS: The present study conducted a review of survey data from families of individuals with 3q29DS in order to characterize their medical and psychological symptoms. An eleven item measure assessing the type and severity of feeding problems were completed by 127 caregivers of children with 3q29DS.

RESULTS: While 21.25% of all individuals reported feeding problems, rates were higher within individuals with comorbid gastrointestinal complications (41.67%), ASD (52.63%), ID (61.53%) and Global Developmental Delay (62.06%). Individuals from all four subgroups also reported higher dependencies on formula supplementation or feeding tube dependence. Specific feeding concerns reported within each population will be described.

DISCUSSION: The initial data indicates that many individuals with 3q29DS experience feeding concerns, but those with comorbid diagnoses of gastrointestinal complications, ASD, ID and GDD report much higher rates. Given that this population is at higher risk for failure to thrive, have reduced birth weights and more significant growth deficits, this information can be used to predict which individuals would most benefit from early childhood feeding treatment to decrease the need for more invasive medical intervention and potential nutrition related deficiencies and diseases.

101. Acoustic Emission Based Assessment of the Knee in Juvenile Idiopathic Arthritis
Whittingslow, Daniel; Jeong, Hyeon-Ki; Orlandic, Lara; Gergely, Talia; Ponder, Lori; Abramowicz, Shelly; Inan, Omer; and Prahalad, Sampath
102. Assessment of Nutrition and Socioeconomics in Pediatric Post-Transplant Kidney Patients (PUSHUP Study)

Wilkerson, Alexandria; Kang, Christy; Wetzel, Martha; McCracken, Courtney; Ohamadike, Onyinye; Kamel, Margret; and George, Roshan

INTRODUCTION: Nutrition and physical activity are important factors for patients with Chronic Kidney Disease (CKD) both pre-and post-transplantation. Specifically, for post-transplant patients, adequate nutrition and physical activity are important for optimal recovery. Nutrition is influenced by several factors such as pre-transplant nutritional status, time after transplant, side effects of immunosuppressive drugs, and current kidney function. Malnutrition and obesity represent a contraindication to transplantation in many cases and may increase the risk of postoperative complications.

METHODS: Data was collected from 62 patients (42 males, 20 females), who were ≥1-year post-transplant with functioning allograft. Nutrition and physical activity were assessed using the Family Nutrition and Physical Activity Screening Tool (FNPA), which is designed to assess family, environmental, and behavioral factors that may predispose a child to becoming overweight. Patients’ Body Mass Index (BMI) and Percentage Body Fat (PBF) was measured using Bioelectrical Impedance Analysis (BIA) Family economic status information was collected via self-report.

RESULTS: Patients’ ages ranged from (9 -21) with a median age of 15. 68% were males; 48% Caucasian, 37% African American, 14% other. Reported household incomes show 50% (n=29) with an annual income of <$50,000. Among those respondents, nutrition significantly varied between low and high-income groups (> $50,000 vs < $50,000; p=0.022). Additionally, BMI (p<.001) and PBF (p=0.027) scores were significantly higher for patients that reported an income < $50,000. Further analysis shows that female patients (n=10) in lower income ranges reported a higher PBF (p=0.023) and FNPA: Nutrition (p=0.010).
CONCLUSION: Nutrition impacts outcomes post-transplant including BMI and PBF. Patients of lower socioeconomic status are more at risk to have higher BMI. Higher BMI has traditionally been associated with delayed post-transplant function. These findings provide important implications for how healthcare providers can educate transplant recipients about the impact of appropriate nutrition on post-transplant outcomes.

103. Meniscus Root Tears in Children and Adolescents
Willimon, S. Clifton; Busch, Michael T.; and Perkins, Crystal A.

BACKGROUND: The medial and lateral menisci function to optimize force transmission across the knee by increasing contact area between the femur and tibia, absorbing shock, and transmitting loads. Injuries to the meniscus root attachments result in extrusion of the meniscus, impaired distribution of hoop stresses, and degenerative articular wear. The purpose of this study is to describe meniscus root tears, associated injuries, and treatment in a series of pediatric patients

METHODS: A single-institution retrospective review was performed of consecutive patients less than 19 years of age treated for meniscus root tears with minimum 12 month follow-up. The primary outcome was surgery for revision meniscus repair.

RESULTS: 23 patients were identified, including 12 males and 11 females with a mean age of 15.4 years (range 7 – 18 years). Basketball, soccer, and football accounted for the majority (56%) of injuries. The lateral meniscus was involved in 17 patients (74%) and the medial meniscus in 6 patients (26%). The posterior meniscus root was torn in 22 patients (96%) and anterior meniscus root in 1 patient (4%). The most common injury pattern was a lateral meniscus posterior root tear (16 patients, 70%). Associated injuries included an ACL tear (15 patients, 65%), PCL tear (5 patients, 22%), and a tear of the opposite meniscus (6 patients, 26%). Two root tears occurred in isolation, and both were of the posterior root of the medial meniscus.

All patients were treated surgically with transosseous root repair in addition to treatment for their associated injuries. Mean follow-up was 27 months (range 12 – 62 months). No patients required additional surgery for their meniscus root tear. Two patients had a second surgery on the affected knee: one for revision ACL reconstruction 2 years following the primary procedure and the other for chondroplasty of the patella 2.5 years following the primary procedure.

CONCLUSIONS: Meniscus root tears occur in pediatric and adolescent patients, most commonly affecting the posterior root of the lateral meniscus and occurring in association with ACL tears. In our case series, transosseous root repair resulted in successful outcomes in all patients without need for any additional meniscus treatment.

104. Twitter Users’ Opinion and Reactions Toward Autism Awareness Campaign Messages
Yin, Jingjing; Kaleta, Jeffrey; and Khan, Sushmita

Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder that has gradually increased in the last few years globally and in the US, from 0.47% to 1.46%. Given the rate of increase of ASD, the first autism awareness month started in April 1970 and is practiced till date. Every year during this month, Autism campaigns are launched on social media platforms, like Twitter, to draw general public's attention,
awareness and acceptance to those diagnosed of autism and their families. In this study we evaluate tweets pertaining to autism collected during the autism awareness month in 2015. We investigate social media users’ attitude and opinions via sentiment analysis and utilize term frequency analysis and association mining to locate and uncover the relationship between recurring hashtags, mentions and keywords. Finally, to measure the influence of such messages a survival analysis is conducted. The results suggest that messages over Twitter are, for the most part, in support of those living with autism or for the campaigning of autism awareness. We also see a counter campaign expressed via negative messaging trying to perpetuate the myth linking vaccines to autism. Our analysis results suggest the positive ‘support’ messages on social media platform is effective in delivery thus health care supporters of autism awareness should continue with such campaign on social media. In the meantime, counter messaging is necessary, which will alleviate incorrect information and beliefs from public.

105. Examining the Monkey Fetal Brain in Uterus with Advanced MRI Techniques on a 3T Clinical Scanner
Zhang, Xiaodong; Li, Chun-Xia; Kempf, Doty; Milla, Sarah; and Chan, Anthony

BACKGROUND: As reported by CDC, birth defects affect about 3% of all babies born in the United States each year, and are the leading cause of infant deaths. Nonhuman primates (NHP) share high similarities with humans including brain anatomy, physiology, immunology, reproduction, cognitive capacity and social behaviors, and are widely used in biomedical research including congenital malformations induced spontaneously or experimentally by drugs, toxic chemicals, ZIKA viral infection, et al. In this study, we exploited the advanced MRI techniques to examine rhesus monkey fetus in uterus on a clinical 3T clinical scanner.

METHODS: Monkey fetal MR images were collected with a Siemens 3T TIM Trio clinical scanner. A 4-channel Siemens FLEX coil was placed around the abdomen of the pregnant dam. The T2-weighted structural images, diffusion MRI images with b=1000 s/mm² and 30 directions, resting state functional MRI (rsfMRI) were collected by using parallel imaging technique for accelerating the data acquisition. The animal was breathing spontaneously and anesthetized with ~1.5% isoflurane during scanning. The structural, diffusion tensor imaging (DTI), rsfMRI data were processed and evaluated using Siemens Syngo software, FSL and AFNI software respectively. The multi-parameter MR images of fetal brain of a pregnant rhesus monkey (2nd trimester) were demonstrated.

RESULTS: The cerebral gyrus and sulcus of the fetal brain are well-delineated using T2-weighted images. The grey matter and white matter (corpus callosum) of whole fetal brain are clearly demonstrated on fractional anisotropy (FA) and diffusivity (mean, axial, radial) maps. Functional connectivity between medial prefrontal cortex and posterior cingulate cortex is observed and illustrated. Also, it is seen that proper administration of anesthesia reduced the fetus motion dramatically, allowing for improvement of DTI image quality with high resolution.

CONCLUSION: Our preliminary MRI data of monkey fetus in utero demonstrate that MRI is a robust approach to examine the fetal brain structural anatomy, grey matter and white matter abnormality, and functional connectivity non-invasively and longitudinally. As NHP highly mimics the reproduction of human in comparison to rodents, application of the contemporary high-field MRI techniques in monkey fetus in utero can facilitate translational research of birth defect substantially.
### Abstract Author Index

Authors are listed in alphabetical order by last name.

<table>
<thead>
<tr>
<th>A</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abramowicz, Shelly, 75</td>
<td>Boyd Barr, Dana, 10</td>
</tr>
<tr>
<td>Agalliu, Dritan, 21</td>
<td>Bradley, Heath L., 2</td>
</tr>
<tr>
<td>Agalliu, Ilir, 21</td>
<td>Braz Gomes, Keegan, 38</td>
</tr>
<tr>
<td>Agosto, Katelyn, 68</td>
<td>Bricker, Katherine, 19, 53</td>
</tr>
<tr>
<td>Albu, Vanessa, 68</td>
<td>Brown, Lou Ann, 31, 48</td>
</tr>
<tr>
<td>Allen, Sarah, 14</td>
<td>Burrell, T. Lindsey, 7, 52, 74</td>
</tr>
<tr>
<td>Alqasemi, Rama, 69</td>
<td>Busch, Michael T., 58, 59, 60, 76</td>
</tr>
<tr>
<td>Alvarado, Maria, 13</td>
<td>Bushehri, Yousef, 23</td>
</tr>
<tr>
<td>Amanso, Angelica, 38</td>
<td>Calamarno, Christina, 22</td>
</tr>
<tr>
<td>Ammar, Zena, 14</td>
<td>Camacho-Gonzalez, Andres, 32</td>
</tr>
<tr>
<td>Ampatey, Nicole, 21</td>
<td>Cammarata, Alexandre, 30</td>
</tr>
<tr>
<td>Anderson, Larry J., 20, 33, 35</td>
<td>Caprio, Sonia, 7</td>
</tr>
<tr>
<td>Arafat, Dalia, 72</td>
<td>Casero, Robert, 27</td>
</tr>
<tr>
<td>Areces, Marcos Bouza, 43</td>
<td>Caspary, Tamara, 61</td>
</tr>
<tr>
<td>Aumann, Waitman, 6, 34, 39</td>
<td>Castellino, Sharon, 3</td>
</tr>
<tr>
<td></td>
<td>Chahroudi, Ann, 13, 19, 53</td>
</tr>
<tr>
<td></td>
<td>Chan, Anthony, 78</td>
</tr>
<tr>
<td></td>
<td>Chandler, Joshua D., 2, 42</td>
</tr>
<tr>
<td></td>
<td>Chandrakasan, Shanmuganathan, 33, 54, 72</td>
</tr>
<tr>
<td></td>
<td>Chappell, Sarah, 67</td>
</tr>
<tr>
<td></td>
<td>Chatterjee, Paramita, 46</td>
</tr>
<tr>
<td></td>
<td>Chen, Dongdong, 21</td>
</tr>
<tr>
<td></td>
<td>Chew, Siaw Yee, 73</td>
</tr>
<tr>
<td></td>
<td>Chirkova, Tatiana, 20</td>
</tr>
<tr>
<td></td>
<td>Chitre, Neha, 36</td>
</tr>
<tr>
<td></td>
<td>Christina, Melissa A., 58, 59, 60</td>
</tr>
<tr>
<td></td>
<td>Cioffi, Catherine E., 7</td>
</tr>
<tr>
<td></td>
<td>Cirrilo, Piera, 9</td>
</tr>
<tr>
<td></td>
<td>Cohenour, Jessica, 7, 52</td>
</tr>
<tr>
<td></td>
<td>Cohn, Barbara, 9</td>
</tr>
<tr>
<td></td>
<td>Collin, John, 49</td>
</tr>
<tr>
<td></td>
<td>Conneely, Karen, 8, 24, 74</td>
</tr>
<tr>
<td></td>
<td>Conway, Amanda, 6</td>
</tr>
<tr>
<td></td>
<td>Cormier, Stephanie, 4, 15</td>
</tr>
<tr>
<td></td>
<td>Costello, Kaitlin, 20</td>
</tr>
<tr>
<td></td>
<td>Costa, Megan, 31, 37</td>
</tr>
<tr>
<td></td>
<td>Cunningham, Charlene, 22</td>
</tr>
<tr>
<td></td>
<td>Curtis, Sarah, 8</td>
</tr>
</tbody>
</table>
Cutforth, Tyler, 21
Cutler, David, 24, 57, 74
Cuya, Selma M., 21, 67

Dalal, Nupur, 22
Darling, Thomas, 61
Dave, Ishaan, 25, 29
Davis, Michael, 38
Dawson, Paul, 61
Daya, Joshua, 69
Dealmeida, Mary, 68
DeBarros, Berhane, 74
Delgado, Carlos, 25
Denham, Megan, 23
Denson, Lee, 24, 51
DeRyckere, Deborah, 11
Desai, Prateen V., 71
Devincenzo, John, 4, 15
Dickinson-Copeland, Carmen M., 23
Dinh Vu, Luân, 15
Do, Priscilla, 58
Doan, Sylvia, 24
Dobosh, Brian, 2, 30
Dodd, Anne, 51
Dreaden, Erik C., 11, 58
Drissi, Hicham, 38
Duan, Mexiue, 72
Dunlop, Anne L., 10
Dyjak, Patrick, 68

Edwards, Nicole, 37
Eggleston, Emma-Morton, 49
Ernest, Timothy, 69
Escoffery, Cam, 19
Espinoza, Hillary, 33
Estroff, Brandon, 25
Ettel, Donna, 68, 69, 70

Fagin, David, 25
Ferdous, Salma, 29
Fernández, Facundo M., 4, 35, 43, 71
Finlon, Elizabeth, 31
Fitch, Vincent, 40
Ford, Aiden, 9
Forghani, Parvin, 26, 63
Fouda, Genevieve, 53
Frediani, Jennifer K., 27
Fu, Haian, 44, 62

Gala, Rikhav, 16
Gambello, Michael, 27
Gandhi, Mayank, 53
Garg, Neha, 46
Garro, Rouba, 29
Gaul, David, 4
Gavegnano, Christina, 28
Gaydos, Laura, 22
Gefke, Isabelle, 29
Geng, Rena, 25
Gentillon, Cinsley, 43
George, Roshan, 29, 76
Gergely, Talia, 25, 30, 72, 75
Ghai, Shweta, 5
Ghazal, Nasab, 57
Ghosn, Eliver, 72
Giacalone, Vincent, 2, 30
Gibson, Greg, 46, 51, 57, 72
Gillespie, Scott, 7, 52
Gladwin, Mark, 48
Gombolay, Matthew, 66
Gonzalez, Jacquelin, 52
Goodly, Destini, 71
Goudy, Steven, 38
Graessle, Shelby, 68
Graham, Douglas K., 11, 12
Greenbaum, Larry, 54
Greenwald, Roby, 31
Grewenow, Stephanie, 61
Guberman, Jaime, 65
Guerra, Karen, 31, 37
Guglani, Lokesh, 2
Gumber, Sanjeev, 13
Gunter, Chris, 11
Gupta, Nitika, 33
Gutman, Colleen, 32

Ha, Binh, 33, 35
<table>
<thead>
<tr>
<th>Hadley, Timothy, 33</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hanberry, Bradley, 29</td>
</tr>
<tr>
<td>Harrington, Amanda, 6, 34</td>
</tr>
<tr>
<td>Harris, Alan, 11</td>
</tr>
<tr>
<td>Harris, Kamryn, 23</td>
</tr>
<tr>
<td>Hartert, Tina V., 20, 35</td>
</tr>
<tr>
<td>Harvey, Samuel, 23</td>
</tr>
<tr>
<td>Hayat, Matthew, 31</td>
</tr>
<tr>
<td>Henry, Curtis J., 42</td>
</tr>
<tr>
<td>Hesselgesser, Joseph, 19</td>
</tr>
<tr>
<td>Higgins, Melinda K., 27</td>
</tr>
<tr>
<td>Hogan, Scott, 35</td>
</tr>
<tr>
<td>Horati, Hamed, 2</td>
</tr>
<tr>
<td>Howarth, Robyn, 64</td>
</tr>
<tr>
<td>Hu, Xin, 9</td>
</tr>
<tr>
<td>Huang, Danning, 4, 43</td>
</tr>
<tr>
<td>Hyams, Jeffrey, 24, 51</td>
</tr>
</tbody>
</table>

**I**

<table>
<thead>
<tr>
<th>Immergluck, Lilly, 25</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inan, Omer, 75</td>
</tr>
<tr>
<td>Ingle, Alexis, 68</td>
</tr>
<tr>
<td>Ishikawa, Tomoko, 9</td>
</tr>
<tr>
<td>Ivanova, Anna, 10</td>
</tr>
</tbody>
</table>

**J**

<table>
<thead>
<tr>
<th>Jadhao, Samadhan, 33, 35</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jagannath, Vybhav, 70</td>
</tr>
<tr>
<td>Jain, Shabnam, 68</td>
</tr>
<tr>
<td>Janssens, Hettie M., 2</td>
</tr>
<tr>
<td>Jeong, Hyeon-Ki, 75</td>
</tr>
<tr>
<td>Jha, Rajneesh, 43, 66</td>
</tr>
<tr>
<td>Jin, Lingtao, 12</td>
</tr>
<tr>
<td>Johnson, Sofie, 69</td>
</tr>
<tr>
<td>Jones, Dean P., 9, 21, 31, 48, 61</td>
</tr>
<tr>
<td>Jones, Warren, 9, 14, 45, 47, 55, 63</td>
</tr>
<tr>
<td>Joshi, Devyani, 36</td>
</tr>
<tr>
<td>Junker, Anne K., 73</td>
</tr>
</tbody>
</table>

**K**

<table>
<thead>
<tr>
<th>Kaiser, Eileen, 31, 37</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kale, Akanksha, 38</td>
</tr>
<tr>
<td>Kaleta, Jeffrey, 77</td>
</tr>
<tr>
<td>Kamalakar, Archana, 38</td>
</tr>
<tr>
<td>Kamel, Margret, 76</td>
</tr>
<tr>
<td>Kang, Christy, 76</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Kang, Sang-Moo, 48</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kang, Sumin, 12</td>
</tr>
<tr>
<td>Kapfhammer, David, 27</td>
</tr>
<tr>
<td>Kartavenka, Kostya, 31</td>
</tr>
<tr>
<td>Kato, Gregory, 48</td>
</tr>
<tr>
<td>Kazi, Rafi, 39</td>
</tr>
<tr>
<td>Ke, Zunlong, 33</td>
</tr>
<tr>
<td>Kelvin, James M., 11, 58</td>
</tr>
<tr>
<td>Kempf, Doty, 78</td>
</tr>
<tr>
<td>Kendrick-Allwood, Salathiel R., 23</td>
</tr>
<tr>
<td>Khan, Sushmita, 77</td>
</tr>
<tr>
<td>Kilaru, Varun, 8, 24, 74</td>
</tr>
<tr>
<td>Kilgore, Matthew B., 2</td>
</tr>
<tr>
<td>Killingsworth, Kayla, 40, 41</td>
</tr>
<tr>
<td>Kim, Baek, 28</td>
</tr>
<tr>
<td>Kim, Hye Ryong, 58</td>
</tr>
<tr>
<td>Kim-Hoehamer, Young-In, 15</td>
</tr>
<tr>
<td>Klaiman, Cheryl, 45, 47, 63</td>
</tr>
<tr>
<td>Klin, Ami, 9, 14, 45, 47, 55, 63</td>
</tr>
<tr>
<td>Knight, Jessica, 16</td>
</tr>
<tr>
<td>Kochilas, Lazaros, 16</td>
</tr>
<tr>
<td>Kolachala, Vasantha, 33</td>
</tr>
<tr>
<td>Kosters, Astrid, 72</td>
</tr>
<tr>
<td>Kovacs-Balint, Zsofia, 13</td>
</tr>
<tr>
<td>Krigbaum, Nickilou, 9</td>
</tr>
<tr>
<td>Kubanek, Julia, 71</td>
</tr>
<tr>
<td>Kugathasan, Subramanium, 24, 51, 57, 74</td>
</tr>
<tr>
<td>Kumareswaran, Mitra, 5</td>
</tr>
<tr>
<td>Kwong, Hiu Sze, 41</td>
</tr>
<tr>
<td>Kwong, Jennifer Q., 57</td>
</tr>
</tbody>
</table>

**L**

<table>
<thead>
<tr>
<th>La Merrill, Michele, 9</th>
</tr>
</thead>
<tbody>
<tr>
<td>LaPlaca, Michelle, 35</td>
</tr>
<tr>
<td>Lark, Catherine, 55</td>
</tr>
<tr>
<td>Lavau, Catherine, 6, 34</td>
</tr>
<tr>
<td>Lee, Alexander, 70</td>
</tr>
<tr>
<td>Lee, Brian, 29</td>
</tr>
<tr>
<td>Lee, Miyoung Lee, 42</td>
</tr>
<tr>
<td>Leone, Brianna, 70</td>
</tr>
<tr>
<td>Li, Chun-Xia, 78</td>
</tr>
<tr>
<td>Li, Dong, 26, 43</td>
</tr>
<tr>
<td>Li, Longchuan, 9</td>
</tr>
<tr>
<td>Li, Shiyong, 12</td>
</tr>
<tr>
<td>Li, Shuzhao, 9, 48</td>
</tr>
<tr>
<td>Li, Yafeng, 43</td>
</tr>
</tbody>
</table>
Li, Zhiguo, 12
Lilly, Christa L., 49
Lin, Yangjin, 3
Liu, Jingbo, 4, 44
Liu, Rui, 62, 66
Lopez-Ferrer, Daniel, 65
Lyn, Stephanie, 70

M
Ma, Chunyu, 48
MacDonald, Tobey, 4, 44
Mainou, Bernardo A., 18, 65
Makani, Dylan, 21
Malhotra, Anshu, 44
Mallory, Michael, 25
Malone, Tamika, 61
Marcus, Michele, 8
Margaroli, Camilla, 2
Marigorta, Urko M., 51
Marion, Chelsea, 54
Markert, Sarah, 45
Matthews, Jason, 51
Mavinger, Maud, 13
Maxwell, Joshua, 26, 62, 66
McAvoy, Andrew, 46
McCracken, Courtney, 7, 25, 29, 35, 52, 76
McKenna, James, 27
Medrano-Trochez, Camila, 46
Mejias, Arnaldo, 68, 69, 70
Mendez, Adriana, 47, 63
Michopoulos, Vasiliki, 11
Middlebrooks, Lauren, 32
Milla, Sarah, 78
Milligan, Kyle, 35
Mitchell, Rebecca M., 3, 27
Mitchell, Russell, 23
Mo, Angela, 51
Monasterio, Julia, 69
Morris, Claudia, 32, 48
Mosimah, Charles, 49
Mosley, Trenell, 50
Mulle, Jennifer, 61, 74
Murnane, Kevin, 36, 38
Murray, Pamela, 49
Murray-Stewart, Tracy, 27
Mustafa, Aziza, 25

Mynatt, Elizabeth, 19

N
Nagpal, Sini, 51
Narayan, K. M. Venkat, 7
Ni, Fang, 12
Nickerson, John, 29
Niklinska-Schirtz, B. Joanna, 51
Nouraei, Seyed, 48
Nuhu, Nadratu, 7, 52, 74

O
Oberfeld, Austin, 53
Obregon-Perko, Veronica, 19, 53
Ogbo, Ekemini, 54
Ogle, Molly, 46
Ogunyankin, Forest, 55
Oh, Melissa, 38
Ohamadike, Onyinye, 76
Okou, David, 57
Orlandic, Lara, 75
Orlund, Eric A., 10
Oster, Matthew, 16
Oza, Vishal H., 56

P
Pachura, Kimberly, 61
Panuwet, Parinya, 31
Paredes, H., 71
Park, Jaekeun, 44
Patel, Shiv, 67
Patterson, Alexandria, 17
Pelia, Ranjit, 57
Peng, Limin, 2
Peoples, Jessica, 57
Perdue, Lacey A., 11, 58
Perkins, Crystal A., 58, 59, 60, 76
Perlow, Gabriell, 16
Perrmar, Sallie, 53
Pierpont, Bridget, 7
Pilgrim, Adeiye, 21
Pithadia, Deeti, 61
Pollak, Rebecca, 61, 74
Ponder, Lori, 25, 30, 75
Ponzo, Tristan, 47, 63
Potlapalli, Sindhu, 67
Prahalad, Sampath, 25, 30, 54, 72, 75
Prince, Chengyu, 72
Prince, Jarod, 51
Purcell, Ryan, 61
Qu, Cheng-Kui, 12
Rampoldi, Antonio, 62, 66
Ramsay, Gordon, 5
Raper, Jessica, 11, 13
Ratcliffe, Lauren, 53, 64
Rathi, Komal, 21, 67
Raveendran, Muthuswamy, 11
Reed, Laura K., 56
Reeve, Bryce, 3
Rezinciuc, Svetlana, 4, 15, 65
Robinson, Brittany, 53
Rodriguez Steward, Roxana M., 65
Rogers, Jeffrey, 11
Roman, John, 61
Rossi, Michael R., 12
Rouster-Stevens, Kelly, 54
Roy, Krishnendu, 46
Rutkowski, Timothy, 61
Salaita, Khalid, 63
Sanchez, Mar, 11, 13
Santoro, Nicola, 7
Sanz, Iñaki, 54
Saraf, Anita, 44, 66
Sarkar, Surupa, 2
Scahill, Larry, 7
Scahill, Lawrence, 55
Scheel, Lynn, 14
Scheithauer, Mindy, 55
Schnepp, Robert W., 21, 67
Scholte, Bob J., 2
Schrum, Mariah, 66
Sears, Dorothy, 3
Sepehri, Farrah, 70
Shah, Yash, 68
Sharp, William, 7, 52, 74
Shaw, George, 53
Shepard, Caitlin, 28
Shields, Cara, 67
Shildt, Nataly, 68
Shulkin, Barry, 4
Shultz, Sarah, 9, 14, 45, 47, 55, 63
Sidhom, Natalia, 69
Silva, George L., 2
Silver, Susan, 68, 69, 70
Silvestri, Guido, 53
Simkins, James, 49
Singer, Karl, 54
Skulkin, Barry, 15
Smallwood, Heather S., 4, 15, 65
Smith, Alicia, 8, 24, 74
Smith, Matthew Ryan, 21
Somineni, Hari, 24, 57, 74
Sookyong, Koh, 19
Soorneela, Shrawani, 71
Stapel-Wax, Jennifer, 31, 37
Stecenko, Arlene A., 43
Stillman, Mark, 41
Sun, Elizabeth, 29
Sutcliffe, Jane, 53
Swedo, Susan, 21
Sweeney-Jones, Anne Marie, 71
Taneja, Angela, 72
Taylor, Morrisa, 14
Terrell, Metrecia, 8
Thakral, Amit, 72
Thomas, Amanda, 16
Thomas, Laurel, 70
Thomas, Paul G., 65
Tiddens, Harm A.W.M., 2
Tirouvanziam, Rabindra, 2, 30
Tope, Donald, 39
Tope, Robert, 34
Tran, ViLinh, 9
Treadway, Nicole, 72
Tsang, Vivian W. L., 73
Uddin, Ferzan, 53
Uppal, Karan, 4, 7
<table>
<thead>
<tr>
<th>Vargas, Wendy, 21</th>
<th>Williams, Abby, 25</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vega-Fernandez, Patricia, 30</td>
<td>Willimon, S. Clifton, 58, 59, 60, 76</td>
</tr>
<tr>
<td>Veltman, Mieke, 2</td>
<td>Withycombe, Janice, 3</td>
</tr>
<tr>
<td>Venkateswaran, Suresh, 24, 57, 74</td>
<td>Wright, Elizabeth, 33</td>
</tr>
<tr>
<td>Vos, Miriam B., 7, 27</td>
<td>Wu, Changsheng, 43</td>
</tr>
<tr>
<td>Wachtel, Eleva V., 71</td>
<td>Wynn, Grace, 61</td>
</tr>
<tr>
<td>Walters, Thomas, 51</td>
<td>X</td>
</tr>
<tr>
<td>Wang, Chuan-En, 50</td>
<td>Xiong, Niya, 2</td>
</tr>
<tr>
<td>Wang, Ruoning, 65</td>
<td>Xu, Chunhui, 26, 43, 44, 62, 63, 66</td>
</tr>
<tr>
<td>Wang, Zhong Lin, 43</td>
<td>Y</td>
</tr>
<tr>
<td>Warren, Stephen, 61</td>
<td>Yang, Jae, 33</td>
</tr>
<tr>
<td>Warshaw, Barry, 29</td>
<td>Yeago, Carolyn, 46</td>
</tr>
<tr>
<td>Wasilewski-Masker, Karen, 23</td>
<td>Yin, Jingjing, 77</td>
</tr>
<tr>
<td>Wawrzzonek, Addam, 7, 52, 74</td>
<td>Yu, Wen-Mei, 12</td>
</tr>
<tr>
<td>Wechsler, Daniel, 6, 34, 39</td>
<td></td>
</tr>
<tr>
<td>Weinshenker, David, 61</td>
<td>Zaman, Rokon Uz, 38</td>
</tr>
<tr>
<td>Wetzel, Martha, 76</td>
<td>Zhang, Xiaodong, 78</td>
</tr>
<tr>
<td>Whiting, Victoria, 69</td>
<td>Zhang, Yingze, 48</td>
</tr>
<tr>
<td>Whittingslow, Daniel, 75</td>
<td>Zhong, Julia, 14</td>
</tr>
<tr>
<td>Wilcox, William, 50</td>
<td>Zmitrovich, April, 32</td>
</tr>
<tr>
<td>Wilkerson, Alexandria, 76</td>
<td>Zughaier, Susu, 16</td>
</tr>
<tr>
<td>Willett, Nick, 38</td>
<td>Zwick, Michael, 50, 61</td>
</tr>
</tbody>
</table>