2020 SOUTHEASTERN PEDIATRIC RESEARCH CONFERENCE

Frontiers in Child Health Research

ABSTRACT & POSTER BOOK
Greetings!

The 2020 Southeastern Pediatric Research Conference was set to be an exciting day of research and networking, with prominent national and local speakers, poster sessions and ample opportunities to meet new collaborators. While the in-person event was cancelled to keep everyone safe and healthy during the COVID-19 outbreak, we are pleased to share with you the 201 abstracts that were submitted and selected for poster presentation at this conference. These abstracts represent the impressive scope of pediatric research being conducted throughout the southeast and across all 12 pediatric research centers at Children’s Healthcare of Atlanta, Emory University and Georgia Institute of Technology.

We encourage you to peruse the abstracts and take action:

- Contact the corresponding authors with questions and ideas
- Note if the authors provided a poster and check out the accompanying PDF of posters that would have been presented at the event
- Note whether an abstract was related to a pilot grant or trainee award, and learn about funding opportunities you may not have been aware of!

We are so proud of the incredible work being done to transform child health and can’t wait to see you for our next conference on June 11, 2021!

Warm regards,

Ann Chahroudi, MD, PhD
Associate Division Chief for Basic/Translational Research, Division of Infectious Diseases
Associate Professor of Pediatrics, Emory University School of Medicine
Director, Center for Childhood Infections & Vaccines (CCIV)
Medical Director, Ponce Family & Youth Clinic, Infectious Diseases Program, Grady Health System

Wilbur A. Lam, MD, PhD
W. Paul Bowers Research Chair
Associate Professor, Department of Pediatrics
Wallace H. Coulter Department of Biomedical Engineering
Aflac Cancer and Blood Disorders Center of Children’s Healthcare of Atlanta
Chief Innovation Officer, Pediatric Technology Center
Emory University and Georgia Institute of Technology
Abstracts by Pediatric Research Center (click on heading to jump to section)

Aflac Cancer and Blood Disorders Center .......................................................................................................... 2
Center for Childhood Infections and Vaccines (CCIV) ........................................................................................ 36
Center for Clinical and Translational Research (CCTR) ...................................................................................... 50
Center for Cystic Fibrosis and Airways Disease Research (CF-AIR) .............................................................. 80
Center for Drug Discovery (CDD) ...................................................................................................................... 88
Center for Pediatric Cellular Therapies (CPCT) ................................................................................................. 93
Center for Transplantation and Immune-mediated Disorders (CTID) ............................................................... 95
Children's Center for Neurosciences Research (CCNR) .................................................................................. 111
Clinical Outcomes Research and Public Health (CORPH) ............................................................................. 124
Heart Research and Outcomes Center (HeRO) ............................................................................................... 151
Marcus Autism Center .................................................................................................................................... 169
Pediatric Technology Center (PTC) ................................................................................................................ 193
Information About Pilot Grants and Trainee Awards ..................................................................................... 203
Posters ............................................................................................................................................................ 204

Please use CTRL+F to search for individual investigator names or keywords in this document.
Wilms tumor (WT) is the most common renal malignancy of childhood. Previous work has shown that WT affects black children disproportionately in comparison to white children, irrespective of nationality. Genetic aberrations that drive this cancer health disparity between black and white children have not been reported. The Children’s Oncology Group TARGET database was queried for WT patient characteristics and genomic analyses. Clinical and genomic variables were compared between patients registered as black or white. Appropriate statistical tests including Mann-Whitney U and Pearson Chi Square tests were applied to compare continuous and categorical variables. Within the TARGET discovery set (enriched for adverse events; N = 94 white, 19 black patients), no differences in clinical features or outcomes were detected. Within the TARGET validation set (random, one-third sampling of NWTS-5; N = 360 white, 92 black patients), no differences in stage, histology or event-free survival at 5 years were identified. However, black children appeared older at diagnosis (p=0.050) and over-represented relative to the US Census. Five-year overall survival was 85.1% for black patients and 88.1% for white patients (p=0.250). Median follow-up was significantly less for black patients (7.0 years) than white patients (11.0 years, p<0.000). Within the discovery set, differences between black and white specimens for copy number gain at 1q and amplification of 2p24 were not observed. Among all patients, white children were more likely to have one or multiple targeted genetic mutations compared to black children (p=0.026). Mutations in ACTB (p=0.030) and DICER1 (p=0.041) were more common in black patient specimens, whereas DGCR8 (p=0.041) mutations were more common among white patient specimens. In conclusion, black children were older at WT diagnosis and harbored specimens containing more frequent ACTB and DICER1 mutations. WT specimens of white patients more commonly contained multiple mutations in the TARGET genes and in DGCR8. Genetic alterations in WT specimens unique to race may inform mechanisms contributing to Wilms tumorigensis.
Survival rates are suboptimal for pediatric patients with acute myeloid leukemia (AML) and cytotoxic chemotherapies have short and long-term side effects. Targeted agents can reduce toxicity; however, resistance to single-agents often develops, thus combination therapies may provide more durable responses. To increase efficacy and reduce exposure to cytotoxic agents, a targeted combination therapy (MERTK/ROCKi) was utilized alone and with doxorubicin or etoposide, frontline AML cytotoxic chemotherapeutics. MERTK is aberrantly expressed in >80% of AML patient samples and inhibitors are in clinical development. Both MERTK and rho-associated, coiled-coil-containing kinases 1&2 (ROCK1/2) regulate cell cycle progression and shMERTK or siROCK1 induce apoptosis in AML cells. Here, analysis of The Cancer Genome Atlas database revealed poorer overall survival in patients with higher levels of ROCK1 mRNA (p<0.01,n=172). MERTK inhibitors (MRX-2843 or UNC3997) and ROCK1/2 inhibitors (RKI-1447 or GSK269962A) synergized to reduce cell density in 4/6 pediatric AML cell lines and had an additive effect in 2/6. Flow cytometric analysis of cells stained with live/dead viability dyes revealed synergistic induction of cell death in KG-1 (p<0.05), OCI-AML5 (p<0.05), and NB4 (p<0.1) and additive death in Kasumi-1 cultures. Cells treated with the combination therapy failed to expand, even when cultured in the absence of inhibitor(s), and cell density was reduced in response to treatment with the combination therapy compared to single agents (p<0.01). Flow cytometric analysis of DNA content revealed an increased fraction of cells with G2/M DNA content in cultures treated with MRX-2843 (p<0.01). This effect was more pronounced in cultures treated with the combination therapy (p<0.05-p<0.0001) and was accompanied by an accumulation of cells in late S phase. Thus, the combination therapy mediates anti-leukemia activity by multiple mechanisms, including abrogation of cell cycle progression and induction of cell death. Addition of MERTK/ROCKi therapy to doxorubicin or etoposide additively decreased cell density relative to chemotherapy alone in OCI-AML5 and KG-1 cultures (p<0.0001). Co-treatment with doxorubicin and MERTK/ROCKi therapy also decreased OCI-AML5 cell density compared to MERTK/ROCKi therapy (p<0.01). These data indicate that combination therapies targeting MERTK and ROCK1/2, alone or in conjunction with chemotherapy, may be particularly effective for treatment of AML.
Impact of Cryopreservation on Clinical Sample Transcriptomes Assessed by Single Cell Profiling

Bhasin, Swati; Khalsa, Harimander; Thomas, Beena; Theocharidis, Georgios; Veves, Aristidis; and Bhasin, Manoj

Corresponding Author: Swati Bhasin, PhD, Emory University, swati.sharma.bhasin@emory.edu
Center: Aflac Cancer and Blood Disorders Center
Type: Clinical or Translational
Keyword(s): single cell transcriptome analysis of cryopreserved clinical samples
Related to Pilot Grant or Trainee Award: No
Poster Available: Yes - P1

Single cell transcriptome profiling has emerged as a powerful technique for dissecting cellular heterogeneity in many different diseases. Traditional bulk analysis approaches average out the gene expression signals which complicates analysis of diverse cell types present in a given tissue sample. In diseases like cancer, it is highly desirable to identify the extent of immune infiltrate as well as the presence of immunosuppressive cell populations for a timely evaluation of response necessary for determining the course of treatment. A major limitation of single cell transcriptomics is that along with freshly prepared high viability single cell suspensions, the technique requires considerable expertise and specialized equipment which is not readily available. One alternative to using fresh single cell suspensions is to viably freeze single cells harvested from a patient. Frozen samples offer many advantages including flexibility to process at a later time and/or ship to another lab/collaborator who have the capability to process single cell samples. Our group is standardizing the protocol for single cell transcriptomics assay of viably frozen bone marrow single cell suspensions of AML patients at Emory University. The aim of our study is to generate a much more granular map of the cellular landscape in AML critical for understanding the disease progression and response to therapy. We have evaluated the efficacy of using frozen samples by comparing the transcriptomes of fresh and viably frozen cells from skin biopsy samples collected in a clinical setting from diabetic and healthy control subjects. Gene expression profiles, cellular compositions and pathways of matched 6 fresh and 6 frozen samples cryopreserved for over 3 months have been compared. In both fresh and frozen samples, we are able to generate transcriptome landscape of major cell types including fibroblasts, adipocytes, immune cells. Our analysis indicates that overall, the single cell transcriptome landscapes of fresh and viably frozen samples are quite similar. Furthermore, the comparative analysis of transcriptome profiles at genes as well as at pathways level shows significant similarity. In conclusion, cryopreservation has subtle impact on the transcriptome landscape that does not impact biological conclusions of the studies.
LIN28B-PDZ Binding Kinase Signaling Promotes Neuroblastoma Metastasis

Chen, Dongdong; Cox, Julie; Annam, Jayabhargay; Weingart, Melanie; Essien, Grace; Rathi, Komal; Rokita, Jo Lynne; Khurana, Priya; Cuya, Selma; Pilgrim, Adeiye; Li, Daisy; Shields, Cara; Laur, Oskar; and Schnepp, Robert

Corresponding Author: Julie Chen, MS, Emory University, dongdong.chen@emory.edu
Center: Aflac Cancer and Blood Disorders Center
Type: Basic
Keyword(s): Neuroblastoma, Metastasis
Related to Pilot Grant or Trainee Award: No
Poster Available: No

LIN28B is an RNA binding protein that plays key roles in normal development and, when deregulated, oncogenesis; mechanistically, it blocks the processing of the let-7 family of tumor suppressors and binds mRNAs directly. We previously demonstrated that LIN28B induces neuroblastoma proliferation, in part by regulating the expression of RAN GTPase and Aurora kinase A. Given the widespread metastases seen within neuroblastoma, we investigated whether and how LIN28B influences neuroblastoma metastasis. We generated GFP-luciferase expressing neuroblastoma cell line models in which LIN28B levels were manipulated, injected these models into the tail veins of NSG mice, and tracked dissemination using an IVIS Spectrum system. We used gain and loss of function approaches to manipulate transcripts of interest in neuroblastoma cells and measured effects on self-renewal, invasion, and downstream signaling. To discover LIN28B-associated pathways, we assessed clinically annotated mRNA expression datasets. Mice injected with LIN28B-depleted neuroblastoma cells exhibit delayed onset of tumor metastasis, reduced tumor burden, and extended survival (103 days vs. 50 days, p<0.0001), compared to mice bearing neuroblastoma cells expressing control scrambled shRNA. We next demonstrated that LIN28B promotes, and let-7 opposes, self-renewal and migration, two hallmarks of metastasis. In addition, we evaluated the TARGET dataset of neuroblastoma tumors and found LIN28B mRNA expression to be robustly correlated with PBK (PDZ-binding kinase) mRNA expression (PBK; r=0.67; p=3.2x10^-33), a kinase with roles in cell proliferation/survival, self-renewal, and metastasis that is overexpressed in multiple malignancies. We demonstrated that LIN28B directly promotes, and let-7 opposes, the expression of PBK protein, and, indeed, that PBK is a novel and direct let-7 target. Moreover, we revealed that MYCN binds to the promoter of PBK and positively regulates PBK RNA and protein expression. Finally, PBK depletion mimics the effects of LIN28B depletion, with respect to self-renewal and invasion. Our findings suggest that LIN28B/let-7 shapes neuroblastoma metastasis, in part through influencing PBK, a kinase not previously implicated in the pathogenesis of aggressive pediatric solid tumors. Current studies are defining whether PBK, a therapeutically tractable target for which clinically relevant inhibitors exist, represents a novel therapeutic vulnerability in metastatic neuroblastoma.
Child Life Specialist Videoconference Support of Siblings of Pediatric Stem Cell Transplant Recipients: A Pilot Study

Pentz, Rebecca; Thomson, Mary; Schuetz, Rebecca; Dixon, Margie; Bryson, Elyse; Hianik, Rachel; and Height, Ann

Corresponding Author: Margie Dixon, BS, Emory University, mddixon@emory.edu
Center: Aflac Cancer and Blood Disorders Center
Type: Clinical or Translational
Keyword(s): Siblings, Child Life Services
Related to Pilot Grant or Trainee Award: No
Poster Available: Yes - P2

BACKGROUND: Siblings of children undergoing hematopoietic stem cell transplant (HSCT) have reported experiencing difficult emotions, feeling negative effects of separation from the patient and caregiver(s), being faced with additional responsibilities or burdens, and lacking information about the patient’s medical situation. A certified child life specialist (CCLS) is trained to address such concerns, but has limited interactions with siblings at home. This pilot study aimed to provide siblings with certified child life services through videoconferencing.

METHODS: Families of children undergoing HSCT who had siblings (7-18 years old) living at home were eligible. All participants provided consent or assent. Weekly videoconference sessions between the sibling and the CCLS were attempted for 100 days post-transplant. The CCLS completed usefulness evaluations after each session. After 100 days, parents and siblings were interviewed. Interviews were qualitatively coded.

RESULTS: Nine families (13 parents; 5 siblings, 4 half siblings and 1 adopted sibling) participated. Average sibling age was 10.5 years, ranging from 7-15 years. In total, 62 of 136 (46%) attempted videoconference sessions were completed. 69% of missed sessions were due to the sibling not answering the call. Suggestions for improvement included text message reminders and meeting with sibling non-parent caregivers who could facilitate the sessions. Sessions lasted on average 20.3 minutes. Overall impressions of the intervention were positive with all but 1 parent and all siblings stating in the post-intervention interviews that the intervention went well, was enjoyable, or was helpful. The one parent reported the intervention was okay. Sibling quotes show the helpfulness: “I actually felt like I didn’t have so much worries, and I didn’t feel left alone.” “The best part was being just having someone to talk to.” On a 10-point scale of usefulness, with 10 being most useful, siblings rated the intervention 8.6; parents 9.6; CCLS 8.6. The most commonly cited positive outcomes were the sibling receiving medical education (9 families) and 1-on-1 attention from the CCLS (8 families).

CONCLUSIONS: Providing child life services via videoconferencing to siblings of children undergoing HSCT is possible and considered useful by parents, siblings and in the professional judgement of the CCLS. Implementing suggested improvements may increase the completion rate.
Promoting Anti-Tumor Immunity via Bispecific T Cell Engaging Cytokine (BiTEokine) Therapy in Pediatric and Adult Acute Lymphoblastic Leukemia

Do, Priscilla; Perdue, Lacey A.; Chyong, Andrew; Henry, Curtis J.; Porter, Christopher C.; and Dreaden, Erik C.

Corresponding Author: Erik Dreaden, PhD, Emory University, e.dreaden@emory.edu
Center: Aflac Cancer and Blood Disorders Center
Type: Technology
Keyword(s): Immunotherapy, Cancer
Related to Pilot Grant or Trainee Award: No
Poster Available: No

The FDA-approved bispecific T cell engager (BiTE) therapy, blinatumomab (Blin), provides an alternative to conventional chemotherapy in pediatric and adult patients with relapsed/refractory or MRD+ acute lymphoblastic leukemia (ALL). Blin has recently outperformed conventional chemotherapy in two phase III clinical trials in pediatric patients following first relapse, leading to an early endpoint for both trials due to positive outcomes. BiTE therapy bypasses the need for natural recognition of tumor cells by cytotoxic T cells, inducing potent anti-tumor immunity. Specifically, Blin targets malignant B cells using the antigen binding domain of an αCD19 antibody linked to the antigen binding domain of a T cell-activating αCD3 antibody. Despite impressive results with Blin clinically, complete response rates are 39-69%, and a significant proportion of patients are refractory or still relapse. To improve this outcome, we have engineered a nanoparticle-based assembly and screening approach to capitalize on cytokine-enhancement of BiTE activity. We found that IL-12 significantly improves Blin-induced B-ALL cell lysis in vitro and, using nanoparticles capable of displaying ~142 antibodies/particle, we generated a library of 47 unique drug candidates with varying densities of antibodies to target CD19, activate CD3, and localize IL-12. We screened these particles in parallel using a co-culture assay for target cell lysis and T cell expansion by flow cytometry. Through regression modeling of these data, we identified lead candidates that maximize leukemia cell lysis and T cell proliferation. These results indicate that optimally lytic architectures favor high αCD3 to αCD19 ratios and are improved by IL-12 in a dose-dependent fashion. Future studies aim to characterize the activity of lead candidates from this screen in humanized mouse models of leukemia. Overall, this work aims to improve the therapeutic potential of bispecific T cell engager therapy using a modular drug scaffold that can be rapidly deployed for the discovery of treatments for other cancers or autoimmune diseases in both pediatric and adult patients.
OBJECTIVES: Intravenous immunoglobulin (IVIG) is used for infection prevention in pediatric B-cell acute lymphoblastic leukemia (B-ALL), but evidence for this is lacking. We describe the prevalence of hypogammaglobulinemia in pediatric B-ALL, predictors of IVIG use and its efficacy for infection prevention.

METHODS: We will conduct a retrospective review of children age 1-21 years with B-ALL treated at Aflac Cancer and Blood Disorders Center from 2010 to 2017. The cohort was identified through the cancer registry. Demographics, disease factors, laboratory values, medications and infection outcomes were linked between the electronic medical record and an institutional database. Outcomes of interest include emergency department (ED) visits, hospitalization days, and episodes of infection. Descriptive statistics will be performed. Outcomes will be compared between IVIG recipients and non-recipients. Univariable and multivariable logistic regression models will assess predictors of IVIG administration.

ANTICIPATED RESULTS: We identified 443 patients with B-ALL during the study period who met inclusion criteria. Exclusion criteria included receipt of IVIG or hematopoietic stem cell transplant prior to diagnosis. The average age at diagnosis is 6.5 years (standard deviation 4.8 years); 52.6% are male; 61.6% are white; 61.0% are standard risk per National Cancer Institute criteria. Among eligible patients, 137 (31.1%) received IVIG. We hypothesize that IVIG initiation is associated with hypogammaglobulinemia and history of severe infection. We also anticipate that frequency of emergency department visits, hospitalization days, and episodes of infection will decrease after IVIG initiation.

DISCUSSION: The immunological profile of children with B-ALL and factors influencing their susceptibility to infection are still incompletely understood. The benefits of IVIG are unknown. This study will provide evidence for IVIG prophylaxis recommendations in pediatric leukemia patients.
A Role for the NUP214 Nuclear Pore Protein in Leukemogenesis

Harrington, Amanda; Aumann, Waitman; Tope, Donald; and Wechsler, Daniel

Corresponding Author: Amanda Harrington, Emory University, amanda.harrington@choa.org
Center: Aflac Cancer and Blood Disorders Center
Type: Basic
Keyword(s): Leukemia
Related to Pilot Grant or Trainee Award: No
Poster Available: No

BACKGROUND: The t(10;11) CALM-AF10 translocation is a recurrent abnormality in 5-10% of T-cell acute lymphoblastic leukemias. CALM-AF10 leukemias are associated with elevated HOXA gene expression. However, the mechanism by which CALM-AF10 targets and activates the HOXA locus is unclear. We previously determined that CALM-AF10-mediated leukemogenesis requires interaction with the CRM1/XPO1 nuclear export receptor protein. Importantly, CRM1 can substitute for CALM: we have shown that a CRM1-AF10 fusion activates HOXA genes and is leukemogenic in vivo. Since neither CALM nor CRM1 contains a DNA binding domain, another protein must be involved in recognizing the HOXA locus. We hypothesize that NUP214, a protein known to interact with CRM1, mediates the ability of CRM1-AF10 to activate HOXA genes. NUP214 is involved in leukemogenic translocations that are associated with increased HOXA expression, and NUP214 can localize to Hoxa DNA. Furthermore, we have found that mutating CRM1 residues that mediate binding to NUP214 abrogates CRM1-AF10 leukemogenesis. We hypothesize that NUP214 can substitute for CRM1, and that a NUP214-AF10 fusion will be leukemogenic.

OBJECTIVE: To determine whether a NUP214-AF10 fusion can activate HOXA gene expression and promote leukemogenesis.

DESIGN/METHODS: A NUP214-AF10 fusion plasmid was created using PCR cloning. Expression of NUP214-AF10 protein in transiently transfected cell lines was assessed by western blot and confocal microscopy. The ability of NUP214-AF10 to activate HOX genes was measured by RT-qPCR. Self-renewal potential of NUP214-AF10-transduced hematopoietic progenitors in vitro will be assessed using methylcellulose assays.

RESULTS: NUP214-AF10 protein expression was verified in transiently transfected HEK293 cells by western blot and immunofluorescence. We used RT-qPCR to show that NUP214-AF10 is associated with changes in Hoxa and Hoxb gene expression in stably transfected murine NIH3T3 cells.

DISCUSSION/SIGNIFICANCE: We have demonstrated that CRM1 is required for CALM-AF10 leukemogenesis, and that interaction between NUP214 and CRM1 is important. We are investigating the importance of NUP214 in CALM-AF10 leukemogenesis by fusing it to AF10. Here we show that the NUP214-AF10 fusion protein can be expressed in two different cell lines, and that NUP214-AF10 expression is associated with changes in HOX gene expression. These studies will help establish the CRM1/NUP214 interaction as a potential therapeutic target.
Mesenchymal stromal cells (MSCs) are spindle-shaped, plastic-adherent *in vitro* with remarkable immunosuppressive activity *in vitro* and *in vivo*. MSCs have been employed as a cellular immunotherapy in many preclinical and clinical studies, especially focused on the treatment and prophylaxis of graft versus host disease (GVHD) during hematopoietic stem cell transplantation (HSCT). Although MSCs present a promising immunosuppressive cell therapy for GVHD, the importance of intravenously-infused MSC trafficking to secondary lymphoid organs (SLOs) and the chemotactic axis for GVHD prophylaxis after HSCT remains poorly understood. We show that interferon-γ-primed MSCs (γMSC), as well as MSCs, migrate to activated T cells but not non-activated T cells *in vitro*, consistent with the notion that inflammation chemoattracts MSCs. Protein array analysis of non-activated and activated T cell conditioned media revealed candidate chemokines that may mediate γMSC trafficking. Antibody blocking of both CCL3 and CCL5, but neither alone, abrogated *in vitro* migration to activated T cell conditioned media. Interestingly, pharmacologic antagonism of CCR1 and CCR5 significantly reduced (by 50%) but did not abolish migration, suggesting that while CCL3 and CCL5 mediate γMSC trafficking, they may act through receptors other than their classically recognized partners. *In vivo*, γMSCs migrated to spleen, gut associated lymphoid tissue (GALT) and mesenteric lymph nodes, but not bronchial, or skin draining lymph nodes as determined by qPCR. Migration is significantly reduced after syngeneic HSCT in which mice have condition regimen induced tissue injury but not alloreactivity. γMSCs also localize in non-lymphoid organs that receive a substantial fraction of cardiac output, presumably by capillary lodging. Syngeneic HSCT does not alter this non-lymphoid localization consistent with the idea of nonspecific sticking in capillaries. These migration data suggest a novel paradigm for γMSC prophylaxis of GVHD without impairing graft-vs-leukemia, anti-viral immunity or immune reconstitution after HSCT.
Prostaglandin Receptor EP2 Is a Novel Molecular Target for High-risk Neuroblastoma

Hou, Ruida; Li, Lexiao; Yu, Ying; and Jiang, Jianxiong

Corresponding Author: Ruida Hou, MD, University of Tennessee Health Science Center, rhou@uthsc.edu
Center: Aflac Cancer and Blood Disorders Center
Type: Clinical or Translational
Keyword(s): Neuroblastoma Prostaglandins
Related to Pilot Grant or Trainee Award: No
Poster Available: No

As the third-most common type of cancers in infants and young children, neuroblastoma accounts for almost 10% of all childhood cancers. Despite remarkable advances in tumor diagnosis and management during the past decades, the five-year survival rates for patients with high-risk neuroblastoma remain below 50%. Developing new therapeutics for this devastating type of childhood cancer is an urgent unmet need. Cyclooxygenase (COX) via synthesizing prostaglandin E2 (PGE2) promotes tumor cell proliferation, survival, migration and invasion, and fosters an inflammation-enriched microenvironment that can facilitate angiogenesis, immune evasion and treatment resistance. However, which downstream PGE2 receptor subtype - namely EP1, EP2, EP3 and EP4 - is directly involved in COX activity-promoted neuroblastoma growth remains elusive. Analyzing a neuroblastoma patient database (N = 88), we show that COX-1/microsomal prostaglandin E synthase-1 (mPGES-1)/EP2 signaling axis is highly associated with the aggressiveness of human neuroblastoma. PGE2 signaling-associated genes including its synthetic enzymes and receptors are ubiquitously expressed in human neuroblastoma cells with various backgrounds. A time-resolved fluorescence resonance energy transfer (TR-FRET) method reveals EP2 as a key Gαs-coupled receptor that mediates PGE2-initiated cAMP signaling in neuroblastoma cells. Taking advantage of novel selective bioavailable brain-impermeable smallmolecule antagonists that we recently developed to target the peripheral PGE2/EP2 signaling in vivo, we further demonstrate that pharmacological inhibition of the EP2 receptor substantially delays the growth of tumors formed by human neuroblastoma cells in mice. Collectively, our results suggest that PGE2/EP2 pathway contributes to the growth and malignant potential of human neuroblastoma cells; EP2 receptor inhibition by our pharmacological compounds might provide a novel therapeutic strategy for this deadly pediatric cancer.
Myeloid Cells Contribute to An Effective Immune Response Against B Lymphoblastic Leukemia Cells

Hunter, Rae; Henry, Curtis; and Porter, Christopher

Corresponding Author: Rae Hunter, MS, Emory University, ahunte8@emory.edu
Center: Aflac Cancer and Blood Disorders Center
Type: Basic
Keyword(s): leukemia, innate immunity
Related to Pilot Grant or Trainee Award: No
Poster Available: No

Pediatric patients diagnosed with acute lymphoblastic leukemia (ALL) have a 15-20% mortality rate, in spite of current advances in therapies, including immunotherapies. Despite the use of immunotherapies in patients with relapsed or refractory ALL, relapse rates post-treatment remains over 50 percent. These observations highlight our lack of understanding of immune evasion mechanisms used by leukemia cells.

Our group has previously published that IL-12 promotes a very effective immune response to B-cell ALL cells in vivo. While T cells are required to maintain remission, we observed prolonged survival in IL-12 treated Rag1-/- mice with leukemia as compared to untreated mice, suggesting a major role of the innate immune response in ALL cells. Depletion of NK cells with anti-NK1.1 did not abrogate the elimination of an immunogenic model of leukemia, in which calcineurin has been knocked down by shRNA. However, depletion of myeloid cells from immunocompetent mice using colony-stimulating factor-1 receptor (CSF1R) depleting antibodies promoted the progression of this leukemia, which was eliminated in all isotype treated, immune-competent recipients, indicating myeloid cells play a critical role in the immune response to ALL cells. Ongoing studies suggest dendritic cells (DCs), specifically CD11b+CD11c+ DCs, are reduced in mice transplanted with parental relative to immunogenic, Cn-deficient ALL cells. Furthermore, macrophages cultured in vitro with parental cells expressed significantly lower surface levels of CD80, CD40 and CD86 compared to levels found on the surface of cells cultured with Cn-deficient leukemia cells. Overall, our data suggest that ALL cells may compromise the T-cell priming capacity of innate immune cells.
Acute lymphoblastic leukemia (ALL) is the most prevalent cancer diagnosed in children, affecting about 3,000 people below the age of 20 each year in the United States. The most common first step of treatment is induction, and how the patient responds to this step is highly associated with their subsequent outcome. In order to understand how immune and leukemic cells are affected by induction in pediatric ALL, we have performed a pilot investigation utilizing single-cell RNA-sequencing technology (scRNA-seq) on the 10X Genomics Chromium platform. Samples from seven patients were collected, four of whom exhibited measurable residual disease (MRD) after induction, and three patients who did not. Leukemic cells were separated from peripheral immune cells using flow cytometry. From each patient, scRNA was sequenced from ~1000 cells each from peripheral immune cells before treatment, peripheral immune cells after treatment, leukemic cells before treatment, and leukemic cells after treatment, where applicable. The goal of this study is to discern how the immune and leukemic cell populations vary both before and after treatment and between those patients who did respond to induction and those who did not, and the manner in which the gene transcript expression within those cell populations differ. Results will be presented highlighting the identification of distinct clusters of leukemic cells with differential engagement of pathways related to cell adhesion, cell division, metabolism, and specific signaling mechanisms. Profiling of healthy immune cell populations is also expected to highlight differences in the abundance and gene activity of specific lymphoid and possibly myeloid subsets in the response to induction therapy.
Biopsychosocial Factors Associated With Parenting Stress in Pediatric Sickle Cell Disease

Johnson, Yelena L.; Woodward, Kerri E.; Cohen, Lindsey L.; Dampier, Carlton; and Sil, Soumitri

Corresponding Author: Yelena Johnson, PhD, Emory University, yelena.johnson@choa.org
Center: Aflac Cancer and Blood Disorders Center
Type: Clinical or Translational
Keyword(s): sickle cell disease; parenting stress
Related to Pilot Grant or Trainee Award: No
Poster Available: No

BACKGROUND: Caregivers of youth with sickle cell disease (SCD) play an essential role in their child’s disease management. Caregiving responsibilities can contribute to significant parenting stress, which may adversely affect children’s and caregivers’ quality of life. However, existing literature on parenting stress in pediatric SCD is limited. This study aimed to identify biopsychosocial factors related to parenting stress and associations with healthcare utilization among youth with SCD.

METHOD: Participants were youth with SCD (9-20 years old, 14.33 ± 2.46) and their caregivers (n=80). Youth were primarily African American (88.0%), with genotype HbSS (73.5%), and prescribed hydroxyurea (77.1%); caregivers were primarily mothers (%). Caregivers completed the Pediatric Inventory for Parents (PIP) and 36-Item Short Form Health Survey (SF-36) to assess parenting stress and parent health, respectively, and provided sociodemographic information (i.e., family income, youth disease phenotype, treatment type, missed school days) during an outpatient comprehensive SCD clinic. Healthcare utilization data were extracted via medical chart review.

RESULTS: Caregivers of youth with HbSC reported higher parenting stress difficulty than those of other SCD phenotypes F(4, 73)=2.50, p=.049, and caregivers of youth prescribed hydroxyurea reported greater parenting stress frequency than those not prescribed hydroxyurea, F(1, 77)=4.48, p=.037. Parenting stress frequency and difficulty were inversely correlated with caregiver Physical and Emotional Role Functioning, Vitality, and Social Functioning (all p’s < .05). Parenting stress difficulty was inversely associated with Emotional Functioning, Bodily Pain, and General Health (all p’s < .05). More missed school days were associated with higher parenting stress frequency (p = .02) and difficulty (p = .02). Higher parenting stress frequency (p < .001) and difficulty (p < .001) were correlated with higher frequency of youth inpatient admissions in the following year. Parenting stress frequency and difficulty were not associated with youth age, chronic transfusion treatment, annual family income, caregiver Physical Functioning, or ED visits in the following year.

CONCLUSIONS: Higher parenting stress was associated with poorer caregivers’ health, and youths’ disease phenotype, treatment type, missed school days, and healthcare utilization. Future studies and interventions aimed to address parenting stress in pediatric SCD are needed and may improve both caregiver and youth outcomes.
BACKGROUND: Childhood brain tumors are the second most common pediatric malignancy in the United States. Although survival rates after treatment are steadily improving, survivors continue to experience a host of medical, neurocognitive, and psychosocial late effects that can negatively impact health-related quality of life (HRQOL). Given that reports of HRQOL can influence important treatment decisions throughout survivorship, agreement between parent and child perceptions of HRQOL is critical. The aim of the present retrospective study was to identify statistical associations between parent-proxy and child measures of HRQOL among pediatric brain tumor survivors.

METHODS: HRQOL and medical data were extracted via systematic medical chart review. Child-parent dyads followed in an outpatient integrated brain tumor survivor clinic across a 15-month span were previously administered a measure of HRQOL (Pediatric Quality of Life™ Inventory, Version 4.0 [PedsQL]).

RESULTS: A total of 149 patients (55% female; Mage=14.73) and their primary caregivers completed the PedsQL. Statistical analyses revealed significant positive correlations between child and parent-proxy reports of HRQOL (e.g., Parent and Child Total Score, r=.665, p<.001). However, paired samples t-tests also revealed statistically significant differences between child and parent-proxy reports of HRQOL across all PedsQL scales: Physical Functioning (t[148]=-2.36, p=.02), Emotional Functioning (t[148]=-2.78, p=.02), Social Functioning (t[148]=-4.56, p<.001), School Functioning (t[147]=-2.36, p=.02), Psychosocial Functioning (t[148]=-3.99, p=.001), Total (t(148)=-3.92, p<.001). Parents reported lower scores than children across all scales. Additional multivariate analyses will further examine how demographic and medical covariates may influence parent-child concordance.

CONCLUSIONS: Findings suggest that parents of pediatric brain tumor survivors report significantly lower HRQOL than the survivors themselves. The present results are consistent with existing literature indicating that parents of children with cancer tend to underestimate HRQOL, while parents of healthy controls typically overestimate HRQOL. These data also underscore the value of obtaining self-report measures of HRQOL from patients whenever possible. Future studies are needed to examine these correlations longitudinally in order to inform guidelines for long-term neuropsychological monitoring in pediatric brain tumor survivors.
Identification of a Potential Role for EPS15 in CALM-AF10 Leukemogenesis

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

Corresponding Author: Rafi Kazi, MD, Emory University, rafi.kazi@choa.org
Center: Aflac Cancer and Blood Disorders Center
Type: Basic
Keyword(s): CALM-AF10 Leukemia
Related to Pilot Grant or Trainee Award: No
Poster Available: No

BACKGROUND: CALM-AF10 leukemias, which account for ~10% of childhood T-ALL and a subset of AML, have a poor prognosis. These leukemias exhibit increased HOXA gene expression, which we have shown is dependent on the interaction between CALM-AF10 and CRM1. Since neither CALM-AF10 nor CRM1 contains a recognized DNA binding domain, the mechanism by which CALM-AF10/CRM1 interacts with HOXA genes is not understood. We used a proximity based-labeling approach dependent on biotin ligase (BioID2) to identify candidate CALM-AF10-interacting proteins that could mediate binding to HOXA genes.

OBJECTIVE: Identify candidate proteins that interact with CALM-AF10 and potentially mediate DNA binding

DESIGN/METHODS: We prepared a BioID2-CALM-AF10 expression plasmid and determined that it is transcriptionally activates HOXA genes and promotes leukemogenesis, similar to CALM-AF10. HEK293 cells were transiently transfected with BioID2-CALM-AF10 and grown in the presence or absence of biotin, and mass spectrometry (MS) was used to identify candidate interacting proteins. Initial validation of candidate proteins was conformed via co-immunoprecipitation (co-IP) in HEK293 cells transiently transfected with CALM-AF10.

RESULTS: Three independent experiments identified 11 biotin-labeled proteins that interact with CALM-AF10. Importantly, the approach was validated through identification of two proteins: DOT1L, a protein known to interact with AF10, and NUP214, a nuclear pore protein with a potential role in CALM-AF10 leukemias. Among the remaining nine proteins, we identified epidermal growth factor receptor substrate 15 (EPS15), a known CRM1-interacting protein that is also involved in KMT2A/MLL translocations. Co-IP studies showed that EPS15 directly interacts with CALM-AF10, validating a potential role for EPS15 in CALM-AF10 leukemogenesis.

CONCLUSIONS: Proximity-based labeling using biotin ligase is a novel approach for identifying proteins that interact with CALM-AF10. Among the proteins identified, EPS15 is an intriguing candidate: KMT2A-EPS15 translocations (t(1;11)(p32;q23)) have been identified in both AML and ALL, and KMT2A-EPS15 is among the more common seen KMT2A rearrangements. Since EPS15 binds to CRM1 and is involved in both signal transduction and transcriptional regulation, our demonstration that it also interacts with CALM-AF10 suggests a possible role in mediating binding to HOXA genes. EPS15 overexpression and knockdown studies in hematopoietic precursors and CALM-AF10 leukemia cells are currently underway to evaluate effects on leukemogenesis.
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 enhance or reduce 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 frontline cytotoxic chemotherapy. Among over 500 unique tumor-toxic drug combinations, we have identified and validated optimal drug ratios that synergize to augment leukemia cell killing in a panel of T-cell and early T-cell precursor acute lymphoblastic leukemia (ALL) cells. We characterize transcriptional changes that occur under selective pressure by these synergistic drug combinations; and, using machine learning, we also identify transcriptomic signatures that may predict leukemia cell responses to distinct drug combinations. In addition, we demonstrate simultaneous, combinatorial drug loading into nanoscale liposomes, retention of drug ratios over time, and ratiometric drug delivery to leukemia cells in vitro. We hypothesize that liposomes conditionally maintaining drug ratios—both in circulation and following leukemia cell delivery—for prolonged periods in vivo will improve treatment outcomes and tolerability when compared with cytotoxic chemotherapy in patient-derived mouse models of leukemia.
Dissecting the Impact of Obesity on T-Cell Acute Lymphoblastic Leukemia Pathogenesis

Lee, Miyoung; Talekar, Ganesh R.; 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 approaching 80% in children while falling to 60% in adult cases. These numbers further decline in patients with primary resistance or 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. Obesity (characterized by a body mass index > 30) has been shown to reduce the survival of patients with B-cell acute lymphoblastic leukemia; however, obese patients with T-ALL exhibit superior survival outcomes. To determine how obesity impacts T-ALL pathogenesis, we analyzed the functional responses of human T-ALL cells exposed to adipocyte-secreted factors and after transplantation into mice fed high-fat diets.

In preliminary studies, we found that adipocyte-conditioned media (ACM) promoted extensive DNA damage, and apoptosis and accumulation in the SubG1 phase of the cell cycle, indicative of apoptotic bodies in T-ALL cells.

Metabolic status of T-ALL cells were evaluated. Cells with high apoptosis in ACM showed lower cell proliferation compared to that of low apoptosis. Interestingly, all three cell lines exhibited lower NAD/NADH ratio in ACM compared to those in control, indicating altered metabolism.

To determine how ACM promoted apoptosis in T-ALL, we assessed the impact of adipocyte-secreted factors on intracellular signaling pathways. We found that phospho-CHK2 was increased in ACM-treated T-ALL cells relative to those cultured control. Furthermore, surface and intracellular levels of Notch1, and its downstream target Myc, were downregulated when T-ALL cells were cultured in ACM. The detrimental impact of adiposity on T-ALL survival in vitro was strengthened by in vivo studies in immune-deficient mice, where greater than 60% of lean mice succumbed to T-ALL progression compared to 20% of obese mice.

In conclusion, our studies reveal a surprisingly detrimental impact of obesity on T-ALL pathogenesis. Importantly, we found that adipocyte-secreted proteins promote T-ALL cell death by abrogating Notch1-mediated signaling. In future studies we will identify the specific adipocyte-secreted proteins that promote T-ALL apoptosis and determine their therapeutic potential.
Defining and Timing of Palliative Opportunities in Pediatric Neuro-Oncology

Massie, A. McCauley; Allen, Kristen; Ebelhar, Jonathan; DeGroote, Nicholas; Wasilewski-Masker, Karen; and Brock, Katharine

Corresponding Author: McCauley Massie, BS, Emory University, mccauleymassie@gmail.com

Center: Aflac Cancer and Blood Disorders Center
Type: Clinical or Translational
Keyword(s): Palliative Oncology
Related to Pilot Grant or Trainee Award: No
Poster Available: No

BACKGROUND: Children with brain tumors experience relatively high morbidity and mortality due to tumor location and general prognosis. These children and their families could benefit from palliative care (PC) aimed at alleviating suffering and improving quality of life. It is recommended to introduce palliative care support early in the disease course, however, this is often not done, and many “palliative opportunities” are missed. The number, type, and timing of palliative opportunities in children with brain tumors are unknown.

METHODS: A single-institution retrospective review was performed on patients diagnosed with a brain tumor between 0-18 years of age who died between 01/01/2012-11/30/2017. Demographic, disease, treatment, palliative opportunity, and end-of-life data were collected. A priori, nine palliative opportunities were defined (disease progression; relapse; hospital admission for severe symptoms; intensive care admission; bone marrow transplant; phase 1 trial enrollment; hospice; do-not-resuscitate status). Descriptive statistics were analyzed and palliative opportunities compared for all variables. Palliative opportunities were evaluated in quartiles from diagnosis to death.

RESULTS: Amongst 101 patients with a median age at death of 8 years (range 0-22), there were 781 palliative opportunities, and a median of 7 (IQR=6) palliative opportunities per patient. Number and type of palliative opportunities did not vary by demographics or diagnosis. Palliative opportunities increased closer to death. Thirty-four (33.7%) patients received PC consultation, a median of 2.24 months before death. Likelihood of PC consultation did not differ by diagnosis category (p=0.59) or total opportunities (p=0.09). PC consultation was associated with having a do-not-resuscitate order (p=0.0028). Hospice was involved for 71.3% of patients.

CONCLUSION: Children with brain tumors often suffer events during their disease course warranting psychosocial or palliative support. These palliative opportunities increase toward the end-of-life. Cumulative tracking of palliative opportunities can be used to map the course and trajectory of disease. Recognition of palliative opportunities should inform clinical care especially at the level of the primary oncologist, such that PC consultation is considered before the majority of palliative opportunities occur. Future research should assess strategies and interventions to optimize care through recognition of palliative opportunities and early introduction of PC for children with brain tumors.
Phosphorylated YB1 Mediates PARP Dependent DNA Repair in SHH Medulloblastoma

McSwain, Leon; Chen, Victor; Dey, Abhinav; Malhotra, Anshu; MacDonald, Tobey; and Kenney, Anna

Corresponding Author: Leon McSwain, BS, Emory University, Lmcswai@emory.edu
Center: Aflac Cancer and Blood Disorders Center
Type: Clinical or Translational
Keyword(s): Medulloblastoma; Radiooncology
Related to Pilot Grant or Trainee Award: No
Poster Available: No

BACKGROUND: The Sonic hedgehog (SHH)-subgroup of the pediatric brain tumor medulloblastoma (MB) maintains a high frequency of radioresistance-mediated recurrence. Targetable DNA repair proteins prevalent in the tumor area, and perivascular stem-cell niche, the putative source of stem cells driving MB recurrence, remain elusive. Y-box binding protein 1(YB1), a DNA repair and RNA binding protein regulated through the YAP-IGF2-YB1 axis, is elevated in the stem cell niche and prevalent throughout the tumor area of SHH murine and human tumors.

METHODS: NeuroD2-SmoA1 SHH mouse medulloblastoma organotypic slice culture served as the primary model for understanding YB1 dynamics in the mouse PVN as it allows for maintaining an intact microenvironment. In experiments requiring single cell analysis, SmoA1 tumors were dissociated to generate medulloblastoma cell (MBCs) cultures and utilized for Comet assays, western blotting, and immunofluorescence.

RESULTS: We found that YB1 responds to radiation through enhanced Serine 102 phosphorylation and translocation to the nucleus. YB1 over-expression increased the rate of DNA repair after radiation, while PARP inhibition abrogates YB1-driven DNA repair. Secondarily, overexpression of phospho-serine 102 YB1 mutant demonstrates a necessity of Serine 102 phosphorylation for YB1 mediated DNA repair.

CONCLUSIONS: Our results suggest that, when combined with radiation, YB1 inhibition could serve as a viable method of targeting MBCs and the perivascular stem cell niche.
Duration of Neutropenia by Course and Demographics for Pediatric Acute Myeloid Leukemia Patients Using Data From the Leukemia Electronic Abstraction of Records Network (LEARN)

Miller, Tamara; Getz, Kelly; Demissei, Biniyam; Rabin, Karen; Daves, Marla; Lupo, Philip; Scheurer, Michael; Burrows, Evanette; Fisher, Brian; Grundmeier, Robert; Lee, Judy; Wilkes, Jennifer; Winestone, Lena; Gramatges; Monica; and Aplenc, Richard

Corresponding Author: Tamara Miller, MD, MS, Emory University, tamara.miller@emory.edu

Center: Aflac Cancer and Blood Disorders Center
Type: Outcomes
Keyword(s): acute myeloid leukemia, toxicity
Related to Pilot Grant or Trainee Award: No
Poster Available: No

BACKGROUND: Children with acute myeloid leukemia (AML) experience periods of prolonged neutropenia after chemotherapy. This study sought to use detailed electronic health record (EHR) data to describe and compare course-specific neutropenia durations by demographic and treatment characteristics.

METHODS: The Leukemia Electronic Abstraction of Records Network (LEARN) comprises data from patients with leukemia treated at Children’s Healthcare of Atlanta, Children’s Hospital of Philadelphia, and Texas Children’s Hospital between 2006 and 2018. Treatment data were collected manually. Absolute neutrophil counts (ANCs) and demographics were extracted from the EHR using ExtractEHR, a software package we developed which requires input of only medical record numbers and chemotherapy course dates. De-identified laboratory data were cleaned to remove erroneous results. Neutropenia duration in each course was computed as the first day post-chemotherapy of ANC <200 cells/µL until ANC >200 cells/µL. Mean durations with standard deviations (SD) were calculated, and unadjusted mean differences (MD) in neutropenia duration were compared by age, sex, race, ethnicity, and chemotherapy regimen using linear regression.

RESULTS: Laboratory results on 251 patients (825 courses) were extracted; 52.2% were female, 18.4% were Hispanic, 63.6% were white, and 25.5% were black. Mean days of neutropenia varied by course (Induction I: 24.0, SD 9.7; Induction II: 21.3, SD 14.7; Intensification I: 18.5, SD 8.8; Intensification II: 21.4, SD 10.5). The largest difference by chemotherapy regimen was during Induction II: neutropenia duration was longer after mitoxantrone/cytarabine than cytarabine/daunorubicin/etoposide (25.4 vs. 16.6 days, p<0.01). Compared to children aged 2-10, children aged 16-20 (MD -5.2, p=0.01) and children aged 0-1 (MD -3.3, p=0.03) had shorter neutropenia duration during Induction I, but not consistently across subsequent courses. There were no statistically significant differences in mean neutropenia duration in any course by sex, race, ethnicity, or for ages 11-15.

CONCLUSION: LEARN provides estimates of neutropenia duration for each frontline AML chemotherapy course. Multivariable analyses and further evaluations of variability in neutropenia duration over the trajectory of frontline treatment are ongoing. These results can be used to counsel patients on risks for complications of prolonged neutropenia, project potential hospitalization duration, and provide a baseline comparison for evaluating experimental agents in future trials.
Developing and Testing a Psychosocial Intervention for Adolescents With Cancer and Sickle Cell Disease

Moran, Sarah; McKee, Laura G.; Cohen, Lindsey L.; Sil, Soumitri; Jones, Brady; Goyer, Meghan; Shneider, Caitlin; Michel, Jena; and Donati, Matthew

Corresponding Author: Sarah Moran, BA, Georgia State University, smoran8@student.gsu.edu
Center: Aflac Cancer and Blood Disorders Center
Type: Clinical or Translational
Keyword(s): psychosocial intervention, cancer, sickle cell disease
Related to Pilot Grant or Trainee Award: 2018, CORPH, FOCUS: Feasibility, Acceptability, and Pilot of an Intervention to Improve Functioning in Adolescents with SCD (PI: Lindsey Cohen, PhD)
Poster Available: Yes - P4

Adolescents with sickle cell disease (SCD) and cancer face unique psychosocial challenges. However, patients have limited access to therapeutic resources, especially following discharge from inpatient status; few portable interventions are available for youth outside the hospital. The current intervention, refined in Phase 1 and tested post-discharge in Phase 2, uses the Hero’s Journey narrative arc and involves taking and writing captions for purposeful pictures to foster healthy coping during difficult moments and focus attention towards positive moments.

In Phase 1, focused on intervention development and modification, inpatient adolescents with SCD (n = 13; Mage = 15.1 years; SD = 2.1; 61.5% male) and cancer (n = 9; Mage = 15.1, SD = 2.0; 55.6% male) were interviewed with semi-structured prompts focused on patient feedback about the intervention, which was presented verbally, via written handouts and video content. Using a flexible, inductive approach, we (1) identified themes that guided intervention refinement, (2) revised materials and presented them in subsequent phases of interviews, and (3) made further adjustments as appropriate. For example, adolescents from the first phases of interviews reported confusion about the 12-stage Hero’s Journey story arc that was presented, which resulted in condensation of the story arc to 3 stages. This change was presented in subsequent interviews. Other modifications to intervention materials will be presented.

Phase 2, a pilot randomized control trial, is underway to determine if the intervention refined in Phase 1 is effective in improving psychosocial functioning in adolescents with SCD and cancer. Participants complete a baseline assessment and are randomly assigned to the intervention (photo-taking and survey) or control (survey only) condition. Intervention participants are oriented to the Hero’s Journey; at discharge, they are instructed to take two photos per day, and write a caption and piece of advice for each photo as if they are communicating with another adolescent with SCD or cancer. After 10 days, participants complete the follow-up assessment. Data collection is ongoing (N=8, 6; intervention, control). A review of the methodological approach to developing the intervention will be discussed, and preliminary results regarding efficacy, acceptability, and feasibility of the intervention will be presented.
Immune Thrombocytopenia Purpura (ITP) is defined by a low platelet count in the absence of any other known causes and affects over 4,000 children and 8,000 adults each year in the United States. While majority of ITP cases resolve themselves, ITP is associated with an enhanced risk of bleeding, as approximately 10% of affected children have major bleeding, and 0.5% of children will have life-threatening intracranial hemorrhage. The treatments for these conditions have significant side effects and no existing diagnostic or biomarker can determine which patients will develop life-threatening bleeding. This presents a significant challenge for clinicians as they must balance risk-factors associated with ITP with those of available treatments and determine with limited evidence which patients require therapy and which should be monitored. To that end, we developed a novel platelet contraction cytometer (PCC) capable of measuring single platelet function, specifically contractile force, in high throughput. In a study of 49 pediatric ITP patients, we demonstrated that platelet forces are impaired in patients with symptomatic bleeding as opposed to asymptomatic patients and healthy controls, as low average platelet forces strongly correlated with bleeding. Using an average platelet force cutoff of 26nN, we found that low platelet forces were associated with bleeding in ITP patients with a sensitivity of 100% and a specificity of 86.8%. Moreover, when tracking individual patients (n=6) over time, we demonstrated that whenever a patient’s blood sample is associated with increased average contraction force >26 nN or platelet count >40k/μL, bleeding symptoms are alleviated. Conversely, whenever a patient exhibited high platelet contractile force and low platelet count, the onset of bleeding symptoms correlated with a decrease in platelet forces. As such, our work suggests that single platelet forces could be used as a biophysical biomarker to assess bleeding risk in patients with ITP regardless of count.
Osteosarcoma (OS) is the most common primary bone malignancy in adolescents and young adults. Metastatic disease often presents in the lungs and if not able to be surgically removed, is rarely curable. Mesenchymal stromal cells (MSCs) are a cell therapy of intense therapeutic interest as they have been shown to be potentially beneficial in regenerative medicine as well as in cancer. Since MSCs can be genetically modified to secrete various cytokines, there is increasing interest in using MSCs as a cellular therapy to locally deliver cytokines. We have shown that transduced MSCs can locally secrete murine interferon-gamma (mIFNy), a pro-inflammatory cytokine, to alter the immune microenvironment and polarize tumor associated macrophages to induce an anti-tumor effect in a neuroblastoma model. Metastatic OS has a higher infiltration of TAMs compared to localized disease and thus direct delivery of mIFNy-expressing MSCs could provide benefit to this deadly disease as well. To demonstrate migration of MSCs towards OS cell lines in vitro, we utilized the IncuCyte Chemotaxis Cell Migration system to perform real-time live cell imaging of MSCs of OS conditioned media. WE demonstrated significant MSC migration towards two OS cell lines, OS-17 and 143-B, compared to control media. After intravenous infusion, MSCs become lodged in the lungs and subsequently distribute to the body which we anticipate will result in localization to pulmonary OS metastases as well as the primary tumor. We will use the results of our IN VITRO migration assays to develop strategies to deliver transduced MSCs to tumor in a mouse model of metastatic OS.
BACKGROUND: Neuroblastoma (NB) is the most common extracranial solid tumor of childhood and in the high-risk subset, less than 50% of patients survive 5 years after diagnosis. Given the relative paucity of targetable somatic mutations in NB and the long-term negative effects of current treatment modalities, we seek to develop innovative targeted therapies. We hypothesize that altered regulation of transcription and translation are major mechanisms of neuroblastoma oncogenesis. We have shown that one such regulator, the RNA-binding protein (RBP), LIN28B, promotes neuroblastoma aggression, highlighting the role of RBPs in modulating NB tumorigenesis. We hypothesized that subsets of RBPs may serve as drivers of neuroblastoma aggression. Using a candidate-based approach, we identified MSI2.

OBJECTIVE: In an effort to ultimately more effectively treat neuroblastoma, we seek to determine the role of RBPs in NB pathogenesis.

METHOD: We depleted MSI2 in human NB cell lines and examined the impact on proliferation, survival, and expression of downstream target genes.

RESULTS: We interrogated clinically annotated neuroblastoma gene expression datasets and found that increased expression of MSI2 is associated with worse prognosis, higher stage and reduced survival in high-risk NB. Additionally, we observed decreased colony formation, increased apoptosis and decreased proliferation in vitro and in vivo of NB cells with reduced MSI2 levels. We then used unbiased proteomics approaches to identify MSI2-influenced proteins and identified networks implicated in metabolism, translation, and the regulation of oxidative stress. We further explored the role of some of these targets in NB tumorigenesis.

CONCLUSION: Here, we have shown for the first time that higher MSI2 expression is associated with worse prognosis in NB. In addition, our in vitro and in vivo results support a role of MSI2 in mediating multiple hallmarks of cancer. Proteomics analysis revealed that many metabolic pathways and networks known to lead to clinical aggression of tumors are downstream of MSI2. These results are indicative that MSI2 and many of its targets are important in NB tumorigenesis. We will continue to validate these targets with the rationale that this provides a basis for better understanding tumorigenesis in NB; thus, unveiling novel therapeutic vulnerabilities in this disease.
Siglec-15 Is a Novel Immunomodulatory Protein and Therapeutic Target in Childhood Leukemia

Pillsbury, Claire; Fonseca, Jairo; Dougan, Jodi; Abukharma, Hasan; Liu, Linda; and Porter, Christopher

Corresponding Author: Claire Pillsbury, BS, Emory University, claire.pillsbury@emory.edu
Center: Aflac Cancer and Blood Disorders Center
Type: Basic
Keyword(s): immunosuppression leukemia
Related to Pilot Grant or Trainee Award: No
Poster Available: No

Despite advances that have greatly improved the overall mortality of pediatric B cell acute lymphoblastic leukemia (B-ALL), it remains one of the leading causes of cancer-related death in children. Recent therapeutic developments in the field of immunotherapy have shown efficacy in treatment of refractory disease and highlight the need for greater understanding of the immune evasion mechanisms underlying this disease.

Our group has begun characterization of a novel immunomodulatory agent, Siglec-15, in the context of pediatric hematological malignancies. We have queried patient databases to show SIGLEC15 is overexpressed at the RNA level in primary B-ALL and acute myelogenous leukemia (AML) samples as compared to healthy donor controls. We have also validated the higher expression of Siglec-15 as compared to healthy donor PBMCs at the RNA and protein levels through RT-qPCR, immunoblotting, and flow cytometry across a panel of human B-ALL cell lines. Mechanistic studies suggest that SIGLEC15 expression is positively regulated by PKC and calcineurin. As immunofluorescence indicated Golgi localization of Siglec-15 in leukemia cells, raising the possibility of release from leukemia cells, we measured soluble Siglec-15 in patient plasma and found significantly higher detectable levels in children with B-ALL as compared to healthy individuals. Finally, CRISPR-Cas9 knockout of Siglec-15 in an aggressive model of BCR-ABL+ murine B-ALL results in lymphocyte-dependent prolongation of survival.

Together, these results suggest Siglec-15 is a novel and potent immunosuppressive molecule active in leukemia progression with potential for therapeutic targeted intervention.
Subtype and Grade-Dependent Spatial Heterogeneity of Immune Architecture in Pediatric Glioma

Robinson, Hope; Kaushal, Akhilesh; Velazquez Vega, Jose E.; MacDonald, Tobey; Schniederjan, Matthew J.; and Dhodapkar, Kavita M.

Corresponding Author: Hope Robinson, PhD, Emory University, mhrobin@emory.edu
Center: Aflac Cancer and Blood Disorders Center
Type: Clinical or Translational
Keyword(s): Glioma, Immune Microenvironment
Related to Pilot Grant or Trainee Award: No
Poster Available: No

Brain tumors are a leading cause of cancer-related mortality in children. The presence and spatial features of infiltrating immune cells has been shown to impact outcome in several adult tumors. However, pediatric tumors typically express a lower mutational burden and properties of immune cells in these tumors and particularly their spatial features are less studied. We utilized multiplex immunofluorescence immunohistochemistry coupled with machine learning and single cell mass cytometry (CyTOF) to evaluate T cells infiltrating 18 pediatric low grade gliomas (LGG) and 8 pediatric high grade gliomas (HGG). We find that LGG are characterized by greater T cell density compared to HGG. Even among low-grade tumors, T cell infiltration can be highly variable and subtype-dependent, with greater T cell density in pleomorphic xanthoastrocytoma and ganglioglioma. CD3+ T cell infiltration correlates inversely with the expression of SOX2, an embryonal stem cell marker commonly expressed on glial tumors. T cells within both HGG and LGG tumors exhibit phenotypic heterogeneity and include subsets that express markers of tissue residence and stem-like memory T cells. Interestingly, these subsets exhibit distinct spatial patterns with TCF1+ stem-like T cells exhibiting greater proximity to vasculature compared to tissue-resident memory T cells. Comparison of these subsets by CyTOF also identified several differences in phenotype and functional properties of these distinct T cell subsets. These data provide several novel insights into the subtype- and grade-dependent changes in immune architecture in pediatric gliomas and suggest that exclusion of tumor-resident T cells may be an important feature of high-grade tumors, which may be targeted to improve immune control in glioma.
Obesity Attenuates T-Cell Function Which May Impact the Efficacy of T-Cell Based Immunotherapies in Pediatric Leukemia

Ross, Anthony; Lee, Miyoung; Hamilton, Jamie; Talekar, Ganesh; Story, Jamie; Raikar, Sunil; and Henry, Curtis

Corresponding Author: Anthony Ross, MD, MS, Emory University, anthony.ross@choa.org
Center: Aflac Cancer and Blood Disorders Center
Type: Basic
Keyword(s): Leukemia, CAR T
Related to Pilot Grant or Trainee Award: 2019, Warshaw Fellow Research Award, Obesity Attenuates T-Cell Function Which May Impact the Efficacy of T-cell based Immunotherapies in Pediatric Leukemia (PI: Anthony Ross, MD, MS)
Poster Available: No

BACKGROUND: Acute lymphoblastic leukemia is the most common malignancy in childhood. Despite vast improvements in treatment, recent studies have shown that children who are obese at diagnosis have poorer survival. The mechanisms of decreased therapeutic responses in obese individuals are not well understood. Changes in pharmacokinetics are partially responsible; however, there is growing interest in understanding how obesity-associated changes to the leukemia microenvironment impacts therapeutic responses. Compromised immune responses have been observed in obese patients, which is of particular interest given the increasing use of immunotherapy in hematologic malignancies. An immunotherapy that has shown success in refractory/relapsed B-ALL is chimeric antigen receptor T-cell therapy (CAR-T) which requires functional T-cells to target and effectively eliminate leukemia cells. The impact of obesity on the efficacy of CAR-T cell function is currently unknown.

OBJECTIVE: Determine the effect of obesity on non-engineered T-cell and CAR T-cell function

DESIGN/METHOD: We developed an in vitro model of obesity utilizing a protocol that differentiates murine bone marrow stromal cells into adipocytes. Primary mouse T-cells were stimulated for 72 hours in the presence of either unconditioned media or conditioned media (stromal cell-conditioned media or adipocyte-conditioned media) followed by flow cytometry to determine the expression of activating (CD44) and inhibitory (PD-1) surface proteins, intracellular cytokine production (IFN-γ and TNF-α), and levels of cytolytic machinery (Perforin and Granzyme B). Similar experiments were conducted on ex vivo T-cells isolated from leukemia patient samples. We are currently examining the impact of adipocyte secreted factors on CAR-T function.

RESULTS: Compared to T-cells activated in control media, T-cells activated in the presence of adipocyte-secreted factors exhibited an exhausted phenotype highlighted by the failure to produce the effector mediators IFN-γ, TNF-α, Perforin, and Granzyme B. Similar compromised responses were seen T-cells isolated from obese children with leukemia. In preliminary studies we also found that human CAR T-cells stimulated in the presence of adipocyte-secreted factors exhibited increased PD-1 expression and reduced TNF-α production, as well as, compromised killing of B-ALL cells.

CONCLUSION: T-cells activated in the presence of adipocyte-secreted factors have reduced functional potential which may compromise the efficacy of CAR-T cell therapies moving forward.
Deciphering the Oncogenic Potential of the LIN28B RNA-Binding Protein in Group 3/4 Medulloblastomas

Shahab, Shubin; Mukarram, Muhammad; Rokita, Jo Lynne; Juraschka, Kyle; Kumar, Sachin; Taylor, Michael; and Schnepp, Robert

Corresponding Author: Shubin Shahab, MD, PhD, Emory University, sshahab@emory.edu
Center: Aflac Cancer and Blood Disorders Center
Type: Basic
Keyword(s): medulloblastoma, RNA-binding proteins, LIN28B, let-7
Related to Pilot Grant or Trainee Award: No
Poster Available: No

Medulloblastoma (MB) is the most common pediatric malignant brain tumor and is currently divided into WNT, SHH, group 3 and group 4 subtypes. Even with multimodal chemotherapy, radiotherapy and surgery, many children with group 3/4 MBs do not survive. While the molecular aberrations underlying WNT- and SHH-driven MBs are relatively well understood, the oncogenic drivers that lead to group 3/4 MBs are poorly defined, limiting therapeutic progress. Beyond somatic dysregulation, cancers display deregulated transcription and translation. RNA-binding proteins (RBPs) play key roles in both transcription and translation, and a subset of RBPs are deregulated in many different malignancies. Indeed, we have previously demonstrated an oncogenic role for the RBP LIN28B in neuroblastoma and it is known to be upregulated in Wilms tumor, hepatoblastoma, germ cell tumors, leukemia, among others. LIN28B is a key regulator of let-7 family miRNAs, which in turn inhibit LIN28B and many other proteins. We postulated that LIN28B plays an important role in MB and that a better understanding of LIN28B and LIN28B-influenced networks may reveal novel therapeutic vulnerabilities.

In support of our hypothesis we find that LIN28B overexpression is associated with significantly worse survival in MB (50% of patients alive at 20 months for high LIN28B vs >140 months for low LIN28B; log-rank test p = 2.604e-03). Accordingly, we demonstrate that LIN28B is very highly expressed only in patients with group 3/4 MBs (p = 4e-07) and in group 3/4 cell lines. We engineered cell lines in which LIN28B is depleted and demonstrated that down-regulation of LIN28B results in significant reduction in cell proliferation by CellTiter-Glo assay and induces apoptosis (induction of cleaved PARP). In contrast overexpression of LIN28B increases MB growth. We plan to inject these cells orthotopically to evaluate effects on tumor growth and survival in vivo. Additionally, to robustly define the signaling networks downstream from LIN28B and identify novel targets, we will perform RNA-seq profiling of group 3/4 cells following LIN28B depletion and validate a subset of these. This work will help define the role for LIN28B in group 3/4 MB aggressiveness and pave the way for similar studies in other cancers.
BMI1 Is a Therapeutic Target in Rhabdomyosarcoma

Shields, Cara; Cuya, Selma; Potlapalli, Sindhu; Chappell, Sarah; Chen, Dongdong; Martinez, Daniel; Pogoriler, Jennifer; Sarah; Rathi, Komal; Patel, Shiv; and Schnepp, Robert

Corresponding Author: Cara Shields, Emory University, ceshiel@emory.edu
Center: Aflac Cancer and Blood Disorders Center
Type: Clinical or Translational
Keyword(s): epigenetics, sarcoma
Related to Pilot Grant or Trainee Award: No
Poster Available: No

Rhabdomyosarcoma (RMS) is an aggressive soft tissue sarcoma which affects mainly children. There are two subtypes: fusion-positive (FP-RMS) and fusion-negative (FN-RMS). FP-RMS is characterized by PAX-FOXO1 fusion proteins and has a worse overall outcome. There is an urgent need to identify targets for this aggressive cancer. The epigenetic complexes PRC1 and PRC2 are overexpressed in a variety of sarcomas and are associated with poor survival. We discovered that BMI1, a protein member of PRC1, is overexpressed in FP-RMS cells. BMI1 is a known oncogene in other cancers, but its role in FP-RMS has not been interrogated; thus, we aim to study it within this context.

First, we analyzed the function of BMI1 in FP-RMS, we depleted BMI1 in FP-RMS cell line models by both shRNA/siRNA knockdown and measured expression, cell proliferation and apoptosis. We then utilized two small molecule inhibitors, PTC-209 and PTC-028, to obtain IC50s in these cell lines and determined effects on cell proliferation and apoptosis in vitro and in vivo. We assessed the effects of BMI1 loss on candidate oncogenic signaling networks.

Initially we examined RNA-seq tumor datasets and determined that BMI1 is robustly expressed in FP-RMS tumors. We confirmed that BMI1 is also overexpressed in FP-RMS cell lines at the levels of RNA and protein. We depleted BMI1 using multiple shRNAs and siRNAs and found that this led to striking decreases in cell growth and an increase in apoptosis. Pharmacologic inhibitors PTC-209 and PTC-028 mediated similar phenotypes. As PTC-028 is more effective in vitro, we used this drug in vivo by utilizing a subcutaneous xenograft model of FP-RMS. We observed a decrease in tumor burden and an increase in progression-free survival in mice treated with PTC-028. We further investigated the molecular impact of BMI1 loss and found that BMI1 inhibits the Hippo pathway.

BMI1 supports proliferation and survival in in vitro and in vivo models of FP-RMS. Inhibition of BMI1 decreases cell proliferation, increases apoptosis, and additionally promotes activation of the tumor suppressive Hippo pathway. Targeting BMI1 pharmacologically may provide a novel treatment option for patients with FP-RMS and potentially other sarcomas.
MERTK Tyrosine Kinase Is a Potential Therapeutic Target in Pediatric Bone Sarcomas

Yeung, Jessica; Santos, Olivia; Smart, Sherri; Wang, Xiaodong; Frye, Stephen; Earp, Shelton; Graham, Douglas; and DeRyckere, Deborah

Corresponding Author: Sherri Smart, MD, PhD, Emory University, sherri.k.smart@gmail.com
Center: Aflac Cancer and Blood Disorders Center
Type: Clinical or Translational
Keyword(s): cancer therapy
Related to Pilot Grant or Trainee Award: No
Poster Available: Yes - P5

Osteosarcoma (OS) and Ewing’s Sarcoma (EWS) are the most common pediatric bone tumors, yet treatment options are limited. MERTK receptor tyrosine kinase is aberrantly expressed and promotes tumorigenesis in numerous cancer types. We investigated MERTK as a potential therapeutic target in OS and EWS. Publicly-available data were used to demonstrate MERTK RNA expression in OS and EWS cell lines, MERTK mutations in OS patient samples, and functional dependence on MERTK in CRISPR-based screens. MERTK protein was also expressed in three of four OS and both EWS cell lines tested. Treatment with MRX-2843, a MERTK tyrosine kinase inhibitor that is currently in clinical development, decreased cell density in cultures of MERTK-expressing OS and EWS cell lines, with EC50 values ranging from 180-1176 nM. The SAOS-2 OS, RD-ES EWS, and SK-ES-1 EWS cell lines were most sensitive, with EC50 values less than 250 nM. Although the remaining MERTK-expressing OS cell lines were less sensitive to MRX-2843 alone, MERTK inhibition enhances chemosensitivity in numerous cancer types and may enhance the response to chemotherapy in OS and EWS as well. The SJSA-1 OS cell line did not express MERTK and was not sensitive to MRX-2843. These data implicate MERTK as a promising therapeutic target in OS and EWS and support continued investigation of MRX-2843 alone and with cytotoxic chemotherapy for treatment of pediatric bone tumors.
Next-Generation Sequencing Reveals Increased Prevalence of PICALM-MLLT10 Fusions in T-ALL and T-LLy

Summers, Ryan J.; Aumann, Waitman K.; Pauly, Melinda; Goldsmith, Kelly; Porter, Christopher C.; Worsley, Randolph; Graham, Douglas K.; Castellino, Sharon M.; and Wechsler, Daniel S.

Corresponding Author: Ryan Summers, MD, FAAP, Emory University, rjsumme@emory.edu
Center: Aflac Cancer and Blood Disorders Center
Type: Clinical or Translational
Keyword(s): T-ALL, PICALM-MLLT10, next-generation sequencing
Related to Pilot Grant or Trainee Award: No
Poster Available: No

The genomic landscape of pediatric and young adult T-cell acute lymphoblastic leukemia (T-ALL) has been described. However, prospective sequencing of newly-diagnosed pediatric T-ALL patients has not been routinely conducted. The Aflac Precision Medicine Program (APMP) uses comprehensive tumor profiling to identify molecular targets with the aim of improving outcomes of children with de novo or recurrent high-risk tumors. The APMP targets newly-diagnosed T-ALL and T-lymphoblastic lymphoma (T-LLy) because of higher relapse rates, dismal salvage rates post-relapse, and inferior overall outcomes compared to pediatric B-ALL.

Pediatric patients with newly diagnosed T-ALL or T-LLy enrolled on the IRB-approved AflacPM1702 protocol between 5/17/2018-12/20/2019. Tumor (bone marrow, peripheral blood, soft tissue) and germline samples (saliva, peripheral blood) were analyzed using Ashion’s GEM Extra® Platform, including full transcriptome (RNASeq) and whole-exome (WES) sequencing coupled with germline subtraction.

A total of 14 patients with newly-diagnosed T-ALL or T-LLy enrolled during the study period. One patient was excluded due to inadequate sample for sequencing. Overall, the mutational landscape in de novo T-ALL/LLy was similar to published reports, with comparable mutation rates in the JAK/STAT, NOTCH, and cell cycle regulation pathways, among others. Most interestingly, however, PICALM-MLLT10 (CALM-AF10) fusions were detected in 4/13 cases (30.8%), compared to a published rate of 5-10%. Importantly, only 2/4 patients with PICALM-MLLT10 fusions detected had t(10;11) present on karyotype. Additionally, RAS pathway mutations were present in 4/13 cases (30.8%), compared to a published rate of 14 %. There was no difference in WBC count at diagnosis, CNS status, or end-induction MRD status in patients with or without PICALM-MLLT10 fusions.

Our results demonstrate that prospective comprehensive tumor profiling utilizing a combination of WES and RNASeq is feasible in pediatric T-ALL and T-LLy, and that sequencing results generally reflect the published literature. The use of RNASeq revealed an increased frequency of PICALM-MLLT10 fusions that were not detectable by karyotype. Recent data suggest that MLLT10 fusions carry an adverse prognosis in pediatric and young adult acute myeloid leukemia, regardless of fusion partner. The increased prevalence of PICALM-MLLT10 fusions in our cohort provides a rationale for their further study in T-ALL and T-LLy.
INTRODUCTION: Youth with sickle cell disease (SCD) have high rates of healthcare utilization and challenges in accessing care. Psychosocial screening has been found useful in predicting increased service utilization in pediatric oncology populations, which can help guide allocation of resources and treatment needs, thus improving access to care. This study aimed to investigate the utility of psychosocial screening in predicting healthcare utilization for youth with SCD.

METHODS: Children and adolescents with SCD (n=80; Mage=14.30, SD=2.46) and their parents were recruited from comprehensive SCD clinics. Youth were primarily African American (n=72; 92.30%), female (n=41; 56.20%), and with hemoglobin type HbSS (n=61; 76.25%). As part of a larger battery of surveys, parents completed the Psychosocial Assessment Tool (PAT), a psychometrically sound measure of psychosocial risk in pediatric populations. The PAT categorizes psychosocial risk into 1 of 3 categories: Universal (minimal distress), Targeted (elevated distress, risk factors present), and Clinical (persistent distress, high risk factors). Healthcare utilization one year before and one year after PAT completion was determined through retrospective chart review.

RESULTS: One-year pre-PAT completion, participants had an average of 1.99 (SD=3.18) admissions and 1.56 (SD=1.80) emergency department visits. One-year post-PAT completion, participants had an average of 2.38 (SD=3.14) admissions and 1.56 (SD=3.13) emergency department visits. Based on ratings from the PAT, 60% (n=48) of families were categorized within the Universal group, 27.5% (n=22) within the Targeted group, and 12.5% (n=10) within the Clinical group. Healthcare utilization varied significantly across PAT groups one-year post-PAT completion (padmissions=0.02; pED=0.04). The Targeted group demonstrated significantly higher admissions (p<0.01) and emergency department visits (p=0.02) one-year post-PAT completion compared to the Universal group. The Clinical group did not differ significantly from the Targeted or Universal groups (p’s>.05).

CONCLUSIONS: Higher psychosocial risk is related to more healthcare utilization within the subsequent year for youth with SCD. Integrating the PAT into routine clinical care may help providers identify individuals in need of greater psychosocial or medical support to further optimize SCD management. Future studies with larger sample sizes are needed to evaluate the feasibility and efficacy of preventative interventions for individuals with SCD who are at higher psychosocial risk.
Genetically Engineered T Cells Using CRISPR and Mechanoporation Show Therapeutic Potential Against Childhood Acute Lymphoblastic Leukemia/Lymphoma

Yu, Tong; Raikar, Sunil; Spencer, Trent; and Sulchek, Todd

<table>
<thead>
<tr>
<th>Corresponding Author:</th>
<th>Tong Yu, BS, Georgia Tech, <a href="mailto:tyu44@gatech.edu">tyu44@gatech.edu</a></th>
</tr>
</thead>
<tbody>
<tr>
<td>Center:</td>
<td>Aflac Cancer and Blood Disorders Center</td>
</tr>
<tr>
<td>Type:</td>
<td>Clinical or Translational</td>
</tr>
<tr>
<td>Keyword(s):</td>
<td>Chimeric Antigen Receptor T cells therapy (CAR-T), microfluidic mechanoporation</td>
</tr>
<tr>
<td>Related to Pilot Grant</td>
<td>2018, Aflac, Microfluidic Platform to Deliver mRNA Knockout Reagents for T-cell</td>
</tr>
<tr>
<td>or Trainee Award:</td>
<td>Malignancy-directed CAR T-cell Manufacturing (MPI: Sunil Raikar, MD and Todd Sulchek, PhD)</td>
</tr>
<tr>
<td>Poster Available:</td>
<td>No</td>
</tr>
</tbody>
</table>

Survival for children with relapsed T-cell acute lymphoblastic leukemia or lymphoblastic lymphoma (T-ALL/T-LLy) is extremely poor when treated with chemotherapy alone. Recently the approved cell therapy based upon chimeric antigen receptor (CAR) T cells, offers an effective immunotherapy for childhood hematological malignancy. The therapeutic T cells are modified to express tumor targeting receptors derived from antibodies. However, CAR-T cell therapy has been difficult to use in T-cell disease because of the lack of a cancer specific T-cell antigen. CAR-T cells developed against T-cell malignancies undergo fratricide and are less effective.

To address this problem, we have designed a CAR against the pan T-cell antigen CD5, while using CRISPR-Cas9 editing to knockdown endogenous CD5 expression in CAR T-cells to improve therapeutic function. To deliver the cas9/gRNA complex into gene-edited T cells, we applied a new microfluidic mechanoporation device. After optimizing the device and operational parameters, we achieved high delivery efficiency comparable to standard electroporation (58% vs 57% delivered population) and reached over 50% CD5 knockout in a cell line and >30% CD5 reduction in primary T cells. Most importantly, cells processed by microfluidic devices maintained high viability (>90%) and unaltered proliferation rate (30 fold growth over 5 days) in Jurkat, while cells processed by electroporation showed reduced viability (50-60%) even after 5 days of recovery and impaired proliferation (5 fold growth). Electroporation also increased PD-1 expression in T cells, which could lead to T cell exhaustion in patients. We now transduce CD5 CAR to the CD5 negative primary T cells and compare the tumor targeting efficiency in the T cell leukemic mouse model.

To summarize, we showed an alternative delivery method for safe and robust genetic engineering of primary T cells. This method preserves T cell viability and proliferative ability, and will increase the targeting efficiency against T-ALL. Our result is directly translatable to CAR-T therapy development for childhood hematological malignancies.
Comparison of the Gut Microbiome Between Children With Solid Tumors Post-Chemotherapy and Age-, Gender-, and Race-Matched Healthy Controls

Zhou, Shuqi; Martin, Melissa; Powell, Christie; Olson, Thomas; Bruner, Deborah; and Bai, Jinbing

BACKGROUND: The human body hosts trillions of microbes in the gastrointestinal (GI) tract. Cancer therapies can alter healthy diversity and composition of the gut microbiome (GM); however, characteristics of the GM in children with solid tumors receiving chemotherapy are still unknown. Studying the GM in children with solid tumors post-chemotherapy could help identify potential gut microbes associated with treatment-related GI symptoms and toxicity.

PURPOSE: Profile the GM in children (aged 7-18 years) with solid tumors post-chemotherapy and compare the GM profiles among these children and age-, gender-, and race-matched healthy controls.

INTERVENTIONS: A case-control study was conducted, with 27 cancer cases and 22 healthy controls enrolled. Children with solid tumors post-chemotherapy within 1 year were recruited from Children’s Healthcare of Atlanta (CHOA) and healthy controls were recruited via flyers in CHO. GM was assessed using stool specimens collected at home following a revised Human Microbiome Project protocol. Children’s demographics and clinical information were collected. The bacterial 16S rRNA V4 gene region was extracted and sequenced using standardized 16S metagenomic sequencing protocol. All bioinformatic analyses (diversity, taxonomy, and abundance) were conducted using QIIME 2.

EVALUATION: No significant differences were found between cancer cases and healthy controls in age (p=0.053), gender (p=0.774), race (p=0.172), and BMI (p=0.346). There were no significant differences in alpha-diversity metrics: Observed operational taxonomic units (OTUs), Shannon, Faith’s Phylogenetic Diversity, and Pielou_e (all p>0.05). Two study groups significantly differed in gut microbial beta-diversity based on Jaccard distance (p=0.009) and a trend in Unweighted UniFrac distance (p=0.074). Taxonomic analysis showed that the dominant bacterial phyla were Bacteroidetes, Firmicutes, Proteobacteria, Verrucomicrobia, and Actinobacteria. The dominant bacterial genera were Bacteroides, Faecalibacterium, Prevotella, Roseburia, and Ruminococcus. Abundance analysis showed that children with solid tumors had lower phylum abundances of Firmicutes, Bacteroidetes, and Proteobacteria.

DISCUSSION: Children with solid tumors showed different GM profiles in beta-diversity and taxa abundance compared to healthy controls, probably associated with cancer pathogenesis and treatment toxicities and symptoms. Future studies should confirm our findings in a larger sample and understand the impact of the GM alterations on cancer treatment toxicities and symptoms.
BACKGROUND: The 2019-2020 influenza season is amongst the most deadly flu seasons within the last decade, resulting in over 10,000 deaths domestically thus far. Based on the performances of the marketed flu vaccines of previous years, there is a need for a more cross-protective or universal flu vaccine. The aim of our research was to investigate the efficacy and protectivity of a cross-protective matrix-2 protein virus-like particle (M2e VLP) vaccine, which would be administered transdermally (through the skin using laser ablation) in a pre-clinical mouse model for influenza.

METHODS: The M2e VLP and adjuvants Alhydrogel® and MPL-A® were encapsulated into a sustained-release polymer matrix and spray dried to produce microparticles that were incorporated into the influenza vaccine. The particles were assessed for their in vitro immunogenicity (ability to stimulate immune cells) and cytotoxicity (toxicity in cells) before being assessed in an in vivo pre-clinical influenza mouse model. 4-6 week old CFW (Swiss Webster) mice were vaccinated with one prime and two booster doses transdermally using laser ablation. Next, the mice were challenged with live influenza virus (A/Philippines/2/82 strain). Blood samples were collected for determination of antibody titers throughout the animal study.

RESULTS: The vaccine microparticles were shown to produce high levels of nitric oxide indicating a strong innate immune response. The vaccine particles were also shown to be non-cytotoxic across a range of concentrations for up to 4 days in cell culture. The mice vaccinated with M2e VLP + adjuvants microparticles demonstrated elevated levels of Immunoglobulin G (IgG), an indicator of adaptive immunity, beginning at week 7, which were maintained through week 10 compared to the unvaccinated groups.

CONCLUSION: Since the current licensed vaccines against influenza may facing numerous challenges associated with production time, antigenic changes and route of administration, we developed a potentially universal flu vaccine with the M2e VLP that was easy to formulate, immunogenic, safe, and easy to administer. Additionally, in the future, fast-dissolving microneedle patches will be explored as an alternate, economical formulation for transdermal delivery of the microparticulate vaccine.
Antiretroviral therapy (ART) improves outcomes for the 2.1 million children living with HIV globally but is not a cure. Therefore strategies to reduce, eliminate, or control the persistent HIV reservoir would be highly beneficial. In this study, we sought to evaluate the impact of a therapeutic vaccine and TLR7 agonist in SIV-infected, ART-treated infant rhesus macaques (RMs). Four-week-old RMs (n=16) were infected orally with SIVmac251 then after an additional 4 weeks placed on a daily ART regimen. After 22 weeks post-infection (wpi) 8 RMs received 2 doses of Ad48 vectors expressing SIVgag-pol-env 8 weeks apart and 2 doses of MVA vectors expressing SIVgag-pol-env at 38 and 50 wpi; 10 oral doses of the TLR7 agonist (GS-986) were administered every other week beginning at 40 wpi. The remaining 8 RMs served as experimental controls. The magnitude of SIV-specific cellular immune responses, measured by IFN-γ ELISPOT and multiparametric intracellular cytokine staining, was increased in vaccinated RMs following Ad48-prime and was boosted by MVA. GS-986 induced an elevation of CD14+CD169+ monocytes 24 h after doses 1, 5, and 10. Four weeks after the last GS-986 dose daily ART was stopped to assess efficacy and animals were evaluated for 8 weeks. A similar time to rebound was observed in treated and control RMs. Despite the fact that SIV-infected ART-treated infant RMs are capable of mounting a robust vaccine response and immune activation by the TLR7 agonist this regimen was not sufficient to impact viral rebound. These results are distinct from previous studies performed in acutely infected adult RMs and highlight the importance of a pediatric model to evaluate HIV cure interventions during the unique period of immune development.
The Utility of a Travel Screen at Triage in Pediatric Emergency Medicine

Greenky, David; Gillespie, Scott; Levine, Aly; and Murray, Brittany

Corresponding Author: David Greenky, MD, Emory University, dgreenk@emory.edu
Center: Center for Childhood Infections and Vaccines (CCIV)
Type: Clinical or Translational
Keyword(s): travel emergency department
Related to Pilot Grant or Trainee Award: 2018, Buchter Resident Research Award, The Global Health Population of CHOA: Impact of Interpreter Use and Recent Travel in the Emergency Department (PI: David Greenky, MD)
Poster Available: No

BACKGROUND: The travel screen was implemented by Emergency Departments (ED) across the country in 2014 to detect patients exposed to Ebola early and prevent local outbreaks. It remains part of the triage protocol in many EDs to detect communicable disease from abroad, and has become a de facto screen for other travel-related illness. Its utility has not been studied in the pediatric ED.

METHODS: This was a retrospective review of electronic medical records across three EDs from 1/1/16-12/31/16. The screening question reads, “Has the child or a close contact of the child traveled outside the United States in the past 21 days?” A follow-up question requesting travel details is included for positive screens. We compared length of stay, return-visit rates and differences in disposition between patients with positive and negative travel screens using generalized linear regression. Matched regression estimates, 95% confidence intervals, and p-values were reported.

RESULTS: The study population included 152,945 patients with a total of 322,229 encounters in 2016, of which 232,787 encounters had a travel screen documented during triage. There were 2,258 patient encounters that had positive travel screens. Only 201 (8.9%) of these encounters had further description of the travel in the comments box. The odds of hospital admission for patients with positive travel screens were 1.76 (95% CI: 1.54, 2.01; p<0.001) times the odds of hospital admission for patients screened negative. The significance of this finding was largely driven by general hospital admission. Other metrics did not differ significantly between the groups.

CONCLUSION: While a positive travel screen was mildly predictive of inpatient admission, information is not available to providers about travel-related risk. Recent literature suggests integrating a travel history with presenting symptoms and region of travel and could produce a more specific travel screen. A revised travel-screen should be implemented and studied in the pediatric ED.
Background: Hundreds of children die in the United States each year due to seasonal influenza infection, but little is known about risk factors related to childhood mortality from influenza. This study seeks to expand what is known about children who die related to complications of influenza infections in Georgia.

Methods: This retrospective case study included children (birth through 18 years of age) who died from complications of influenza-related illness at a single healthcare system in the state of Georgia between April 1, 2009 and April 30, 2019. Demographic and clinical data from patient medical records were collected using a standard case report form. Descriptive statistics were performed.

Results: Twenty-four pediatric deaths were identified. The median age was 8.9 years (IQR 2.3-13.4) and 50% were female. The largest number of deaths occurred in the 2009-10 influenza season (n=8, 33%). Twenty-one (88%) children had no documented influenza vaccine for the season of death. All children had received some routine childhood vaccines, and 17 (71%) had received all recommended vaccines for age with the exception of influenza. Most children had a chronic medical condition (n=15, 63%), with cardiovascular disease (29%), neurologic disorder (33%), gastrointestinal disorder (33%), and genetic conditions (25%) most commonly represented. The majority of children (58%) had a healthcare encounter within 7 days of the hospitalization in which they died, and 21% had been hospitalized within 2 weeks prior. The median number of days between symptom onset and hospital admission was 3 (IQR 2-5). Respiratory co-pathogens were identified in most patients (n=16, 67%), with Staphylococcus aureus detected in 7 children.

Conclusions: In this analysis of children with influenza-related mortality in Georgia, patients were older than those typically thought to be at highest risk and most had comorbid conditions. Many patients sought care prior to hospitalization and the median duration of symptoms prior to presentation was only 3 days suggesting that access to care did not contribute substantially to death. Most children did not receive seasonal influenza vaccine but had received other routine childhood vaccines. Strategies to improve influenza vaccine uptake should be prioritized, especially among school-aged children with chronic medical conditions.
RSV Antibodies as Correlates of Protection in Hospitalized Adults With Acute Respiratory Tract Illness or CHF or COPD

Jadhao, Samadhan; Ha, Binh; Lee, Chun Yi; Hussaini, Laila; Bristow, Laurel; Tippett, Ashley; Gibson, Theda; Hart, Mari; Salazar Luis; Gaffney, Michelle; Benyeogor, Ifeyinwa Kanayo; Cheng, Andrew; Drobeniuc, Ana; Traenkner, Jessica; Stephens, Kathy; Swerdlow, David; Hubler, Robin; Agosti, Yasmeen; McCracken, Courtney; Rostad, Christina A.; Kao, Carol; Yildirim, Inci; Rouphael, Nadine; Anderson, Evan J.; and Anderson, Larry J.

Corresponding Author: Samadhan Jadhao, Emory University, Samadhan.Jadhao@emory.edu
Center: Center for Childhood Infections and Vaccines (CCIV)
Type: Clinical or Translational
Keyword(s): Respiratory Syncytial Virus
Related to Pilot Grant: No
Poster Available: No

Respiratory Syncytial Virus (RSV) is estimated to cause 50,000 – 125,000 hospitalizations/year in U.S. children <5 years of age. RSV is also an important pathogen later in life with severe disease also occurring in the elderly and in those with congestive heart failure (CHF) or chronic obstructive pulmonary disease (COPD). Data are limited on RSV hospitalizations and correlates of antibody titers to the protection in adults. Correlates of antibody dependent protection are important to evaluate the quality and efficacy of vaccines and define the protective antibody titers vaccines would need to induce to protect against RSV. Level of antibody titers in human can also influence protective response of RSV vaccines due to interference of pre-existing immunity to mount vaccine immune response. We prospectively enrolled (Oct 2018–Mar 2019) 596 patients (pts) ≥50 years of age hospitalized with acute respiratory tract infections or of any age with chronic obstructive pulmonary disease or congestive heart failure and from Metropolitan Atlanta. NP and OP swabs were tested for RSV using BioFire® FilmArray® and sera for antibodies (ab) against RSV lysate. Asymptomatic adults ≥50 years of age were controls. Of 596 study pts, 36 (6%) were RSV PCR+. Three of 311 controls, 8/210 RSV PCR- pts, and 11/17 PCR+ pts with acute- and convalescent sera had a >2-fold rise in lysate ab. For correlates of protection, we compared antibodies in acute sera from 17 pts with >2-fold titer rise to: 1) 20 PCR- pts with <2-fold titer rise (matched by age, sex and specimen collection date (Control 1)) and 2) 21 PCR- pts or controls with <2-fold titer rise (matched by age and sex and with serum specimens collected early in the RSV season (Control 2)). The lysate ab in RSV-infected patients (geometric mean titer (GMT) 15,484) was significantly lower than Control 1 (GMT 61,353; P<0.01) and Control 2 (GMT 88,146; P<0.01). The F ab and neutralizing ab tended to be lower but not significantly. The low rate in controls, suggests that a 2-fold increase in lysate ab indicates RSV infection. Differences in acute lysate titers may correlate with protection from RSV hospitalization.
Bacteria utilize a complex chemical communication system—a process known as quorum sensing. This mode of intercellular communication affords bacteria the ability to assess densities of chemical signaling molecules, also known as autoinducers. In turn, autoinducers target specific chemical receptors, and through signal transduction cascades, promote changes in gene expression. When bacterial signaling molecules reach a concentration threshold, one particular response is the formation of a biofilm. Biofilms are extracellular matrices that provide bacteria with important metabolic resources, and perhaps most importantly, act as virulence factors against host immune systems. Our research seeks to disrupt the process of quorum sensing via the competitive inhibition of bacterial chemical signaling molecules with a new class of lead antibiotics. By targeting bacterial communication through a process that does not yield bacteriostatic or bactericidal effects typical of traditional antibiotics, yet downregulates biofilm gene expression, we are able to proactively safeguard against the selection of antibiotic resistant strains of bacteria. The desired result of this approach is to decelerate the rapidly progressing global issue of antibiotic resistance.

A dehydration coupling of carboxylic acid derivatives to select amino acids was used to produce families of novel lead compounds. Biofilm inhibition was measured via a traditional crystal violet assay. Compounds containing variations of halogenated functionality centered around aromatic rings, in addition to trans-cinnamic acid derivatives, were found to possess anti-biofilm properties in *Bacillus subtilis*, *Staphylococcus aureus* and *Streptococcus mutans*. All compounds were then tested in triplicate with inhibition percentages ranging from 33-89 %. Successful biofilm inhibitors were also found to neither kill nor inhibit the growth of each bacterial species, thus verifying their alternative action to traditional antibiotics. In the future, additional work will focus on the isolation of biofilm regulatory genes and the biochemical pathways of action taken by each compound.
Measles, a highly contagious infection was responsible for high worldwide morbidity before introduction of measles vaccine. Currently this vaccine is administered via regular hypodermic needles. Since children are primary recipients of the vaccine, we are interested in examining the possibility of delivering vaccine via needle-free oral dissolving films (ODFs). In order to achieve this, we are experimenting the fabrication of ODF containing microparticulate measles vaccine with the use of a fully automated 3D printing technology. Oral cavity is rich in dendritic cells which traffic antigenic material to the mucosal associated lymphoid tissue (MALT).

The measles vaccine microparticles will be first formulated using a specialized three-fluid nozzle using a Buchi spray dryer B-290. Using this, the microparticles encapsulating the antigen embeded in a biodegradable polymer will be surface coated with a biopolymer that can provide the sustained release of antigen.

ODFs will be then be formulated using completely automated 3D printing technique. UV curing of the polymer will allow for the formation of the films in situ in few seconds, as compared to several hours using the traditional formulation method. The ODFs will be formulated in the 3 different layers such that the middle layer containing the vaccine microparticles will be protected by the top and bottom layers of the film which will also contain a muco-adhesive biopolymer such as chitosan. The formulated ODFs will be evaluated for thickness, tensile strength, Young’s modulus (to measure elasticity and stiffness of the film), percent elongation and dissolution of the vaccine microparticles.

The ODFs containing the measles vaccine will be further tested in a pre-clinical mouse model for induction of immune response by determining the specific antibody responses to the measles vaccine antigen.
The Role of Maternal Vaccination on Healthcare Visits for Acute Respiratory Infections in HIV-Exposed, but Uninfected (HEU) Infants

Kao, Carol; Thomas, Amanda; Camacho-Gonzalez, Andres; and Sheth, Anandi

Corresponding Author: Carol Kao, MD, Emory University, carol.kao@emory.edu
Center: Center for Childhood Infections and Vaccines (CCIV)
Type: Clinical or Translational
Keyword(s): Vaccine-preventable infection
Related to Pilot Grant or Trainee Award: No
Poster Available: No

BACKGROUND: HEU infants remain at higher risk for hospitalization and severe infections, with worse outcomes from common childhood illnesses. Maternal immunization during pregnancy with influenza and tetanus, diphtheria, pertussis (Tdap) vaccine is recommended and effective at protecting infants from vaccine-preventable infections.

METHODS: We conducted a retrospective cohort study of HIV-positive women who delivered and received prenatal care at Grady Memorial Hospital (GMH; Atlanta, GA) between November 1, 2012 and June 30, 2018. Vaccination history was ascertained through the Georgia Registry of Immunization Transactions and Services or by review of electronic medical record. Mother and infant charts were reviewed. We defined acute respiratory infection (ARI) as infants who presented with symptoms or an admitting diagnosis suggestive of an ARI. Relative risks (RR) of identified care visits (clinic, ED/urgent care, hospitalization) in the first six months post-partum between HIV-positive mothers with varying vaccinations were compared with 95% confidence intervals.

RESULTS: Two-hundred-thirty-six HIV-positive women who delivered at GMH were identified for our study. Of those, 66 (28%) received only influenza, 32 (14%) received only Tdap vaccine, 64 (27%) received both and 74 (31%) did not receive any vaccines during pregnancy. Infants born to mothers vaccinated at all tended to have higher gestational age than those that did not (p-value 0.057). There was a trend towards decreased risk of a clinic visit (RR 0.77, 95%CI: 0.27-2.20), emergency department/urgent care visit (RR 0.79, 95%CI: 0.53-1.29) or any healthcare-associated visit (RR 0.86, 95%CI: 0.60-1.26) in the first six months of life for an ARI in infants born to mothers who received any vaccine during pregnancy versus none although not reaching statistical significance. There was a trend towards decreased risk of hospitalization for an ARI in the first six months of life in infants born to mothers who received both influenza and Tdap vaccines during pregnancy versus unvaccinated (RR 0.55, 95%CI: 0.14-2.22).

DISCUSSION: There was a lower risk of healthcare visits for ARI in the first 6-months of life in HEU infants born to mothers who received antepartum vaccinations. Although not statistically significant, larger studies are needed to fully characterize the immune responses in this unique population.
Targeting Novel Inhibition of the Enterovirus 71 RNA-Dependent RNA Polymerase

Kirby, Karen; Emanuelli, Andres; Du, Haijuan; Montero, Catherine; Zandi, Keivan; Tedbury, Philip; Schinazi, Raymond; and Sarafianos, Stefan

Enterovirus 71 (EV71) is a picornavirus that is an etiologic agent of Hand, Foot, and Mouth Disease (HFMD), which predominantly affects young children. In some cases, EV71 infection can result in severe neurological or cardiopulmonary effects in children, and even death. There are currently no antiviral agents approved for the treatment of EV71, however, a vaccine has recently been approved for use in China. While there is extensive variability among picornaviruses in terms of pathogenesis, the type of disease they cause, and the molecular differences among their structural proteins, there is remarkable conservation in some regions of the RNA-dependent RNA polymerase (RdRp), which is absolutely required in viral replication. Specifically, the Sarafianos laboratory has previously identified a highly conserved binding pocket in the RdRp of a related picornavirus, Foot and Mouth Disease Virus (FMDV), which affects cattle and can cause wide-spread devastation to livestock populations. Preliminary inhibitors of FMDV targeting this pocket were identified through screening of a small subset (~13%) of an in-house compound library. It was also demonstrated that targeting this pocket, which is conserved among multiple picornaviruses, leads to potent antiviral activity in the FMDV picornavirus. Hence, our overarching hypothesis is that compounds that bind at this novel pocket possess broad antiviral activity against multiple picornavirus RdRps. Preliminary experiments have shown that two of the antiviral agents targeting FMDV also inhibit EV71 at low micromolar concentrations, albeit with moderate cytotoxicity in human rhabdomyosarcoma cells. This study focuses on validation of the antiviral inhibition mechanism of the preliminary compound hits in EV71, and screening for compounds with improved antiviral and cytotoxicity profiles against EV71.
Specificities of the Viral Reservoir in Naïve CD4+ T-cells During Pediatric HIV/SIV Infection

Mavigner, Maud; Obregon-Perko, Veronica; Horner, Anna; Vanderford, Thomas; Kulpa, Deanna; Kearney, Mary; Permar, Sallie; Silvestri, Guido and Chahroudi, Ann

Corresponding Author: Maud Mavigner, PhD, Emory University, maud.mavigner@emory.edu
Center: Center for Childhood Infections and Vaccines (CCIV)
Type: Clinical or Translational
Keyword(s): HIV; naive CD4+ T-cells
Related to Pilot Grant or Trainee Award: 2017, JFF, Characterization of SIV Latency in Rhesus Macaque Infant Naïve CD4+ T Cells (PI: Maud Mavigner, PhD)
Poster Available: No

Of the estimated 37.9 million people worldwide infected by HIV, 1.7 million are children. A critical obstacle to curing HIV is the reservoir of latently-infected CD4+ T-cells that persists during antiretroviral therapy (ART). Naïve T-cells are long-lived, multipotent and represent a larger proportion of the lymphocytes in children than in adults. Using a model of SIV infection in infant rhesus macaques (RMs) on ART, we previously showed that naïve CD4+ T-cells (TN) were the main contributor to the viral reservoir, emphasizing the need to characterize this population in HIV pediatric infection.

In the current studies, we further evaluated the viral reservoir in TN during infancy and childhood in both RMs and children. Six 4-week-old infant RMs were orally challenged with SHIV CH505.375H.dCT then initiated on ART 8 weeks post-infection. Reservoir analyses were performed in peripheral blood (PB) and lymph nodes (LN) after 12 months of ART. We also identified 29 ART-suppressed children infected with HIV-1 and treated at Grady Ponce Center in Atlanta. PB was collected from 9 patients and the enrollment is ongoing. Naïve and memory CD4+ T-cells from children and RMs are sorted by FACS. The size of the viral reservoir is estimated by quantifying cell-associated DNA in sorted cells by qPCR. Additionally, proviruses sites of integration into the host genome and full-length sequences are determined by multiple-displacement amplification single genome sequencing (MDA-SGS).

In our animal model, plasma SHIV RNA reached undetectable levels after 10-20 weeks of ART. In line with our previous study, after sustained viral suppression, similar levels of SHIV DNA were found in naïve and central/transitional memory CD4+ T-cells. Our results also showed that TN account for the majority of the CD4+ T-cell reservoir in both PB and LN in these infant macaques on ART for an extended period of time. Preliminary data obtained in ART-suppressed HIV-infected children showed lower levels of HIV DNA in naïve than in memory CD4+ T-cells. However, TN represented up to 79.4% of the CD4+ T-cells.

Our work suggests that the TN compartment is of critical importance for HIV persistence on ART in infants and children.
Spray Dried Microparticles Boosts *In Vitro* and *In Vivo* Immunogenicity of RSV F-VLP Vaccine

Menon, Ipshita; D'Sa, Sucheta; Kang, Sang-Moo; and D'Souza, Martin

**Corresponding Author:** Ipshita Menon, Mercer University, Ipshita.jayaprakash.menon@live.mercer.edu

**Center:** Center for Childhood Infections and Vaccines (CCIV)

**Type:** Clinical or Translational

**Keyword(s):** Particulate vaccine, ablative laser, Microparticles, RSV

**Related to Pilot Grant or Trainee Award:** No

**Poster Available:** No

**INTRODUCTION:** Vaccine-enhanced respiratory disease has thwarted attempts to develop a vaccine for Respiratory Syncytial Virus (RSV) using the inactivated form of the virus. The objective of this study was to evaluate the immunogenicity of encapsulated fusion protein virus like particle (F-VLP) vaccine. Furthermore, this study aims to use Precise Laser Epidermal System (P.L.E.A.S.E) laser to administer the vaccine via the transdermal route of administration to employ the rich presence of Langerhans cells in the skin.

**METHODS:** The F-VLP was incorporated into a biodegradable polymer matrix and spray dried to form microparticles (MP). The *in vitro* innate immunity of the F-VLP MP along with adjuvant monophosphoryl lipid A (MPL) MP was investigated using Greiss’ nitrite assay. Furthermore, *in vitro* adaptive immunity was studied by evaluating the expression of major histocompatibility complex (MHC) I and II and co-stimulatory molecules CD40 and CD80 by DC 2.4 using flowcytometry. Subsequently, vaccine-adjuvant combination was administered to Swiss Webster mice via the transdermal route using P.L.E.A.S.E laser. The mice were bled at regular intervals during the study to determine immunoglobulin G (IgG). The mice were then challenged with RSV A2 virus at week 13 and post challenge the viral load in the lungs was analyzed using an immune plaque assay.

**RESULTS:** Nitric oxide is a marker for innate immunity, we observed that, the F-VLP MP + adjuvant (Adj) stimulated significantly higher nitrite release from RAW 264.7 macrophage cells. The F-VLP MP+Adj also significantly enhanced expression of MHC I and II as well as CD40 and 80 (cellular immunity). Serum analysis using ELISA indicated significantly higher levels of RSV specific IgG levels (humoral immunity) in the mice immunized with F-VLP MP+Adj. Furthermore, the mice immunized with F-VLP MP+Adj had negligible viral plaques, proving that the vaccine was able to clear the infection from the lungs.

**CONCLUSION:** The F-VLP MP+Adj vaccine was able to produce a balanced humoral as well as cellular immune response. The microparticulate vaccine was also able clear the virus from the lungs. Thus, the novel microparticulate F-VLP MP+Adj vaccine, administered transdermally using ablative laser shows promise for a vaccine for RSV.
Breastfeeding transmission accounts for the majority of new pediatric HIV-1 infections and commits infants to lifelong antiretroviral therapy (ART), as interruption is typically followed by return of replication. A better understanding of viral rebound during analytical treatment interruption (ATI) could inform the development of alternatives to ART-based strategies to achieve long-term viral remission in the pediatric population. In this study, we orally-infected 10 rhesus macaques at 4 wks of age with SHIV.CH505.375H.dCT and placed them on daily ART at 8 wpi. ART was interrupted after 1 yr to assess viral rebound. During ATI, rebound viremia was detected within 7-35 d, with variable peak viral loads that reached levels seen at time of ART initiation. Post-treatment control of viremia was seen in 5/5 macaques expressing the MHC Mamu A01+ allele. Various parameters were evaluated for their ability to predict the outcome of ATI. In our model, we do not see an association between PD-1 expression on CD4+ T cells and time to rebound, as previously reported for HIV-1 infection in adults, but higher frequencies of this cell type were associated with higher viral burden post-rebound. SHIV-DNA and -RNA persistence in blood, lymph node, and colorectal CD4+ T cells was also evaluated. Just prior to ATI, the highest levels of SHIV-RNA were found in the colorectal compartment, suggesting this region could be a site of ongoing replication on ART and a source of rebound viremia. To explore this, we used ImmunoPET to visualize whole-body SHIV replication after discontinuing long-term ART. Longitudinal imaging of SHIV envelope expressing cells in tissues before and immediately following ATI shows a dramatic expansion of infected cells in the GI tract, prior to SHIV RNA reaching detectable levels in the plasma, supporting this region as an early site of viral reactivation. In conclusion, this work provides novel insight into the kinetics, origin, and predictors of viral rebound in a pre-clinical macaque model of pediatric HIV infection. Our preliminary data implicates the GI tract as a key site to be studied for the development of remission strategies and one to be monitored in HIV-infected children being considered for ATI.
Quality Improvement in Molecular Microbiological Diagnosis: Evaluation of Pathogen Detection by Broad-Range PCR Amplification and DNA Sequencing and Clinical Outcomes

Quincer, Elizabeth; Gonzalez, Mark D.; and Yildirim, Inci

Corresponding Author: Elizabeth Quincer, MD, Emory University, equince@emory.edu
Center: Center for Childhood Infections and Vaccines (CCIV)
Type: Outcomes
Keyword(s): Broad-Range PCR; Quality Improvement
Related to Pilot Grant or Trainee Award: No
Poster Available: No

BACKGROUND: Recovery of pathogens from clinical specimens can be hampered by prior antimicrobial treatment, fastidious microorganism growth requirements, or lack of specimens for microbiologic testing. The potential use of broad-range PCR and subsequent DNA sequencing (BR-PCR-Seq) of conserved taxonomic markers could allow for detection of bacterial, fungal or mycobacterial pathogens directly from clinical specimens. The purpose of this quality improvement analysis is to determine the impact of BR-PCR-Seq for pathogen detection performed at a reference laboratory on clinical outcomes at a pediatric tertiary care facility.

METHODS: A retrospective chart review was conducted of 19 patients who had BR-PCR-Seq testing performed from 2015 to 2019 at a pediatric tertiary care facility. BR-PCR-Seq results were correlated with culture, pathology, and other microbiological testing, when available. Clinical outcome data including length of hospital stay, length of time to result, infectious disease consultation, and impact on management were analyzed.

RESULTS: A microorganism was detected from BR-PCR-Seq testing in 7 of 19 (7%) cases. Of these 7, none were confirmed with culture, but two results were consistent with pathology and Gram-stain results. The remaining positive results (n=5) were of unclear clinical significance. A consult to the infectious diseases team was made at the time testing was ordered in 16 of 19 (84%) cases; however, their involvement in ordering the test was often unclear. A change in antimicrobial regimen was made in 2 of 19 (11%) and another change in management was made in 1 of 19 (5%) cases. Average length of hospitalization was not decreased when a result was detected. The length of time for the test to result varied.

DISCUSSION: BR-PCR-Seq testing has been employed in a number of clinical contexts at CHOA. Although, BR-PCR-Seq may prove useful in its ability to identify microorganisms directly from clinical specimens, its ability to make an impact on clinical outcomes should be weighed with practical considerations, including cost and turnaround time. A consult to the infectious diseases service is warranted to ensure BR-PCR-Seq testing is ordered in an appropriate manner and that results, positive or negative, are interpreted correctly in light of the patient’s clinical state.
The Effects of RSV Attachment (G) Protein Mucin Domains on Viral Growth Kinetics, Thermostability, and Steric Shielding of the Fusion (F) Protein

Roe, Molly; Lapp, Stacey; and Rostad, Christina

Corresponding Author: Christina Rostad, MD, Emory University, christina.rostad@emory.edu
Center: Center for Childhood Infections and Vaccines (CCIV)
Type: Basic
Keyword(s): RSV and vaccine
Related to Pilot Grant or Trainee Award: 2019, JFF, The Effects of RSV Attachment (G) Protein Mucin Domains on Steric Shielding of the Fusion (F) Protein, Pre-F Stability, and Immunogenicity of a Live-Attenuated Vaccine Candidate (PI: Christina Rostad, MD)
Poster Available: No

BACKGROUND: Respiratory syncytial virus (RSV) is a leading cause of lower respiratory tract infections in infants, with cases ranging from minor upper respiratory tract infections to pneumonia with respiratory failure, yet there remains no vaccine. RSV G, a surface glycoprotein which consists of a small, central conserved domain and two variable mucin domains, facilitates viral attachment to the host cells. However, its contribution to viral growth kinetics, thermostability, and steric shielding of RSV F (a second surface glycoprotein that facilitates viral fusion) is unclear. Here we explore the phenotype of viruses expressing deletions of their mucin domains or complete deletions of G protein.

METHODS: To explore the effects of the G mucin domains, RSV viruses were generated in strain A2-line19F with mKate2 label with either wild type full-length G (membrane-bound and secreted, AZ), truncated G with removed mucin domains (membrane-bound and secreted, AZ-G155), and truncated G with removed mucin domains (secreted only, AZ-G155S), and protein expression was confirmed via western blotting. ELISAs were also performed to explore the effects of G mucin domains on antibody binding to F. Viral growth kinetics and thermostability were measured in HEp2 and Vero cells.

RESULTS: Removal of G mucin domains (AZ-G155) led to significantly higher F binding by ELISA despite similar F expression levels by western blotting. AZ-G155 was the most attenuated, followed by AZ-G155S and then AZ respectively. No significant differences in growth kinetics were apparent in Vero cells, making this cell line likely suitable for rescue of a vaccine candidate. Thermostability at 37°C was likewise measured, with AZ having the highest stability, followed by AZ-G155 and AZ-G155S. Viral growth kinetics and thermostability were also measured in two thermosensitive (unstable) strains with G-null mutations: A2 and 220. In these cell lines, significant differences were found in viral growth kinetics, but not in thermostability.

CONCLUSION: Removal of the RSV G mucin domains increased antibody binding to RSV F without affecting F surface expression, suggesting the presence of antigenic shielding. The presence or absence of RSV G and its mucin domains modulated viral growth kinetics and thermostability in a strain-specific manner.
Suicide is the third leading cause of death for individuals aged between 15 and 19 years. There is increasing interest among scientific communities to better understand the neuroanatomical factors associated with suicidal ideation (SUI). Due to a variety of complicated interactions among variables which are associated with SUI and as per the suggestions proposed by the Research Domain Criteria framework for suicide, there is currently a lack of consensus pertaining to underlying neural mechanisms of SUI. A better understanding of the neuroanatomical basis of SUI remains an important challenge in ongoing attempts to treat such behavior. Due to significant involvement of the prefrontal cortex (PFC) in cognitive reframing, reappraisal, and action-planning, we hypothesized that the cortical structure of regions within the PFC will be associated with SUI.

We recorded neuroanatomical data from 110 adolescents (mean age = 16.02±1.45 years, 28 F) recruited from the Boys Town residential care facility, where participants are housed in strictly controlled family homes. All of the participants had significant incidence of SUI documented in their electronic youth record in the last 90 days. The Suicide Probability Scale was administered to quantify the severity of SUI, hopelessness (HOP), negative self-evaluation (NEG), and hostility (HOST). A vertex-wise analysis using FreeSurfer was conducted to identify the brain regions with significant associations between cortical volume (CV) and SUI at a cluster-forming threshold (non-parametric permutation method with 10,000 permutations) and a cluster-wise threshold of 0.05, while controlling for age, sex, HOP, NEG, and HOST. We found a significant negative association between SUI and CV within the left lateral orbitofrontal cortex. The observed cluster was extended to the medial orbitofrontal cortex, superior frontal cortex, rostral middle frontal cortex, and orbitalis.

Increasing levels of SUI are associated with decreases in CV within several critical brain regions, which are known to be associated with cognitive reappraisal, decision-making, and impulsivity. The observed areas should further be investigated to better understand the basis of neuropathophysiology of SUI in suicidal attempters. Our findings provide a scientific platform for future studies to investigate whether brain morphometry could aid in predicting the likelihood of future suicide attempts.
An Observational Pilot Study of Resident Handoff Practices and Values in the Intensive Care Unit

Bederman, Leonid; De, Subhendu; Chico, Suhasini; and Basu, Rajit

Corresponding Author: Leonid Bederman, MD, Emory University, lbederm@emory.edu
Center: Center for Clinical and Translational Research (CCTR)
Type: Clinical or Translational
Keyword(s): patient safety, patient handover
Related to Pilot Grant or Trainee Award: No
Poster Available: No

BACKGROUND: Patient handoffs represent a significant proportion of clinical miscommunication and have been identified by various national regulatory bodies as a patient safety priority. We identified a paucity of standardization and clarity during our resident shift-change handoff in the pediatric intensive care unit (PICU) and conducted an observational study to characterize this deficiency.

METHODS: Pediatric residents (PGY1 – PGY3) were surveyed regarding the importance of commonly understood “best practices” for patient handoff. Responses were tracked using a Likert scale. A “handoff assessment tool” was designed based on these responses and utilized to observed resident evening shift-change handoff in the PICU. Handoff events were observed by three independent observers and elements of handoff were tracked using the handoff assessment tool.

RESULTS: 59 (72%) residents completed the handoff survey. The five highest-scoring handoff elements – description of patient illness severity, explicitly stated action items, reason for PICU admission, contingency planning, and ongoing medical issues – were chosen for further analysis. 104 patient handoff events were observed across 8 different PICU shift changes. A description of patient illness severity received the highest Likert scale score but was only present in 43% of total patient handoffs. This percentage did not change significantly when adjusted for new patients, cross cover patients, intubated patients, or residency specialty (range 40% - 53%). Action items were conveyed in 41% of handoffs, reason for PICU admission in 54%, and contingency planning in 46%.

CONCLUSIONS AND FUTURE DIRECTIONS: In this pilot analysis, we identified a discordance between handoff elements deemed important by pediatric residents and those discussed in real practice. This project is ongoing. Next steps include expanding a baseline validation data set to inform QI based interventions and improve handoff in the intensive care unit.
BACKGROUND: Gut microbial development in preterm infants is affected by environmental and genetic factors. However, the extent to which genetic factors play a role in shaping the preterm infant gut microbiome is poorly understood. Studying the gut microbiome of multiplets in relation to their environmental exposures, such as enteral feeding type, can provide insight into genetic vs environmental influences.

OBJECTIVE: We hypothesized that genetically related preterm infants share more commonality in their gut microbiomes than non-related infants independent of different environments. We also aimed to study the association between enteral feeding type and the gut microbiome in preterm infants with different genetic distances.

DESIGN/METHODS: 50 sets of multiplets (twins and triplets) and 67 singleton controls, matched for birth gestational age and weight, sex, and mode of delivery, were recruited from the NICUs of Tampa General Hospital (TGH) and Carle Foundation Hospital in Illinois. Two stool samples, between day of life (DOL) 4-18 and DOL 18-53, from each infant were analyzed for stool microbiome using 16S rRNA amplicon sequencing on Illumina Miseq. Multiplet and singleton stool samples were matched by postnatal age. Proportions of feeding type from the week prior to each stool sample were recorded. Microbiome composition and diversities were calculated using QIIME2 and statistical testing was performed in R.

RESULTS: Microbial beta diversity distances between genetically related infants were lower than genetically unrelated infants at different time points from two different geographically located NICUs. Enterobacteriaceae and Escherichia abundances correlated in multiplets but not in singletons. The percentage of formula consumed positively correlated with Clostridioides and negatively correlated with Staphylococcus in the Carle singletons, but not in the Carle multiplets. On the other hand, the percentage of formula consumed positively correlated with Clostridioides in the TGH multiplets, but not in the TGH singletons.

CONCLUSIONS: Our results show that preterm multiplet infants consistently shared more similarities in their gut microbiomes than unrelated individuals and that the differences in the gut microbiome between multiplets and singletons were not consistent in relation to enteral feeding type. We will explore the gut microbiome development in relation to different clinical outcomes in multiplets.
PRESENTATION: Eight-year-old female presents with dysuria, back pain, and 5 episodes of gross hematuria within 5 days. She has a history of proteinuria on urinalyses and culture-negative UTIs. She saw physicians for the urinary complaints, including nephrology 2 days prior. She denies fever or upper respiratory symptoms but reports a strep throat infection 5 months ago.

PHYSICAL: She is well appearing and conversant with normal vitals. She has moist mucous membranes, no conjunctival injection or pallor, and no tonsillar exudates. Benign cardiopulmonary and abdominal exams. She exhibits costovertebral tenderness. Normal genitourinary exam with no fissures, urethral trauma, blood or discharge. She is nonedematous with no rashes.

EVALUATION: Urinalysis of a home sample revealed pH 5.0, specific gravity 1.055, 2+ glucose, 1+ blood with no RBCs or WBCs, nitrite positive, and negative leukocyte esterase. The consistency, and odor of the sample were concerning for contamination. Renal ultrasound was normal. Clean-catch urinalysis obtained in the ED had 1+ blood but no RBCs, 5 WBCs, 1+ leukocyte esterase, and positive nitrites. Hgb was normal. The patient admitted to repeatedly adding ketchup to her urine to avoid school after arguing with a friend. Psychiatry did not suspect major psychological stressors or physical or sexual abuse.

DIAGNOSIS: Factitious gross hematuria secondary to ketchup contamination in the setting of unspecified adjustment disorder.

Discussion: Factitious gross hematuria is described as a manifestation of Munchausen syndrome (MS). While ketchup has been documented as a mechanism of pediatric Munchausen by proxy, to our knowledge, this is the first reported pediatric case of factitious gross hematuria secondary to ketchup contamination. Children with factitious gross hematuria use creative methods to mimic hematuria, such as: traumatic bleeding from placing gravel in the urethra; biting one’s cheek, obtaining blood, and introducing it to the urine; and urethral self-catheterization. New social stressors are often present, which may help in the diagnosis of hematuria secondary to MS. Many reported cases resolved with psychiatric therapy. Our case teaches us to appreciate the diagnostic value of home-collected specimens, to maintain a broad differential for hematuria, and to examine urine samples in patients with urinary complaints.
The Importance of Expert Reviewers in Research Intervention Development: A Qualitative Analysis

Chambers, Rebecca; Spratling, Regena; and Lawrence, Patricia

| Corresponding Author: | Rebecca Chambers, RN, Georgia State University, beckchambers@gmail.com |
| Center: | Center for Clinical and Translational Research (CCTR) |
| Type: | Clinical or Translational |
| Keyword(s): | expert reviewers |
| Related to Pilot Grant or Trainee Award: | No |
| Poster Available: | No |

PROBLEM STATEMENT: Using experts allows researchers to create study interventions that are derived from experience, not just evidence. When creating an intervention, caregivers with prior experience should be included in the review process, as they are not only experts in their child’s care, but stakeholders in their child’s outcomes. In a systematic review, several studies found that stakeholder involvement not only improved the relevancy and acceptance of research, but increased the level of trust, understanding, and transparency between researcher and potential user. Before implementing an intervention, evaluating the qualitative feedback from expert reviewers should be considered in order to strengthen not only the intervention, but the study’s outcomes as well.

PURPOSE: The purpose of this study is to describe the expert reviewer feedback on six web-based modules to help caregivers of medically complex children provide home care.

METHODS: A total of 13 expert reviewers, composed of both healthcare professionals and caregivers with personal experience, were asked to review six web-based modules and provide feedback through five open-ended questions. Qualitative content analysis was used to group the expert’s feedback into specific themes.

RESULTS: Based on the qualitative feedback, three themes emerged: module strengths, areas for content expansion, and areas for presentation enhancement. While the analysis revealed opportunities for improvement, the overall consensus was that they are not only helpful, but should be expanded upon for caregivers of medically complex children.

IMPLICATIONS AND CONCLUSIONS: While being a parent is hard, the difficulties of being a parent to a medically complex child is magnified. These difficulties can include health complications, psychological issues, and economic hardships. Having first-hand experience, as either an expert with a clinical or academic background, or as a caregiver who has endured the same hardships, is invaluable when creating interventions. By utilizing these experts and evaluating their responses, researchers are able to create custom interventions that are tailor made to provide a higher quality of care.
BACKGROUND: There is inadequate guidance on the use and dosing of clonidine for withdrawal from opioids and benzodiazepines, using the WAT-1 scoring system in intensive care units (ICUs).

OBJECTIVES: The purpose of this analysis is to evaluate doses of oral and transdermal clonidine that improve withdrawal symptoms in patients receiving sedation or analgesia in the ICU.

METHODS: A retrospective chart review was conducted of children receiving clonidine in the ICUs at Children’s Healthcare of Atlanta (CHoA) between 2016 and 2018. Patients were included if they received ≥ 5 days of sedation/analgesia, and were given oral or transdermal clonidine during weaning. Patients were excluded if methadone and clonidine were initiated on the same day, if clonidine was a home medication, or used for hypertension. Data collection included demographics, sedation duration, clonidine doses, and patient hemodynamics.

RESULTS: A total of 66 patients met inclusion criteria. Median age was 0.4 years (IQR 0-1.4), with most patients <1 year of age [n = 47, 71%]. The medication was delivered via suspension [n = 39, 59%] or transdermal patch [n = 27, 40%], with an average starting dose of 7 mcg/kg/day (IQR 5-9.7). None of the patients had clinically significant hypotension or bradycardia. Clonidine improved withdrawal symptoms in 47 patients (71%) and 19 patients (29%) had complete resolution of symptoms.

CONCLUSIONS: Clonidine was effective in decreasing withdrawal symptoms without causing adverse effects in patients weaning off of sedation. Application of these findings could lower the use of opioid agonists in managing withdrawal in the ICU.
Universal Adolescent HIV Screening in the Pediatric Emergency Department: Barriers & Successes in a Region of High HIV Prevalence

Duda, Elizabeth; Camacho-Gonzalez, Andres; Morris, Claudia; Newton, Naomi; Alevy, Ryan; Palmer, Katherine; Middlebrooks, Lauren; Griffiths, Mark; and Gutman, Colleen

Corresponding Author: Elizabeth Duda, BA, Emory University, esduda@emory.edu
Center: Center for Clinical and Translational Research (CCTR)
Type: Clinical or Translational
Keyword(s): Adolescents, HIV
Related to Pilot Grant or Trainee Award: 2019, Warshaw Fellow Research Award, Pilot Implementation of Universal Adolescent HIV Screening in the Pediatric Emergency Department (PI: Colleen Gutman, MD)
Poster Available: No

CDC recommends universal HIV screening >13, which has been implemented in adult emergency departments. We have previously demonstrated potential missed cases of adolescent HIV in the pediatric emergency department (PED) in the absence of universal screening.

1) To assess the feasibility of routine adolescent HIV screening in an urban PED in a region of high HIV prevalence; 2) To determine the unique barriers to HIV screening in adolescents in the PED

Prospective implementation of universal HIV screening in a convenience sample of adolescents aged 13-18 at a PED. Serum-based 4th generation HIV testing was collected via fingerstick (or with other blood products when possible) and run through a central lab. Caregivers were allowed to remain in the room for HIV counseling, enrollment, and testing. Data were collected regarding patient demographics and HIV testing quality metrics.

Over 4 months, 3204 adolescents were seen in the PED (60% female, mean 15.1yrs); 344 consented to HIV screening (57% female, mean 15.1yrs). Adolescents who consented to HIV screening were more likely to be older than those who declined testing (p=0.025). Twenty-one percent of samples were collected with other blood tests, the mean time to test results was 1hr45min, and 79% of patients were discharged before the result was available. Barriers to testing included: fear of needlestick, cost of testing, time to test results, staff availability, and institutional discomfort discussing sexual health with adolescents. Having a caregiver present for enrollment was not significantly associated with adolescent consent to screening (adjusted OR 1.07, 95%CI 0.67-1.70). One of 344 tests was positive in a 14-yr-old male with an initial CD4T-cell count>500 (Stage 1 HIV).

In this pilot, we identified multiple barriers to universal adolescent HIV screening in the PED. Notably, caregiver presence was not one of them. Despite barriers, 11% of adolescents seen in the PED over 4 months underwent HIV screening and 1 adolescent was diagnosed with HIV in the early stage when anti-viral initiation is most effective. Routine HIV screening of adolescents in the PED is feasible and can identify early infection. Addressing the unique barriers to adolescent HIV screening is critical in high prevalence regions.
INTRO: Eosinophilic esophagitis (EoE) is an inflammatory and increasingly common condition of the esophagus that affects adults and children. The demographics of pediatric EoE have not been thoroughly studied but current literature suggests that the disease mainly affects Caucasian males. Our population in Georgia includes a significant proportion of African Americans (30.5% of the population). Here we present data on symptoms, diagnosis, and therapy effectiveness of a preliminary cohort of African American children with EoE.

METHODS: We retrospectively reviewed the charts of 40 pediatric African American patients seen at one of two urban clinics from 2010-2019 who were diagnosed with EoE. EoE diagnosis was determined following current guidelines including clinical symptoms, more than 15 eosinophils/hpf on biopsy as well as exclusion of other causes of esophageal eosinophilia.

RESULTS: In terms of presenting symptoms, 47.5% of our patients presented with vomiting/regurgitation, 42.5% with dysphagia/feeding difficulties, 42.5% with reflux, 20% with abdominal pain, and 5% with food impaction. Half of females presented with reflux compared to 38.5% of males. 34.6% of males and 28.5% of females presented with dysphagia. One male and one female presented with food impaction. Findings at diagnostic endoscopy included abnormal mucosal findings in 57.5%. Remission was achieved in twenty-one patients, of those six achieved via proton pump inhibitor (PPI) alone, one via food elimination alone, nine with proton pump inhibitor (PPI) and food elimination and four with topical corticosteroids and food elimination. In those that did not achieve remission, six had poor compliance with either medical or dietary elimination and ten were lost to follow up.

CONCLUSION: Our results suggest that African Americans are more likely to present with less specific symptoms such as reflux, abdominal pain, failure to thrive and vomiting and/or regurgitation than Caucasians. African American females were more likely to present with reflux than males. There were a higher proportion of males affected than females. Remission was most often achieved via PPI alone or combination of PPI and food elimination/elemental diet. Further study will detail a larger cohort of pediatric African Americans with EoE and compare them to our overall pediatric EoE population.
A 5-Year Comparison of Pediatric Motor Vehicle and Firearm Injury Trends in the U.S.

Fraser Doh, Kiesha; Sheline, Erica; Morris, Claudia R.; Wetzel, Martha; and Simon, Harold K.

Recent evidence from the Centers for Disease Control demonstrate that firearm injuries (FI) have reached a record high. This is juxtaposed against motor vehicle injuries (MVI) which have decreased. Comparing the trends of the leading causes of pediatric death (FI and MVI) can inform the injury prevention communities.

This study compares national trends in pediatric MVI vs. FI for patients presenting to a collaborative network of pediatric hospitals over a 5-year period.

A retrospective review of patients <19 presenting nationally to 34-member hospitals who contribute to the Pediatric Health Information System (PHIS) database from Jan. 1, 2013 to Dec. 31, 2017. PHIS was queried for patients who had billable diagnosis codes for either FI or MVI and analyzed using SAS and modeled separately. Trends were calculated using linear regression models with logged case counts as the dependent variable. Regional trends were assessed using the US Census Bureau regions with data normalized by overall inpatient admissions for each PHIS hospital center per region.

There were 89,145 visits for MVI and 3,247 for FI over the 5-year period. A 5-year time trend was observed for FI inpatient admissions, with the number of FI-related admissions increasing by 15% per year on average (p= 0.008). However, no clear time trend was present for MVI-related inpatient admissions. ICU admissions noted a 13% (p=0.04) increase for FI vs -5.3% (p=0.22) change for MVI. Over the period, FI deaths increased by 21%(p=0.07) vs 1% (p=0.88) increase for MVI. Regional trends showed FI increased nationwide over the study period and are consistently higher in the South and Midwest.

Trends show an increasing number of injuries attributed to FI presenting to a large pediatric database of hospitals while similar increases in MVI are not seen. Regional and national increases in FI related deaths are also noted. Motor vehicles are highly regulated in design, safety features and use. In contrast, firearms are much less regulated, less studied and safety designs are less mandated. These and other differences need further investigation in order to optimally study and effectively intervene on these alarming trends in pediatric related firearms deaths.
Firearm injuries are a public health crisis. In 2017 there were 2583 children killed by firearms. Recent Centers for Disease Control and Prevention data show firearm and motor vehicle fatality rates are converging as the top cause of death for children 1-19 years.

To describe the prevalence and characteristics of firearms-related injuries in pediatric patients in 4 different regional pediatric trauma centers in the south, mid-Atlantic, northeast and west coast of the US.

Data were retrospectively obtained from the trauma registry and electronic medical records of each pediatric trauma center. Patients were included if <19 years old, evaluated in the ED or hospital from 2003-2018 and diagnosed with a ballistic firearm injury. Data included demographic information, intent, and patient’s disposition. Descriptive statistics were calculated for variables of interest.

A total of 984 children were treated for firearm-related injuries during study period. Median age was 14 years and the majority of patients were African-American, male and had public health insurance. The mechanism was assault in 62% of cases. In the south the perpetrator was frequently self/another child and often unintentional compared to the other 3 sites. Only 11% of patients were discharged from the ED: 40% were ward admissions, 16% PICU and 26% to the operating room while 5% died in the ED. An additional 5% died during hospitalization. 12% required massive blood transfusion. Trends of firearm injury fluctuated between sites with an increase in the south over 15 years.

The incidence of pediatric firearm-related injuries seen at trauma centers since 2003 varies by site and region, with an upward trend noted only at the southern site, where injuries are often accidental compared to other sites. These injuries are serious, with most patients warranting admission, and 9% resulting in death. Studies seeking etiology of upward or downward regional trends in firearm injury may identify successful injury prevention campaigns in that region vs. specific risks that can be targeted in future injury prevention campaigns. Potential efforts may reduce the frequency and healthcare impact of firearm injuries.
Effectiveness of a Gamification Intervention to Limit the Hawthorne Effect in Pediatric Gait Analysis

Geil, Mark; Rahnama, Leila; Jarrells, Justin; Poisal, Micah; Sergeant, Erica; and Soulis, Kimberly

Corresponding Author: Mark Geil, PhD, Kennesaw State University, mgeil@kennesaw.edu
Center: Center for Clinical and Translational Research (CCTR)
Type: Clinical or Translational
Keyword(s): gait, Hawthorne Effect
Related to Pilot Grant or Trainee Award: 2018, CTID, Correlation of Clinical Outcome and T Cell Repertoire and Homing in Overlapping Pediatric Autoimmune Diseases (MPI: Nitika Gupta, MD, DCH, DNB, MRCPCH and David Okou, MS, PhD)
Poster Available: No

INTRODUCTION: The Hawthorne Effect occurs when individuals alter their behavior because they are being observed. Anecdotally, the Hawthorne Effect has been observed during gait analysis. Often called “lab gait,” individuals walk differently with markers attached and in the presence of camera arrays. This is problematic in both clinical and research gait analysis, since spontaneous or natural gait provides better knowledge of nervous system function and potential neuromotor abnormalities. Cognitive distraction is sometimes used, but is challenging in many populations. This study tested a low-immersion virtual reality gamification intervention intended to produce more natural gait.

METHODS: Typically developing children aged 4 to 12 were recruited to participate in this IRB-approved protocol. Prior to motion analysis, children were provided a survey of their feelings about the study based on five emotional domains in Walden et al. Next, a standard instrumented 3D motion analysis was conducted with a full-body Vicon Plug-In-Gait marker set and level overground walking and comfortable self-selected speed. After ten trials, we displayed a virtual environment on monitors around the lab using Argonaut software (Idoneus Digital). Children chose an environment (space, park, castle) and an avatar. The software couples with Vicon marker coordinates to create a real-time 3D reconstruction of the markers. Children were free to wander the lab for about ten minutes, seeking objects and exploring the space (Fig. 1). During that time, we collected trials during any straight-line walking in the Vicon system. Next, we turned off the monitors and repeated the initial gait analysis with ten more trials. Finally, we repeated the survey on feelings.

RESULTS: 30 children participated in the study. Following the intervention, children were significantly more happy (p=0.02) and less scared (p=0.01). In general, walking speed and step length were unchanged. However, a significant moderate correlation (p=0.01, r=-0.55) was observed between reduction in "scared" feeling and increase in walking speed post-intervention.

DISCUSSION: In this typically developing sample, not all children were anxious about the analysis. However, the ones who showed changes in gait, suggesting the potential for an intervention to produce more natural gait. Ongoing analysis will assess GDI, arm swing, and variability.
Idiopathic Toe Walking (ITW) is diagnosed in children three and older who walk on their toes despite no apparent contributing disorders. Current treatments address the locomotor presentation, but not any underlying cause. Based upon previous studies, we believe that some children with ITW are sensory-seeking, and may respond well to in-shoe interventions. In this study, we investigated a novel treatment intended to address tactile stimulation of the plantar surface of the foot, without constraining joint movements.

METHODS: Subjects with ITW screened as sensory-seeking were fit with flexible, textured sensory insoles and instructed to wear for a four-month period in this IRB approved study. At both initial fitting and final four-month assessment, instrumented 3D gait analysis was conducted in three conditions: shoes and insoles, shoes only, and barefoot. Additionally, the weight bearing lunge test was conducted at each assessment to assess range of motion, and parental feedback was assessed using the Orthotic and Prosthetic User Survey (OPUS).

RESULTS: Two subjects have completed the trial to date, a five year old male (A) and a seven year old female (B). Parents of both subjects reported 100% favorable results on the OPUS regarding factors related to device fit, appearance, and durability. Temporospatial parameters with respect to footwear condition (barefoot versus shod with insoles) and time condition (baseline versus follow-up). For example, Subject B showed little change in average speed at baseline when the insoles were introduced (1.44 vs 1.40 m/s), but faster walking at follow-up only in the insole condition (1.57 with shoes and insoles, 1.36 barefoot). Gait Variability Index also decreased at follow-up. Ankle range of motion increased at follow-up in both children, by 5.2 and 5.1 degrees. In both subjects, percent of steps with initial heel contact (not toe contact) improved with insoles at baseline, and improved more at follow-up. Subject B showed 100% heel contact with the insoles, with reduced knee flexion angle at initial contact.

DISCUSSION: In this limited sample, sensory insoles were effective at controlling ITW. This is potentially important, since current orthotic treatments are aimed at mechanically limiting joint motion, but this treatment addresses a possible etiology.
Taking the Pulse of POCUS: State of Point-of-Care Ultrasound at a Tertiary Pediatric Hospital System

Gutierrez, Peter

Point-of-care ultrasound (POCUS) is becoming an integral part of daily practice by physician in many specialties, especially those that are adult-focused. In pediatric medical training, pediatric emergency medicine is the only specialty with content outlines that specify knowledge of POCUS modalities, but other specialties at our institution have reported using it during clinical practice. The primary goal of study is to quantify and categorize the use of POCUS by practitioners of different pediatric specialties at our large pediatric institution, and secondarily to assess the degree of interest by pediatric residents, fellows, and program leaders want to integrate POCUS into their training. Data was collected via online survey, which asked about current use of POCUS in clinical decision making, current POCUS training, and desire for further formal POCUS training. In total, 14 program directors/assistant program directors (PD/APDs) representing 10 of 15 fellowship training programs responded to the survey, 30 of 95 fellows representing 9 of 15 fellowship programs, and 32 of 82 general pediatric and general pediatric/neurology residents responded. From PD/APDs, only 2 of the programs report using POCUS for clinical decision making currently (cardiology, rheumatology), but 13 of the fellows (cardiology, emergency medicine, critical care medicine) and 9 of the residents report doing so. With regards to wanting to start a POCUS training program, 35.7% of PD/APDs, 43.8% of fellow (that don’t already have curricula), and 87.5% of residents would be interested in participating a formal POCUS curriculum. When taking specialty into consideration, some non-acute care-based PD/APDs felt that POCUS was important to future practice (rheumatology, nephrology, endocrinology), as did some fellows (hospital medicine, endocrinology). Current fellow most want to learn abdominal, airway, focused cardiac, and vascular/DVT modalities. Half of the residents surveyed felt that POCUS was important to future practice, and most wanted to learn abdominal, airway, eFAST, focused cardiac, soft tissue, and vascular/DVT modalities. In conclusion, pediatric specialty PD/APDs and their fellows have similar outlooks on the importance of POCUS in future practice. More than half of current residents do not perform POCUS in their current clinical practice, but an overwhelming majority expressed a desire to learn.
Human-induced pluripotent stem cell-derived cardiomyocyte (hiPSC-CM) provide an excellent platform for potential clinical and research applications, such as drug discovery. One way to assess cardiomyocyte functions in the lab is calcium transient analysis. Abnormal calcium transients provide clear and direct evidence for cardiac functional abnormality such as arrhythmia, but manually identifying abnormal calcium transients is a labor-intensive and time-consuming process. For analyzing calcium transients, we can first automatically identify calcium transient peaks and determine abnormality of calcium transient peaks based on select peak features, as well as profile peak specific variables to characterize the identified peaks. Second, we will implement a supervised learning model to learn more information from human expert assessment of peak normality and profiled peak variables in training data, and use the trained model to identify abnormal peaks in test data. By implementing the model, we can obtain peak prediction data about the normality of calcium transient peaks and signals by taking these containing at least one abnormal peak as an abnormal signal. Third, we can profile the following signal specific variables. Lastly, we will implement the same model using training data with signal specific variables, normality assessment based on peak prediction values, and normality assessment by experts, to train a machine learning model that can be used to assess test calcium transient signals. We tested the accuracy of identifying abnormal calcium transient signals by applying our machine learning method to 200 calcium transient signals. We obtained 88% accuracy, 79% specificity and 96% sensitivity for the data. Further, we aim to develop an R package for analyzing calcium transient signals that can implement our proposed analysis procedure to first detect peaks, profile peak specific variables, generate peak normality predictions, profile signal specific variables, and then train a supervised learning model for assessing signal normality. Our R package can be employed in calcium analysis to allow throughput analysis for drug discovery and basic disease mechanism study purposes.
Increased IgD and CD27 Double Negative (DN) B Cell Population in Pediatric Onset Autoimmune Hepatitis

Kolachala, Vasantha; Venkateswaran, Suresh; Chungwen, Wei; Latrece Hill, Aisha; Warren, Vivian; Espinoza, Hillary; Sanz, Iñaki; and Gupta, Nitika

Corresponding Author: Vasantha Kolachala, PhD, Emory University, vkolach@emory.edu
Center: Center for Clinical and Translational Research (CCTR)
Type: Clinical or Translational
Keyword(s): Autoimmune Hepatitis, B cell
Related to Pilot Grant or Trainee Award: 2018, CTID, Correlation of Clinical Outcome and T Cell Repertoire and Homing in Overlapping Pediatric Autoimmune Diseases (MPI: Nitika Gupta, MD, DCH, DNB, MRCPCH and David Okou, MS, PhD)
Poster Available: No

BACKGROUND: Autoimmune hepatitis (AIH) is a chronic inflammatory liver disease of unknown etiology. Although AIH presents both in adults and children, several studies have shown that the disease process may actually start early in life. Further dissection of cellular and molecular pathways involved in AIH pathogenesis is likely to lead to the discovery of novel, tailored and better tolerated therapies. AIH is predominantly a T lymphocytes driven disease but there are a few reports implicating B lymphocytes in the immunopathology. However, there is lack of data to understand the functional role of B cells in AIH.

AIM: The aim was to delineate a detailed B cell repertoire in order to assess the functional role of B lymphocytes in pediatric onset AIH.

METHODS: PBMC were isolated from the blood samples collected from patients visiting CHOA, that are diagnosed with AIH (n=25) and non-AIH controls (n =20) processed for flow cytometry. Markers of B cell development, maturation, and activation were assessed using 10 antibodies namely CD19, CD3, CD21, IgD, CD27, CD38, CD11c, CD24, CD138, and 94G. The markers with significantly different level of abundance between the groups were identified using t-test and Wilcoxon tests and the fold-change (FC) calculated using mean differences among the groups.

RESULTS: CD19+ B cell subpopulations were significantly different in AIH patients compared to controls (P<0.05). Of those, 11 of subpopulations CD19, IgD-CD27-DN2, DN1, IgD-CD27-DN3, DN4, IgD-CD27-, aN, DN2, IgD+CD27-aN, DN3, and Plasmablasts were significantly higher in AIH patients (P<0.05). Notably, DN2, IgD+CD27-aN, DN3, and PB showed higher-level differences with a fold-change (FC) greater than 5. We also noticed, 5 markers IgD+CD27+, IgD-CD27-DN1, IgD+CD27-(-T-aN), IgD+CD27-, and T were showed lower-level abundances in AIH patients (P<0.05). Our data showed females have significantly higher B cell activation overall and in particular African American (AA) females (F) had more severe phenotype with significantly higher CD19 (P<0.02) and DN (P<0.04) compared to females of other ethnicities.

CONCLUSIONS: B cells play an important role in AIH with a higher DN and PB population. AA (F) in particular exhibit a more severe phenotype warranting individualized B cell directed therapy.
Cobalamin Deficiency in Children With Sickle Cell Disease: An Unanticipated Risk for Use of Nitrous Oxide Gas

Krieger, Rachel; Brown, Lou Ann; Dampier, Carlton; Harris, Frank; Manoranjithan, Shaminy; Mendis, Reshika; Cooper, Nicholas; Figueroa, Janet; and Morris, Claudia

<table>
<thead>
<tr>
<th>Corresponding Author:</th>
<th>Rachel Krieger, DO, Emory University, <a href="mailto:rkriege@emory.edu">rkriege@emory.edu</a></th>
</tr>
</thead>
<tbody>
<tr>
<td>Center:</td>
<td>Center for Clinical and Translational Research (CCTR)</td>
</tr>
<tr>
<td>Type:</td>
<td>Clinical or Translational</td>
</tr>
<tr>
<td>Keyword(s):</td>
<td>Sickle cell disease, B12/cobalamin</td>
</tr>
<tr>
<td>Related to Pilot Grant or Trainee Award:</td>
<td>No</td>
</tr>
<tr>
<td>Poster Available:</td>
<td>No</td>
</tr>
</tbody>
</table>

BACKGROUND: The prevalence of cobalamin (B12) deficiency in children with sickle cell disease (SCD) is unknown, however B12-deficiency has been reported in 18% of SCD-adults versus 10% in patients without SCD (Kamineni 2006). A higher frequency of B12-deficiency in SCD may be due to higher rates of hemolysis, erythrocyte turnover and folate deficiency. Nitrous oxide gas is commonly used for dental procedures, and is standard therapy for sickle-related vaso-occlusive-pain in France. Recently we reported acute resolution of sickle-related priapism with nitrous therapy (Greenwald 2019). Although nitrous is generally considered safe, patients with B12-deficiency can experience serious neurologic complications, as nitrous impacts cobalamin metabolism. This study evaluates B12 status in children with SCD.

METHODS: Urine samples were prospectively collected as part of a randomized-controlled trial of parenteral arginine therapy in children with SCD requiring admission for treatment of moderate-to-severe pain. Urine methylmalonic acid (MMA) level corrected for creatinine (Cr) reflecting B12 status, was measured via mass spectrometry. B12 deficiency was defined as MMA/Cr of 2.2-5, while severe B12 deficiency was reflected by MMA/Cr>5; MMA/Cr of 1.8-2.2 were considered possibly deficient while an MMA/Cr<1.8 was defined as normal B12 status.

RESULTS: Ninety-four children with SCD and pain were enrolled. Median age was 13 years (Q1, Q3: 10, 16), 51% female, 68% Hb-SS, and 71% were on hydroxyurea. Twenty-six percent (24/94) of patients demonstrated evidence of B12 deficiency, 25% of whom demonstrated a severe B12 deficiency (6/24). Another 7% (7/94) demonstrated possible deficiency. There were no statistically significant differences in age, gender, SCD genotype, hemoglobin levels, MCV or hydroxyurea use in those with and without B12 deficiency.

CONCLUSIONS: Approximately a quarter of children with SCD demonstrated evidence of B12-deficiency, which is higher than expected. Cobalamin deficiency is associated with a constellation of clinically relevant symptoms that may be overlooked in patients with SCD. In addition, these patients may be uniquely at risk for adverse neurological sequelae when receiving treatment with nitrous oxide gas. B12-deficiency is easily corrected with an intramuscular injection of methylcobalamin. Although further study in a larger cohort is needed, screening for B12 deficiency may be warranted in patients with SCD.
Spatial Resolution of Cellular and Gene Expression Heterogeneity Within Colonic Mucosa During Pediatric Ulcerative Colitis

Maddinpatla, Sushma Chowdary; Kolachala, Vasantha; Dodd, Anne; Venkateswaran, Suresh; Matthews, Jason; Nagpal, Sini; Gibson, Greg; and Kugathasan, Subra

Background: Ulcerative colitis (UC) is a chronic disease characterized by remission and relapse of colonic inflammation. There is a need for deep cellular-level characterization of the mucosa in UC. While single-cell RNA sequencing allows for identification of cell types in the colonic mucosa, the spatial context and cellular organization is not captured. However, the use of spatial transcriptomics (ST) allows for sequencing data to be superimposed onto high-definition microscopic images of the mucosa, thus allowing for the in situ capture of both spatial and transcriptomic data. To this end, we have used ST to visually map cell-type organization and gene expression patterns of the mucosa during UC.

Methods: Surgical colonic-tissue obtained from Children’s Healthcare of Atlanta were frozen-fresh in OCT, sectioned to 10µm onto a 6.5x6.5mm capture-area of a 10X Genomics Visium Spatial Gene test-slide containing ~5000 spots with primers: 30nt-poly(dT), 12nt-UMI and spatial-barcodes. Tissue sections were methanol fixed, H&E stained and imaged using brightfield microscope which were used later to superimpose transcription data. After permeabilization, on-slide cDNA synthesis was performed, eluted and libraries prepared. After sequencing on an Illumina-NextSeq, sequence reads were analyzed using Space Ranger for QC, alignment, barcode and image-processing. Loupe browser interactive visualization was used to get spatially resolved clustering based on total gene-expression.

Results: ST analysis revealed 805 spots (1-10 cells-per-spot) occupied by the mucosal section and each spot showed an average of 103,447 reads and 1,519 genes. In total, we obtained 83,275,229 reads for 19,179 protein genes covering 75.5% of the human reference panel-Hg38. Spatial gene-expression-profile was annotated by known markers gene list into 48 subsets: 15 subtypes of epithelial-cells include Stem-cells, 3TA, 2 immature enterocytes, enterocyte progenitor, enterocyte, M-like-cells, BEST4+ enterocytes, secretory TA, Immature goblet, goblet, tuft, enteroendocrine, 12 subtypes of stromal-cells include 8 fibroblasts and 4 endothelial-cells and 21 subtypes of immune-cells include 4 B-cells, 7 myeloid-cells and 10 T-cells.

Conclusion: ST on UC mucosa has given an unprecedented window into the cellular architecture and corresponding gene expression patterns taking place during disease. A comparative analysis of ST data from non-IBD controls tissue will reveal new insight into disease mechanisms of UC.
Missed Opportunities for Adolescent HIV Diagnosis: Targeted Testing vs. Universal Screening in a Pediatric Emergency Department (PED)

Newton, Naomi; Morris, Claudia R.; Camacho-Gonzalez, Andres; Middlebrooks, Lauren S.; Duda, Elizabeth; Alevy, Ryan; Palmer, Katherine; Griffiths, Mark A.; and Gutman, Colleen K.

Corresponding Author: Naomi Newton, MD, Emory University, naomipnewton16@gmail.com
Center: Center for Clinical and Translational Research (CCTR)
Type: Clinical or Translational
Keyword(s): Adolescents, HIV
Related to Pilot Grant or Trainee Award: 2019, Warshaw Fellow Research Award, Pilot Implementation of Universal Adolescent HIV Screening in the Pediatric Emergency Department (PI: Colleen Gutman, MD)
Poster Available: Yes - P9

BACKGROUND: CDC recommends universal HIV screening starting at age 13. This has been implemented in adult EDs, but only few PEDs, where at-risk adolescents may go untested.

OBJECTIVE: 1) Compare characteristics of adolescents tested for HIV in the PED via targeted testing & a universal screening pilot & 2) describe providers’ documentation of HIV risk in PED visits & compare to adolescents’ perception of HIV risk assessment.

METHODS: Prospective study run concurrently with a universal screening pilot. Patients with testing initiated by their provider were defined as having “targeted testing”; those who did not receive targeted testing were eligible for inclusion in the pilot & if tested, were defined as having “universal screening.” Demographics were collected on all tested patients. A subset of adolescents 16-18 years completed a survey on HIV risk factors. All tested patients were asked if their provider had assessed HIV risk during the visit; this was compared to documentation of HIV risk in the chart.

RESULTS: Over 4 months, 107 adolescents received targeted testing & 344 received universal screening. One positive test occurred in a 14-year-old male by universal screening (initial CD4>500); he was missed by targeted testing during 2 prior ED visits within a 60-day period. When compared to those who received universal screening, patients who received targeted testing were more likely to be female (81.6% vs. 56.6%, p<0.001), at least 16 years (71% vs. 44%, p<0.001), or presenting with genitourinary/gynecologic concerns (48% vs. 6%, p<0.001). Many adolescents with HIV risk factors, including one male disclosing male sexual partners, were missed by targeted testing and subsequently tested via universal screening. Adolescents with documented risk assessment were more likely to receive targeted testing; there was low-moderate agreement (k=0.61, 95%CI 0.41-0.74) between providers’ documentation & adolescents’ perception of risk assessment.

CONCLUSIONS: Disparities in HIV risk assessment/testing by PED providers may result in missed opportunities to diagnose adolescent HIV. Adolescent disclosure of HIV risk factors may be unreliable. One HIV+ male was missed by targeted testing in 2 ED visits. Universal HIV screening can address these disparities & expand the group of adolescents tested for HIV in the PED.
Over the past year, approximately 10,000 American teens have died due to a prescription stimulant overdose. A comprehensive, accurate, and easily integrable approach to detect a drug overdose in the environment where it occurs is imperative. This study proposes a multi-factor automated overdose detection system designed to operate in a teen’s living and working space. The eight factors include sweat composition analysis, mood swing detection, measurement of vital signs (heart rate, blood pressure, respiration rate, electrodermal activity), detection of spasms, and consciousness verification. The two-factor reagent strip, consisting of a gold nanoparticle-based diagnostic measure and pH-based control measure, displayed a significant difference in color between simulated case and control sweat. A photometric sensor and hardware apparatus were built and evaluated to integrate the strip onto a laptop mouse. A generalized linear model, gradient boosting machine, and multilayer perceptron trained on over 1.6 million data points were successfully able to detect a mood swing from a baseline emotion. The final model (with an AUC of over 85%) was implemented into the Hero mobile app to monitor a teen’s outgoing SMS messages for a mood swing. A mathematical image processing algorithm to measure vital signs was created, and repeated evaluation resulted in a percent error of <5%. These results, along with external sensors/hardware devices and mobile app features, were incorporated into Hero. In contrast to previous solutions, teens can use the Hero system and mobile app for constant background monitoring of physical, emotional, and biochemical signs of overdose in their daily life.
iSense: Artificial Intelligence Based Early Detection Tool to Identify Linguistic Bio-markers of Mood Disorders and Recognize At-risk Teens

Nori, Divya

Corresponding Author: Divya Nori, Milton High School, divyanori8@gmail.com
Center: Center for Clinical and Translational Research (CCTR)
Type: Clinical or Translational
Keyword(s): Teen Suicide, Mood Disorder Detection
Related to Pilot Grant or Trainee Award: No
Poster Available: Yes - P11

The prevalence of mood disorders has increased rapidly over the past decade, especially in teens. The nature of these disorders is such that most symptoms are in a person’s mind making it harder to diagnose, leading to a mental health epidemic which is difficult to control. This study proposes a mobile application (iSense) powered by an Artificial Intelligence model to identify linguistic biomarkers of mood disorders in a teen’s outgoing SMS messages and send notifications to a parent. The Twitter API was used to create a dataset of over 800 individuals whose language use indicates a mood disorder, based on the DSM-5 Diagnostic Criteria for depressive disorders, and individuals with a healthy mental state. Millions of tweets were collected from these users and filtered to 73,944 tweets based on tweet length (over 50 characters) and dropping retweets. After conducting linguistic analysis, the tweets were used to train/test a generalized linear lasso model (hyperplane-based approach), gradient boosting machine (tree-based approach), and feed-forward multilayer perceptron (neural network-based approach). These models were then compared on several measures such as F1 Score (max 0.717) and accuracy (max 86%). Other features such as notification preferences (parent interface) and a voice-enabled chatbot (child interface) were implemented in the mobile application. iSense has the potential to enable early detection of mood disorders and at-risk individuals, providing a viable solution to combat the increase in suicide cases.
Utilizing Natural Language Processing to Evaluate Sexual History Documentation Among Adolescents Presenting to Pediatric Emergency Departments

Robertson, Caryn; Kandaswamy, Swaminathan; Gooding, Holly; Koyama, Atsuko; Middlebrooks, Lauren; and Orenstein, Evan

Corresponding Author: Caryn Robertson, MD, FAAP, Emory University, crobe30@emory.edu

Center: Center for Clinical and Translational Research (CCTR)

Type: Clinical or Translational

Keyword(s): Informatics and adolescent health

Related to Pilot Grant: No

Poster Available: No

INTRODUCTION: Sexual history documentation (SHxD) in the Pediatric Emergency Department (PED) is associated with higher rates of appropriate STI testing and treatment. However, SHxD occurs in only 18-70% of PED encounters in which adolescents present with a chief complaint potentially related to an STI. The objective of this study was to develop and validate a clinical informatics model for presence of a sexual history using a rules-based natural language processing (NLP). Automated detection of SHxD would facilitate research and quality improvement efforts to improve STI screening and treatment among adolescents presenting to the PED.

METHODS: Medical records from a large pediatric health system with three free-standing PEDs were queried and filtered by emergency department encounters, patient age (13-21 years-old), and potential STI-related chief complaints. We initially created a candidate set of regular expressions to identify SHxD and its key elements. We then utilized a two-phase process of model refinement and model evaluation to compare NLP model detection to manual physician review of provider notes. This two-phase process was then applied to sub-models examining key elements of SHxD.

RESULTS: We identified 814 unique adolescent patient encounters with chief complaints potentially related to STIs. For SHxD and each of the key elements of SHxD, model performance yielded sensitivities >90%, specificities >77%, PPV > 90% (for all except one sub-model), and NPV >97%. We applied the validated NLP model to two years of patient encounters (n=2049) and found SHxD occurred in only 55% of encounters with potential STI-related chief complaints.

CONCLUSION: The development of an NLP model for SHxD and related sub-models allowed for efficient assessment of two years of adolescent patient encounters and analysis of current provider practice habits. In our application of the validated NLP model, we found that SHxD occurred in 55% of patient encounters, indicating clear opportunity for improvement. This new NLP phenotype could produce an automated data feed for quality improvement and research projects aimed at improving the frequency and quality of SHxD with the long-term goal of improving STI screening and sexual health for at-risk adolescents presenting to the PED and other clinical settings.
Human Induced Pluripotent Stem Cell-Derived Cardiomyocytes Exhibit Abnormal Cardiomyocyte Contractility and Calcium Handling After Exposure to TNF-α

Saraf, Anita; Rampoldi, Antonio; Chao, Myra; Li, Dong; Hwang, Hyun; Liu, Rui; Fu, Haian; Fischbach, Peter; Jha, Rajnesh; Maxwell, Joshua T.; and Xu, Chunhui

Corresponding Author: Anita Saraf, MD, PhD, Emory University, anitasaraf@gmail.com
Center: Center for Clinical and Translational Research (CCTR)
Type: Basic
Keyword(s): iPSCs, TNF alpha
Related to Pilot Grant or Trainee Award: 2019, Warshaw Fellow Research Award, The effect of TNF-α on Induced Pluripotent Stem Cells from Hypoplastic Left Heart Syndrome Patients (PI: Anita Saraf, MD, PhD)
Poster Available: No

BACKGROUND: Pro-inflammatory factors such as TNF-α strongly correlate with adverse clinical outcomes in various cardiomyopathies and cardiac arrhythmias including those associated with congenital heart disease (CHD). However, due to limitations in developing animal models for most CHDs, the influence of pro-inflammatory cytokines on disease progression in CHD-associated cardiomyopathy and cardiac arrhythmias is challenging. Cardiomyocytes derived from human induced pluripotent stem cells (hiPSCs) are therefore a practical translational model to study CHD-associated cardiomyopathy and cardiac arrhythmias. METHODS: Cardiomyocytes derived from 2 hiPSC lines were exposed to increasing concentrations of TNF-α (0, 1, 10, 20, 100 ng/mL) at day 18-22 of differentiation for a period of 4 days. Post exposure, we evaluated mitochondrial and cytoplasmic reactive oxygen species (ROS) production, cardiomyocyte apoptosis and cell death, and change in cardiomyocyte markers of differentiation. Additionally, we evaluated the cells for abnormal calcium handling, and generation of calcium sparks and waves. Furthermore, we evaluated calcium propagation through a sheet of cardiomyocytes and changes in cardiomyocyte beat rate, contraction and relaxation velocity. RESULTS: Following exposure to TNF-α, mitochondrial and cytoplasmic reactive oxygen species (ROS) production increased in hiPSC-cardiomyocytes. By day 4, caspase 3/7 production increased in all treated concentrations of TNF-α but more significantly in pathological concentrations of TNF-α (20 and 100 ng/mL). Similarly, higher concentrations of TNF-α also increased cell death as evaluated by live-dead assay. Higher concentrations of TNF-α increased production of calcium sparks and waves, that further led to a statistically significant increase in abnormal calcium transients observed across all concentrations of TNF-α. Furthermore, dyssynchronous cardiomyocyte beating was observed in cell sheets exposed to all concentrations of TNF-α. Lastly, beat rate, and cardiomyocyte contraction and relaxation velocity decreased significantly across all concentrations of TNF-α exposure after 4 days. CONCLUSIONS: Similar to primary cardiomyocytes, and cardiomyocytes in vivo, hiPSC-derived cardiomyocytes exhibit abnormal calcium handling and contractility through ROS-mediated pathways. Hence, hiPSC-derived cardiomyocytes can be used as a translational model to evaluate the effect of pro-inflammatory factors such as TNF-α in cardiomyopathies and cardiac arrhythmias where animal models and primary cardiomyocytes may not be available.
ALT Trends Through Childhood and Adolescence and Their Association With Hepatic Steatosis at 24 Years

Sekkarie, Ahlia; Figueroa, Janet; Northstone, Kate; Welsh, Jean; and Vos, Miriam

OBJECTIVES: Non-alcoholic fatty liver disease (NAFLD) can begin in childhood, although the origins of the disease are not well characterized. ALT is often used to screen for NAFLD. Our objectives are to 1) examine whether elevated ALT at 24 y is associated with hepatic steatosis and 2) describe whether ALT trends from 9-24 y differ by hepatic steatosis grade at 24 y.

METHODS: We used data from a UK population-based birth cohort study (ALSPAC). Patients with 2-4 ALT measurements (at 9, 15, 17, 24 y) were included; pregnant women and high alcohol consumers were excluded. To assess hepatic steatosis, controlled attenuation parameter (CAP) scores from Fibroscan were measured at 24 y. We categorized CAP scores into steatosis grades [none (S0: <248 dB/m), mild/moderate (S1-S2: 248-279 dB/m), and severe (S3: >279 dB/m)]. We used sex-stratified linear mixed models to assess trend differences of log-transformed ALT levels from 9-24 y between the different steatosis grades at 24 y.

RESULTS: The final sample size was 1156 (41.4% male). At 24 y, among those with elevated ALT, 17.5% had severe steatosis (S3), while among those with normal ALT only 4.8% had S3 (p<0.001). In both sexes, there was a more rapid increase in ALT from 9 to 24 y in those with S3 vs S0 at 24 y (p<0.001). In boys and girls at 9 y, there was no significant difference between ALT values for those with subsequent S3 v S0. In boys at 15 y, ALT was mean (95% CI) 19.1 U/L (17.4-20.9) for S3 vs 15.5 U/L (15.0-16.1) for S0; at 17 y, ALT was 25.4 U/L (23.3-27.8) for S3 vs 18.1 U/L (17.5-18.7) for S0; at 24 y, ALT was 45.2 U/L (40.6-50.4) at S3 vs 24.5 (23.5-25.6) at S0 (p<0.001 for all differences). In girls there were smaller but still significant differences in ALT values between those with S3 and S0 at 15, 17, and 24 y.

CONCLUSIONS: Higher ALT levels from childhood to adolescence were associated with severe hepatic steatosis at 24 years. Diverging ALT trends prior to NAFLD diagnosis may allow for the identification and prevention of NAFLD.
Effectiveness of Levetiracetam vs. Phenobarbital for Initial Treatment of Neonatal Seizures

Sewell, Elizabeth K.; Hamrick, Shannon; Patel, Ravi; Bennett, Monica; Tolia, Veeral; and Ahmad, Kaashif

Corresponding Author: Elizabeth Sewell, MD, MPH, Emory University, elizabeth.sewell@emory.edu
Center: Center for Clinical and Translational Research (CCTR)
Type: Clinical or Translational
Keyword(s): Neonatal seizures
Related to Pilot Grant or Trainee Award: 2019, HeRO, Modeling Chemotherapy-induced Cardiotoxicity using Human iPSC-derived Cardiomyocytes (PI: Chunhui Xu, PhD)
Poster Available: No

BACKGROUND: Phenobarbital (PHB) is the most common initial treatment for neonatal seizures, but levetiracetam (LEV) use is increasing despite limited efficacy data to guide optimal pharmacologic treatment.

OBJECTIVE: To compare treatment failure between infants treated first-line with PHB versus LEV, and to describe trends in LEV use.

METHODS: This retrospective cohort study included neonates admitted to the NICU with a diagnosis of seizures from 2009-2018 utilizing the Pediatrix Clinical Data Warehouse. We included neonates admitted for at least 3 days who received PHB or LEV as the initial AED. We excluded patients who received more than one anti-epileptic drug (AED) on the same day due to the inability to discern sequence of administration. Our primary outcome was treatment failure, defined pragmatically as the need for any additional AED. Mixed effect logistic regression was used to compare the risk of AED treatment failure after adjusting for demographic characteristics, comorbid conditions including hypoxic ischemic encephalopathy (HIE), and day of seizure diagnosis.

RESULTS: A total of 8,702 infants with seizures met inclusion criteria, of which 1,860 infants were excluded leaving a study cohort of 6,842 infants from 255 NICUs. Across all years, 6,213 received PHB (91%) and 629 (9%) LEV as first-line therapy. Compared to infants treated first-line with PHB, those treated with LEV were more likely to be term (77% compared to 67%) and weigh more at birth. The incidence of HIE did not vary significantly between AEDs treatment groups, but infants treated with PHB more frequently had severe IVH (15% vs 8%) and meningitis (6% vs 4%). The use of LEV as a first-line treatment for neonatal seizures increased from 11% in 2009 to 18% in 2018. The incidence of need for a second AED was 31% among infants initially receiving PHB, compared to 38% among infants receiving LEV (adjusted odds ratio: 0.70; 95% CI 0.58-0.84).

CONCLUSIONS: Use of LEV for initial treatment of neonatal seizures, compared to PHB, is associated with a higher risk of treatment failure and need for additional pharmacologic treatment. Our findings are of potential concern given the increasing use of LEV as a first-line treatment for neonatal seizures.
Cell-Specific DNA Methylation Patterns of Peripheral Blood Reveals ITGB7 Regulation in CD4+ T Cells Associates With Crohn’s Disease

Shabazz, Kalifa; Venkateswaran, Suresh; Matthews, Jason D.; Cutler, David J.; Conneely, Karen; Smith, Alicia K.; and Kugathasan, Subra

Corresponding Author: Kalifa Shabazz, MS, Emory University, kmshaba@emory.edu
Center: Center for Clinical and Translational Research (CCTR)
Type: Clinical or Translational
Keyword(s): DNA-Methylation, Crohn's
Related to Pilot Grant or Trainee Award: No
Poster Available: Yes - P12

BACKGROUND: Crohn’s Disease (CD) is a chronic, remitting and relapsing disorder of the gastrointestinal tract. Recently we showed that peripheral blood cells of CD patients having distinct DNA methylation (DNAm) patterns. However, this study controlled for differences in cell composition between CD cases and controls, which prevented it from identifying differences in specific blood cell types. Mapping DNAm signatures to specific cell types during CD is fundamental in understanding the role of epigenetics in the onset and progression of disease. Therefore, we sought to distinguish cell-specific methylation signatures to identify immune cell type-specific DNAm differences during CD.

METHODS: Blood samples were obtained from 164 CD pediatric patients and 74 non-IBD controls (RISK cohort). Genome-wide DNAm was profiled at ~850,000 sites using MethylationEPIC array. TOols for the Analysis of heterogeneous Tissues (TOAST) was used to test for cell-type specific DNAm differences in granulocytes, monocytes, B-Cells, T-Cells (CD4 AND CD8), and natural killer cells that associated with CD. The statistically significant sites were identified after multiple test correction with a false discovery rate of <0.05.

RESULTS: CD cases had a higher proportion of granulocytes (P<2.2x10^-16) and a lower proportion of CD4+ T-cells (P<2.2x10^-16) relative to non-IBD controls. While no cell-specific methylation signatures were found for most blood cell types, in CD4+T cells, methylation of the intronic region of ITGB7 (cg04672065) was lower in CD cases (P<5.4x10^-08) when compared to non-IBD controls. Because blood gene expression data is not available in RISK, we evaluated the correlation between DNA methylation and gene expression for ITGB7 in other available datasets. We noted that two distinct tissue types revealed a positive correlation between cg04672065 and ITGB7 expression in blood (R² = 0.46; P = 1.5x10^-13) and rectum (R² = 0.36; P = .03).

CONCLUSION: Epigenetic regulation of CD4+ T-cells during CD leads to lower methylation of ITGB7, likely decreasing ITGB7 expression and down regulating lymphocyte trafficking to/from gut-associated lymphoid tissues. Therefore, our results are providing the further evidence that the dysregulation of epigenetic factors affecting CD4+T cell function may be a contributor to CD-associated gut inflammation.
Pediatric firearm injuries are a public health crisis warranting national attention. Motor vehicle injuries (MVI) involving children are seen in a similar light and therefore provide an effective comparison of resource utilization burden.

This retrospective review of the Pediatric Health Information System (PHIS) database compares resource utilization of patients under 19 years of age presenting to a national network of pediatric emergency departments (EDs) for either motor vehicle or firearm injuries.

PHIS, a national database of resource use for over 45 pediatric hospitals, was queried for patients with billable diagnosis codes for either firearm or MVI from January 1, 2013 to December 31, 2017. Information for patients from the 34 hospital systems which reported the relevant information to PHIS during our study period was analyzed using SAS with descriptive statistics calculated for all variables of interest.

There were 89,145 pediatric ED visits attributed to MVI and 3,247 for firearm injuries within the study time period. Of the patients who presented to the ED for firearm injuries, 48% were admitted to inpatient care versus 14% of patients presenting with MVI (p <0.001). While the majority of patients were discharged home in both categories, 5.1% of patients with a firearm injury expired during the hospital stay compared to 0.3% of patients in motor vehicle accidents. Patients with firearm injuries were more likely to be admitted to an intensive care unit (ICU) and had significantly longer lengths of stay in both the hospital and ICU settings compared to their motor vehicle injury counterparts. Additionally, children presenting for firearm injuries had more imaging per patient, were more likely to both return to the ED and to be readmitted post-discharge at 3 days and 1 year (p <0.001). Finally, the median billed charges were nearly 10-fold higher in the firearm compared to the motor vehicle injuries group.

Markers of resource utilization were on average higher per patient for patients with firearm injuries compared to MVI. Given the high resource burden of pediatric firearm injuries compared to MVI, this study supports comparable national focus and funding on firearm-related injury prevention as given to MVI prevention.
Although many associations between Adverse Childhood Experiences (ACEs) & negative health outcomes in the individual are well-documented, more recent studies have explored whether ACEs have intergenerational consequences that impact the health of children of exposed parents. Current research regarding pediatric treatment adherence has focused on factors relevant to the intergenerational effects of ACEs on child health. While children have lower levels of treatment adherence than adults, studies suggest decreased adherence may be related to parenting stress & other social & emotional conditions linked to parents with a history of ACEs. However, at least one study demonstrated, contrary to its hypothesis, that higher levels of parental distress & child behavioral problems were associated with increased medication adherence.

Participants were recruited using Amazon.com Mechanical Turk, an online crowdsourcing platform. All potential participants were required to have a 99% prior task approval rate & have completed at least 500 prior tasks. 740 participants completed the study between 2/1/19 & 7/9/19, which included a standardized ACE questionnaire. They also shared information about adherence to & monitoring of their child’s medication use. The University of Kentucky Medical IRB reviewed & approved all study procedures.

Of the 740 participants, 483 (65.3%) identified as female & 257 (34.7%) identified as male. Additionally, 617 (83.4%) identified as white, 47 (6.4%) as black, 32 (4.3%) as Asian, & 9 (1.2%) as American Indian, Native Hawaiian, or Alaska Native; 647 (87.5%) reported working or being employed and 511 (69.1%) reported having an Associate’s Degree or higher. 457 (61.8%) of parents reported that their children “always” adhered to medical treatment recommendations & medication administration. Overall, fathers reported higher children’s adherence than mothers (64% vs. 61%). Children’s adherence decreased with the age of the parent & increased with frequency of medications. Our study found no relationship between parental history of ACEs & a child’s medication adherence (p = 0.189; NS). Given the importance of medication adherence, further work is needed to better understand parental & child factors that may contribute.
Expanding the View of Research Impact: Altmetrics of the Georgia CTSA Pediatrics Program

Weber, Amber; Fitzpatrick, Anne; Nehl, Eric; and Llewellyn, Nicole

Corresponding Author: Amber Weber, MPH, Emory University, aaweber@emory.edu
Center: Center for Clinical and Translational Research (CCTR)
Type: Outcomes
Keyword(s): Altmetrics, Publications
Related to Pilot Grant or Trainee Award: No
Poster Available: Yes - P13

The Georgia CTSA Pediatrics program is a longstanding effort to transform scientific discovery into solutions that improve pediatric health. Our program trains researchers, provides pilot funding, and ensures that research projects have the resources needed to move forward.

Previous evaluations of our pediatric research output found a body of work that is highly impactful compared to similar work at other institutions. Over the past eleven years, the program supported 93 Georgia CTSA investigators that authored 250 publications identified as ‘pediatric’ based upon having a pediatric author and clear pediatric content. Top research areas of these articles include immunology, allergies, and hematology.

These 250 articles have accrued over 9,500 academic citations for a mean impact factor of more than 3 times average. However, a new technique for evaluating research impact that is rapidly gaining popularity is to examine “altmetrics” associated with research articles. This study used Altmetrics, drawn from Dimensions, to examine the “big splashes” that garner immediate attention, and the “ripple effects” that have a lasting influence, among articles supported by the Georgia CTSA Pediatrics program.

The 250 pediatric articles have accrued more than 15,000 altmetric citations in media, community and public documents, including: 343 news stories, 72 blog posts, 3,441 tweets, 25 policy documents, and 8 patent applications. Illustrations of big splashes include: a 2019 paper on adolescent fatty liver disease that appeared in the New York Times and 600 tweets to over 2 million followers; and a 2018 paper on T-cell therapy for leukemia that appeared in 8 science blogs, and news outlets around the world (e.g. BBC News, Deutsches Arzteblatt, MedIndia). Examples of ripple effects include: a 2011 article on sugar consumption that is cited in multiple CDC policy reports; and a 2012 article on a new treatment for kidney disease that led to patents on its formulation and manufacture.

The short-term splashes and long-term ripples depicted by altmetrics tell the story of this program’s research impact in ways that go beyond traditional citation measures and inform our understanding of how these research products are being used and translated in the broader community.
PURPOSE/OBJECTIVES: The study objective was to assess the impact of the Strong4Life Motivational Interviewing (MI) Training program, a program developed to improve the weight-management related counseling practices of pediatrician healthcare providers. The training, designed to be brief (2-hours in length), focuses on development of motivational interviewing and patient-centered goal setting skills as well as the promotion of key healthy diet and activity-promoting messages. Between 2011 and 2017, over 1500 clinicians throughout the state of Georgia participated in this training.

DESIGN/METHODS: In 2017, parents of children 6-11 years (n=64) and adolescents 12-17 years (n=57) attending well-child visits with pediatricians in the Atlanta metropolitan-area completed an interviewer assisted questionnaire (in English or Spanish, as preferred) to assess their recollection and their perceptions of the weight-related counseling provided during the visit. This included 62 patients (35 parents; 27 adolescents) of 15 pediatricians who had participated in the Strong4Life training and 59 patients (29 parents; 30 adolescents) of 15 pediatrician who had not participated in the training. Generalized estimating equations were used to account for the lack of independence between providers in the same practice when comparing responses between trained and untrained provider practices

RESULTS: Parents/patients of trained vs. untrained pediatricians were, respectively, more likely to report having been asked about the child’s fruit/vegetable intake (92% vs 75%, p=0.04) and their sugar-sweetened beverage intake (81% vs. 49%, p=0.005). Patients of trained pediatrician were also more likely to recall having been asked during their visit about healthy changes that they wanted to make (76% vs 49%, p=0.005) and to report having worked with their provider to set a healthy behavior goal (58% vs. 40%, p=0.05). There were no difference in the responses of parents/patients of trained vs. untrained pediatricians regarding the proportion who recalled having been counseled regarding the child’s physical activity level (93% vs 86%, p=0.31), screen time (73% vs. 58%. p=0.34), barriers to behavior change (25% vs. 17%, p=0.41) or growth (87% vs. 86%, p=0.10),

DISCUSSION AND CONCLUSION: Participation in the Strong4Life Provider Training program has a positive impact on the weight related counseling practices of pediatricians.
BACKGROUND: Infants with necrotizing enterocolitis (NEC) may require intestinal resection with enterostomy and mucous fistula (MF). They often require prolonged total parental nutrition (TPN) and are at risk for poor growth, cholestasis, and infection. A proposed nutritional strategy of mucous fistula refeeding (MFR) is accomplished by collecting enterostomy output and instilling contents into the MF to mimic the normal intestinal transit and allow for adaptation of the distal bowel. We evaluated the hypothesis that patients with surgical NEC who received MFR had improved clinical outcomes (growth, complications, resource utilization) when compared to patients without refeeds.

METHODS: This retrospective cohort study identified subjects from a single quaternary NICU through Children’s Hospitals Neonatal Database (CHND). Study cohort included patients who underwent NEC surgery with resultant enterostomy and MF and survived to discharge. Demographics, clinical outcomes and resource utilization were obtained from CHND and electronic medical records. Statistical analysis was performed by univariate and bivariate analysis using Wilcoxon Rank-Sum/t-tests or Chi-squared. An adjusted multivariable analysis used logistic and linear regression.

RESULTS: 75 patients were identified; 45 underwent MFR and 30 did not. Reasons for no MFR included distal bowel stricture, recession of MF, and difficulty maintaining cannulation. Demographics were similar between groups. The MFR group reached full feeds after intestinal reconnection 14 days earlier; required 23 fewer TPN days; and had less cholestasis defined as lower peak direct bilirubin and less ursodiol usage (all P<0.02). The MFR group also had higher weight gain between initial surgery and reconnection (P=0.01) and 22 fewer central line (CL) days (P=0.007) with similar length of stay (LOS) and CLABSI rates.

CONCLUSIONS: Infants who underwent bowel resection for NEC and received MFR had better intraoperative growth and less cholestasis without an increased infectious risk when compared to patients that did not receive MFR. Although LOS was not different, the decrease in resource utilization for the MFR cohort is reflected by fewer TPN and CL days by approximately 3 weeks.
The Cystic Fibrosis Transmembrane conductance Regulator (CFTR) is a chloride channel in the ABC Transporter family whose deficiency results in Cystic Fibrosis (CF). Currently, the major cause of death for CF patients is lung failure. Thus, it is important to specifically focus on the structure and function of CFTR in this pulmonary epithelial cell environment. Several high-resolution structures of CFTR have been solved recently, but none of the structures show an open pore that would enable chloride conduction. Our lab believes that this is because these structures are of CFTR in a non-physiological detergent environment, and that lipids are vital to the structure and function of CFTR. This hypothesis is partially supported by these detergent structures, as some copurified lipids are resolved in some of these structures. Our goal is to determine the specific lipid environment CFTR encounters and the effects these lipids have on CFTR activity. We hypothesize that determining these lipid interactions will help the field solve an open-pore structure of CFTR and perform more accurate functional analyses. We will use detergent-purified CFTR as well as styrene maleic acid lipid particle (SMALP)-purified CFTR to test these hypotheses. Detergent purification of proteins leaves only tightly bound lipids, indicating their importance to the protein’s structure and function. Expanding from these lipids to the broader lipid microenvironment, SMALPs require no detergent, but rather “hole-punch” the cell membrane. We plan to isolate the SMALPs containing CFTR and identify the lipids within those SMALPs as compared to the total lipid composition of the cells. After determining which lipids are relevant to CFTR, we will characterize CFTR’s activity in different lipid environments using ATPase and Planar Lipid Bilayer experiments. In a separate yet related project, we are working to determine the mechanism by which a change in ceramide composition of cultured pulmonary epithelial cells from non-CF and CF subjects initiates lipid-mediated signaling that decreases CFTR function. [CFF MCCART17G0, MCCART18G0; NIH F31-HL143863-01].
Nanoflow Cytometry Identifies an Imbalance of Epithelium- and Neutrophil-Derived Extracellular Vesicles in the Airway Environment of Pediatric Cystic Fibrosis Patients

Dobosh, Brian; Giacalone, Vincent; Brown, Milton; Silva, Lucas; Guglani, Lokesh; and Triouvanziam, Rabindra

Corresponding Author: Brian Dobosh, BS, Emory University, bdobosh@emory.edu
Center: Center for Cystic Fibrosis and Airways Disease Research (CF-AIR)
Type: Technology
Keyword(s): Extracellular vesicles, flow cytometry
Related to Pilot Grant or Trainee Award: No
Poster Available: No

INTRODUCTION: Progressive lung disease is the leading cause of mortality in cystic fibrosis (CF), a chronic condition characterized by recruitment of polymorphonuclear neutrophils (PMNs) into the airways. Newly arrived PMNs are exposed to extracellular vesicles (EVs) from the airway epithelium and PMNs recruited before them. In controlled experiments, these EVs were necessary and sufficient to induce pathological changes including reduced bacterial killing and immunosuppressive activities toward macrophages and T-cells. However, children with CF do not always show a high PMN presence in their airways, which suggests that the balance between PMN recruitment and the activity of other cells is still in flux in early stage disease.

METHODS: We utilized spectral nanoflow cytometry to profile the single EV content of the bronchoalveolar lavage fluid (BALF) from 17 CF children (<6 years of age). For nanoflow cytometry, EVs were stained with Di-8-ANEPPs, and with EpCAM, CD66b and CD115 (to ascertain epithelial, PMN, and macrophage origins, respectively). Violet side scatter and/or fluorescence threshold triggering were used for EV detection.

RESULTS: The ratio of neutrophil- to epithelial-derived EVs in CF BALF correlated positively with the percentage of PMNs that are present in the airways (p = 0.003, Spearman’s rho = 0.689). This ratio also correlated with the PRAGMA disease score, which quantifies airway damage by chest computed tomography (p = 0.001, rho = 0.857).

CONCLUSION: Using a method to quantify EVs from specific cell types in vivo, we demonstrated that the ratio of PMN- and epithelial cell-derived EVs tracks with airway damage and neutrophil influx, suggesting a critical interplay between these cells in early CF disease. This EV-focused method can be applied to other diseases in which sampling cells is difficult. Future experiments will use CF BALF biobanks to strengthen data presented here.

FUNDING: CF Foundation (TIROUV15A0), Emory Pediatrics Flow Core.
Obese Asthma in Children: A Unique Phenotype?

Mohammad, Ahamd F.; Stephenson, Susan T.; Grunwell, Jocelyn R.; and Fitzpatrick, Anne M.

Corresponding Author: Anne Fitzpatrick, PhD, Emory University, anne.fitzpatrick@emory.edu
Center: Center for Cystic Fibrosis and Airways Disease Research (CF-AIR)
Type: Clinical or Translational
Keyword(s): asthma, obesity
Related to Pilot Grant or Trainee Award: No
Poster Available: No

BACKGROUND: Obese children with asthma can be difficult to treat. In adult populations, unique immunophenotypes of obese asthma have been described with a greater degree of Th17-mediated inflammation. It is unclear whether a similar phenotype is also present in obese children with asthma.

METHODS: Children 5-18 years with physician-diagnosed asthma and historical evidence of airway lability completed an outpatient research visit consisting of clinical characterization procedures and blood sampling for laboratory studies. Obesity was defined as a body mass index (BMI) at or above the 95th percentile.

RESULTS: 376 children with asthma were enrolled (mean age 11 years, 60% male, 70% underrepresented minorities). Compared to lean children, overweight and obese children received more oral corticosteroid bursts and had more Emergency Department visits for asthma exacerbations in the previous year (p<0.05). Obese children, compared to overweight and lean children, also reported a higher frequency of asthma symptoms (p=0.016) and poorer quality of life (p=0.029). Lung function and features of Type-2 inflammation such as eosinophil counts, IgE and the magnitude of aeroallergen sensitization did not differ between groups and were elevated in nearly all children. However, obese children with asthma with had significantly lower Th1 and Th17 plasma cytokines (p<0.05) and as well as lower mRNA gene expression of IFNy, IL17C and IL33 in peripheral blood mononuclear cells (p<=0.05)

CONCLUSIONS: Obesity in children with asthma is associated with a greater symptom burden. In contrast to adult populations, obese children with asthma have a Type-2 predominant pattern of inflammation, with significantly lower Th1 and Th17 features. Lower Th1 responses may predispose these children to more frequent and severe exacerbations. Alternatively, symptoms in obese children with asthma may be driven by other non-inflammatory physiological factors such as chest wall compression and deconditioning independent of airway tone.
Induced Sputum As an Alternative to Bronchoalveolar Lavage for Evaluation of Airway Inflammation in Young Children With Cystic Fibrosis

Giacalone, Vincent; Margaroli, Camilla; Moncada, Diego; Brown, Milton; Silva, George L.; Peng, Limin; Chandler, Joshua D.; Tirouvanziam, Rabindra; and Guglani, Lokesh

**Corresponding Author:** Vincent Giacalone, Emory University, vincent.giacalone@emory.edu

**Center:** Center for Cystic Fibrosis and Airways Disease Research (CF-AIR)

**Type:** Clinical or Translational

**Keyword(s):** Cystic Fibrosis

**Related to Pilot Grant or Trainee Award:** No

**Poster Available:** No

**RATIONALE:** Newborn screening for cystic fibrosis (CF) has led to improvements in early disease management. While young children with CF generally present with minimal clinical symptoms, monitoring of inflammatory markers in BAL reveals early onset of pathology, but is an invasive procedure.

**OBJECTIVES:** We sought to compare markers of airway inflammation measured from induced sputum (IS), a minimally invasive airway sampling method, vs. matched BAL.

**METHODS:** BAL and IS samples were collected on the same day from 2 year old CF patients. Samples were evaluated for cellular and molecular markers of airway inflammation by cytometry and 20-plex ELISA. Matched blood was used for comparison, reflecting the systemic compartment.

**RESULTS:** We successfully collected 15 IS samples from patients aged 1-6 yrs, including 7 matched with BAL at the same visit. The proportion of immune cells in IS was broadly similar to BAL. We identified similar changes to cellular markers of airway disease in IS and BAL leukocytes compared to blood leukocytes. For neutrophils these included increased surface CD66b and elastase and decreased CD16, as well as increased surface PD-1 for macrophages. Cytokine quantification revealed that BAL and IS contained similar amounts of important proinflammatory cytokines including IL-8 and M-CSF.

**CONCLUSIONS:** IS can be collected successfully from young children with CF and is amenable to the measurement of select cellular and molecular outcomes of early airway inflammation.

**FUNDING:** Pilot grant from the CF@LANTA RDP, funded under CFF Center Grant MCCART15R0, and the Hertz Family Foundation grant from Children’s Healthcare of Atlanta.
Pediatric Emergency Department (ED)-Based Asthma Education Tools and Parent/Child Asthma Knowledge

Goodman, Kina; Arriaga, Rosa; Fitzpatrick, Anne; Kumari, Polly; Cooper, Nicholas; Jayaprakash, Karthika; Stephens, Cal; Figueroa, Janet; and Morris, Claudia

Corresponding Author: Kina Goodman, MD, Emory University, kinale37@gmail.com
Center: Center for Cystic Fibrosis and Airways Disease Research (CF-AIR)
Type: Clinical or Translational
Keyword(s): asthma, education
Related to Pilot Grant or Trainee Award: No
Poster Available: No

BACKGROUND: Asthma is a leading cause of pediatric ED visits and hospitalizations. Retention of asthma education in the acute setting can be challenging. To encourage more engaged learning, educational video games (VG) have been shown to be useful tools for health education.

OBJECTIVES: Determine feasibility of using an asthma VG vs. a standard asthma educational video (SAV) in a pediatric ED setting in children with acute asthma and compare parent/child asthma knowledge after receiving the asthma education intervention.

METHODS: Prospective randomized open label study of asthma education tools (investigational asthma VG vs SAV), comparing parent and child asthma knowledge defined by a modified Newcastle Asthma Knowledge Questionnaire (max score=14) done post-intervention. Children age 6-18 years and their families seen in the ED with an asthma exacerbation were randomized to one of the asthma education tools. 5-point Likert scale satisfaction survey was also performed on those randomized to the video game.

RESULTS: All 53 patient families approached (n=26 VG, n=27 SAV) consented and completed the trial. Demographics, education and clinical history are summarized in Tables 1 and 2; parent/child asthma knowledge, Newcastle Score & VG satisfaction results are in Table 3. There was no difference in parent and child asthma knowledge scores between the VG vs. SAV; however, 96% of parents and 76% of children would rather use the game than watch an asthma education video. There was moderate agreement between parent/child asthma knowledge (Fig 1). Although 57% of children had required past hospitalization for asthma (32% PICU), only 34% were on an inhaled corticosteroid and 40% of parents failed to recognize an asthma exacerbation requiring an urgent ED visit.

CONCLUSION: Our novel intervention using an interactive VG for ED-based asthma education was feasible & accepted by both children & their families, with high satisfaction. Despite relatively high parent education and scores on the knowledge tool, there were select questions that showed lack of understanding for asthma management, particularly with respect to parent/child belief that asthma medications are addictive and poor recognition of progression of asthma symptoms requiring urgent ED evaluation. Identifying gaps in parent/child asthma knowledge is essential to improving asthma education. Further research is warranted.
Cluster Analysis of Children Admitted to the Intensive Care Unit With Critical and Near-fatal Asthma Exacerbations

Kolli, Sneha; Jones, Kaitlin; Mason, Carrie; Opolka, Cydney; Grunwell, Jocelyn; and Fitzpatrick, Anne

Corresponding Author: Jocelyn Grunwell, MD, PhD, Emory University, jgrunwe@emory.edu
Center: Center for Cystic Fibrosis and Airways Disease Research (CF-AIR)
Type: Clinical or Translational
Keyword(s): asthma, pediatric intensive care unit
Related to Pilot Grant or Trainee Award: No
Poster Available: No

INTRODUCTION: Critical asthma, defined by pediatric intensive care unit admission for a life-threatening exacerbation, is a heterogeneous condition. Given this heterogeneity, it remains difficult to predict which children are at highest risk for these critical asthma events. The objective of this hypothesis-generating study was therefore to identify clusters of children with critical asthma with shared clinical features using standardized, population-norm measures of patient-reported physical, emotional and social health for further analysis.

METHODS: Children 6-17 years admitted for critical asthma completed Patient Reported Outcome Measures (PROMIS) questionnaires and the Asthma Control Test (ACT) during their hospitalization to assess their physical, mental, and social health. Demographic, past medical history, and clinical course variables were obtained from the electronic medical record and summarized using descriptive statistics. PROMIS T-scores (norm 50, SD 10) were analyzed by K means clustering and T-tests were used to compare means of two-groups with JMP SAS.

RESULTS: Thirty-four children (average age 10.5 years, 58.9% male) were enrolled; 26 (76.5%) completed both the ACT and PROMIS questionnaires. Thirty-one (91.2%) children were diagnosed with asthma prior to the admission and 76.5% were treated with multiple asthma medications. Two clusters were identified with 5 children (19.2%) in Cluster 1 and 21 children (80.8%) in Cluster 2. Children in both clusters had Asthma Impact Scores that were about 1 SD higher than the population norm T-score of 50 (SD 10) with Cluster 1 and 2 having mean T-scores of 65.7 (SD 8.3) and 58.3 (10.2), respectively. Children in Cluster 1 had lower (worse) mean T-scores for life satisfaction (p < 0.001), meaning and purpose (p < 0.001), positive affect (p < 0.001), and family and peer relationships (p ≤ 0.001) and a higher T-score (worse) for psychological stress (p < 0.001) than children in Cluster 2. Children in Cluster 1 also had lower (worse) ACT scores than children in Cluster 2 (p < 0.05).

CONCLUSION: In a limited cohort of children with a critical asthma exacerbation, a cluster of children distinguished by worse physical, emotional, and social health was identified. Linkages between psychosocial health and asthma warrant further study.
INTRODUCTION: Pediatric acute respiratory distress syndrome (PARDS) is a heterogeneous condition. Although PARDS is associated with neutrophil recruitment into the lungs, the biological mechanisms that contribute to PARDS are unclear. This study determined whether interferon-stimulated genes (ISGs) induced by Type I interferons (IFNs) were elevated in neutrophils from children with PARDS and whether these Type I interferons contributed to neutrophil activation and function as assessed by neutrophil extracellular trap (NET) release.

METHODS: Tracheal aspirate samples from children who were at risk for PARDS or who developed PARDS were collected within 24 hours of intubation. Differential ISG mRNA gene expression, STAT1 phosphorylation and cell surface markers of degranulation were measured in primary airway neutrophils by PCR and flow cytometry, respectively. NET release from primary airway neutrophils was quantified by myeloperoxidase-DNA complexes using an ELISA.

RESULTS: Fifty children were enrolled, including 8 controls (intubated for non-pulmonary issues), 18 children at risk for developing PARDS, and 24 children with PARDS. Compared to children at risk for developing PARDS, children with PARDS had higher ISG mRNA gene expression, greater STAT1 phosphorylation, increased surface expression of CD63, a marker of primary granule exocytosis, and greater NET release from primary airway neutrophils (p<0.05 for each). However, in a subgroup of children with bacterial infection, NET release was impaired despite greater neutrophil ISG gene expression and greater neutrophil degranulation and activation markers (p<0.05).

CONCLUSIONS: Children with PARDS have increased airway neutrophil ISG gene expression and NET release. However, in a subgroup with bacterial infection, neutrophil NET release is impaired and may predispose these children to poorer clinical outcomes including severe and sustained infection.
Miniaturized, High Throughput Assay of Neutrophil Recruitment in Pediatric Lung Diseases

Viola, Hannah; Selva, Cauviya; Grunwell, Jocelyn; Tirouvanziam, Rabindra; and Takayama, Shuichi

Corresponding Author: Hannah Viola, BS, Georgia Tech, hannah.viola@gatech.edu
Center: Center for Cystic Fibrosis and Airways Disease Research (CF-AIR)
Type: Basic
Keyword(s): neutrophil, pulmonary
Related to Pilot Grant or Trainee Award: No
Poster Available: No

Dysregulated airway neutrophil behavior appears in pediatric lung diseases ranging from acute pulmonary infection to cystic fibrosis. Blood neutrophils that transmigrate into the lumen of diseased airways can acquire a pathological, long-lived pathological phenotype with characteristically decreased bacterial killing and increased degranulation that contributes to pathophysiology. Therefore, in vitro models of neutrophil recruitment to the airways are essential for investigating pathophysiology and testing pharmacotherapeutics in pediatric diseases that involve neutrophil recruitment. In prior work, the pathological fate of human airway neutrophils was recapitulated in a six-well plate format enabling transmigration into media containing pooled patient airway fluid (Forrest et al., J Leukoc Biol, 2019; Grunwell et al., Sci Rep, 2019). This original platform is limited in throughput and requires large airway fluid volumes which necessitates pooling pediatric samples for a single assay because sample volumes are limited for infants and small children. Here, we designed a miniaturized neutrophil transmigration platform for high throughput and low-volume assays. The platform features a 96-well format (vs. 6-well originally), requires 75 μL per well (25x volume reduction), eliminating the need to pool samples, and does not require membrane inversion to transmigrate neutrophils. The epithelial layer of NCI-H441 club cell line displays barrier function reflected by high transepithelial electrical resistance (300-350 ohms*cm²) and expression of the ZO-1 junctional protein identified by immunohistochemical staining. This improved platform also requires a fewer neutrophils per well, so that a smaller blood sample is needed for the assay as well. These improvements will accelerate the progress of studies that utilize neutrophil transmigration assays, with the ultimate goal of developing an efficient, point-of-care patient airway environment phenotyping and drug-testing device to target personalized therapies for children with diverse lung diseases involving neutrophils such as acute lung injury and cystic fibrosis.
SAMHD1 is a dNTP triphosphydrolase that functions to keep cellular dNTP levels low when the cell is not dividing. Germline mutations in SAMHD1 have long been associated with Aicardi Goutières Syndrome (AGS), a rare autoimmune disorder characterized by aberrant interferon production. More recently, SAMHD1 mutations have been found in patients diagnosed with leukemia. While several leukemia related SAMHD1 mutations have been identified, the mechanism of their impact on tumorigenesis and therapy resistance remains unknown. We biochemically characterized leukemia-associated SAMHD1 mutants in order to elucidate the mechanistic link between SAMHD1 and leukemia. To do this, we first expressed and purified the HD domain of four mutant proteins (Y155C, P158S, R366C, and R145Q) using a Ni+ column followed by gel filtration. Next, these highly purified proteins were assessed for hydrolysis activity using a HPLC-based assay. All four mutant proteins showed reduced dNTPase activity compared to wild type protein. We then investigated the structural integrity and stability of the mutant proteins using a thermal shift assay. Consistent with in vivo protein levels, Y155C, P158S, and R145Q showed altered thermal shift curves and reduced melting temperatures while R366C had similar protein integrity to wild-type. Indeed, a cross-linking based tetramerization assay found R366C to be capable of forming tetramers similar to wild-type. In conclusion, R366C has unaltered protein stability and no global structural changes, but no longer maintains dNTPase activity. This supports the idea that SAMHD1 mutations raise intracellular dNTP pools, thus allowing for the rapid cell division characteristic of cancer cells.
Targeting Neuropathogenesis by Selectively Killing CNS Reservoirs

Gavegnano, Christina; Shepard, Caitlin; Kleinbard, Ruby; Holler, Jessica; and Kim, Baek

Corresponding Author: Christina Gavegnano, PhD, Emory University, cgavegn@emory.edu
Center: Center for Drug Discovery (CDD)
Type: Clinical or Translational
Keyword(s): HIV macrophage
Related to Pilot Grant or Trainee Award: 2017, CDD, Screening for Agents that Stimulate HIV-1 Replication and Expression in Macrophages for HIV Cure (PI: Christina Gavegnano, PhD)
Poster Available: No

Within the CNS compartment, HIV persists in myeloid sanctuaries, and HIV associated neurocognitive dysfunction (HAND) occurs in up to half of HIV-infected individuals even with well-controlled viremia. Safe, specific agents that selectively eliminate infected myeloid cells driving neuropathogenesis are urgently needed. We have identified two safe, FDA approved agents, rufinamide and bergenin (non-HIV-1 indication), that selectively kill only HIV-infected macrophages (MΦ) and microglia (MG).

METHODS: Cell isolation: Primary human monocytes were isolated from healthy donors; MΦ differentiated with GM-CSF; MG were differentiated with GM-CSF/IL-34-based cocktail. Resting memory T-cells and synchronized non-cycling T cells were isolated with bead cocktails).

INFECTIONS: MΦ or MG were infected (HIV-189.6 MOI 0.5) for 72 hr in the presence of 0.01-10µM rufinamide or bergenin, HIV-1 alone, or HIV+VPX (+ control). HIV-1 infection and cell death was quantified (FACS; p24+/live/dead cells or p24+/death marker+); autophagy, pyroptosis or apoptosis markers). Cell lifespan marker bcl-2 was measured (FACS). HIV-1 acceleration was quantified with RT-PCR for 2-LTR circles and HIV RNA. SAMHD1/pSAMHD1 was quantified (western blot).

RESULTS: Rufinamide and bergenin do not kill uninfected MΦ or MG, and have no impact on HIV-infected resting memory T cells or non-dividing T cells. Both agents demonstrate selectivity for killing HIV-1-infected MΦ/MG, and significantly (p<0.01 t-test) accelerate HIV-1 replication; agents do not alter SAMHD1/pSAMHD1 levels. Cellular lifespan marker Bcl-2 was significantly reduced (p<0.01). Rufinamide and bergenin induce apoptosis-mediated or autophagy-mediated cell death selectively in p24+ cells, respectively.

CONCLUSIONS: Rufinamide and bergenin selectively kill only HIV-1 infected MΦ and MG, do not impact resting HIV-infected T cells, and are not toxic to uninfected cells. Agents accelerate HIV-1 replication in MΦ/MG, and reduce cellular lifespan marker bcl-2, implying acceleration confers selective death of infected myeloid cells; mechanisms are autophagy or apoptosis-mediated, respectively. Acceleration of replication is SAMHD1-independent. Bergenin and rufinamide demonstrate selectivity towards killing of only HIV-1-infected MΦ and MG, warranting further mechanistic studies and eventual studies in humans, to evaluate the use of these agents towards elimination of neuropathogenesis and infection in the CNS.
Phosphodiesterase 4 Inhibitors Attenuate Scratching Behavior

Hanson, Claire; Lawson, Katy; Han, Liang

Corresponding Author: Claire Hanson, BS, Georgia Tech, claireehanson@gatech.edu
Center: Center for Drug Discovery (CDD)
Type: Basic
Keyword(s): atopic dermatitis, itch
Related to Pilot Grant or Trainee Award: No
Poster Available: No

BACKGROUND: Atopic dermatitis (AD) is a common and devastating chronic skin condition affecting approximately 20% of children and 3% of adults worldwide. Sufferers experience chronically dry and itchy skin, and relief generated from scratching typically worsens symptoms and leads to secondary infection. Limitations in AD treatment result in most of the disease’s burden; the two classes of first line therapies, topical corticosteroids and topical calcineurin inhibitors, both have serious side effects that generate a need for novel treatments.

In 2016, the phosphodiesterase 4 inhibitor crisaborole was approved for clinical use in AD therapy. This medication utilizes a new but well-studied approach to regulating inflammatory cytokine production, but it may also reduce disease burden by directly inhibiting the activity itch-sensing sensory neurons within the skin. Demonstrating the effectiveness of PDE4 inhibitor therapies in acute relief from itch would provide an additional compelling reason to further utilize this medication class in clinics.

METHODS: We injected a PDE4 inhibitor or a saline vehicle subcutaneously into the nape of the neck of 2-3 month old C57BL/6 mice. Ten minutes later, we induced itch with chloroquine at the same spot and recorded scratching bouts for 30 minutes.

We also performed RNA-seq analyses and immunohistochemical staining to confirm PDE4 presence in the small diameter pruriceptive neurons of the dorsal root ganglia (DRG).

RESULTS: Three unique pharmacological PDE4 inhibitors significantly decreased scratching bouts in thirty minutes. We additionally observed PDE4A protein expression in small diameter DRG neurons after immunostaining. Finally, we observed that AD-model mice display enriched PDE4A and PDE4B mRNA expression compared to naive controls.

CONCLUSIONS: Our data so far supports the hypothesis that PDE4 inhibitors have a direct effect on neurons. We will continue our analyses by determining if PDE4 inhibition can noticeably suppresses neuronal activation in vitro with calcium imaging.
Interference With LEDGF/p75-Directed Integration Enhances Transcription of HIV-1 Antisense RNA

Mahboubi, Darius; Puray-Chavez, Maritza; Tedbury, Philip; Vanderford, Thomas; Ukah, Obiaara; Achuthan, Vasudevan; Poeschla, Eric; Kvaratskhelia, Mamuka; Engelman, Alan; and Sarafianos, Stefan

Much of the current efforts to understand HIV-1 replication cycle focuses on uncovering the events that take place during viral DNA integration. This stage in the life cycle is a prospective therapeutic target for the development of new antivirals. The interaction between host factor Lens Epithelium-Derived Growth Factor (LEDGF/p75) and HIV-1 Integrase (IN) directs integration into highly intron dense regions of the genome, and towards the center of genes. Disruption of this interaction leads to more random integration, increase in latent infection and, in our data, accumulation of antisense RNA (asRNA). Viral latency is the biggest barrier to complete clearance of HIV infection; the inability to clear HIV infection means that treatment is lifelong. Latently infected CD4+ T cells contain inactive virus and are not killed by antiretrovirals. The origin and role of antisense HIV-1 transcript in latency and infection remains unclear. Understanding the mechanism by which HIV-1 transcription is regulated will advance our knowledge of latency and the role of asRNA. We recently discovered that Jurkat cells that were either genetically engineered using CRISPR/Cas9 to knock out LEDGF/p75 (-/-) or treated with allosteric HIV-1 integrase inhibitor (ALLINI) BI-D, to display an increase in frequency and abundance of HIV-1 asRNA. This effect was accompanied by a reduction in the number of cells expressing the HIV-1 sense RNA. Moreover, the HIV-1 antisense RNA (asRNA) transcripts appeared to reside predominantly in the nucleus of infected cells. To better understand this phenotype, we have initiated characterization of the transcripts by Illumina sequencing. Preliminary data suggest that transcription of the asRNA can originate outside the provirus or within the vDNA and that splicing of HIV-1 transcripts is perturbed following integration in the absence of LEDGF/p75. We hypothesize that accumulation of HIV-1 asRNA is a result of integration site selection or the lack of integration and is associated with latency. Understanding the relationship between LEDGF/p75, HIV-1 asRNA, and latency may help in the discovery of regimens that follow the “shock and kill” or “block and lock” therapeutic strategies.
Optical Control of Immune Cell Signaling via Polymer-Induced Cytokine Latency

Perdue, Lacey A.; Do, Priscilla; David, Camille; Chyong, Andrew; Kellner, Anna; Ruggieri, Amanda; Kim, Hye Ryong; Salaita, Khalid; Lesinski, Gregory B.; Porter, Christopher C.; and Dreaden, Erik C.

Corresponding Author: Lacey Perdue, Emory University, lperdu2@emory.edu
Center: Center for Drug Discovery (CDD)
Type: Basic
Keyword(s): cytokines; immunotherapy
Related to Pilot Grant or Trainee Award: No
Poster Available: No

Cytokine therapies show great potential to induce cancer immune elimination and have demonstrated therapeutic benefit in a subset of patients, for example when combined with dinutuximab in the treatment of pediatric neuroblastoma. However, controlling the pleotropic effects of these immune signal effectors is a challenge, frequently resulting off-target toxic effects in patients. Here, we describe a synthetic approach to precisely target the activity of cytokines via modification with photo-labile polymers. We show that the magnitude, duration, and location of pro-inflammatory cytokine signals can be precisely triggered via LED light exposure of recombinant proteins modified with photo-labile polymers, thus modulating immune cell function ranging from receptor binding affinity, to intracellular T cell signaling, TCR activation, immune cell proliferation, and in vivo cytokine circulation. We demonstrate the generalizability of this strategy using multiple pro-inflammatory cytokines – including IL-2, IL-12, and IL-15 – as well as the potential for transcutaneous photo-activation using this approach. Using these tools, we aim to provide new insights into the mechanics of cancer immune elimination and also to improve the precision of cytokine-enhanced cancer immunotherapies.
Engagement between the natural killer group 2, member D (NKG2D) receptor and its ligands is critical for immune cell targeting of cancer cells. Previous studies have shown bortezomib, a proteasome inhibitor, increases surface expression of NKG2D ligands on cancer cells and enhances their sensitivity to innate immune cell-mediated cytotoxicity. Here, we investigate the combination of bortezomib and γδ T cells as a novel therapy for acute myeloid leukemia (AML), a hematologic malignancy in which CAR-based cellular immunotherapy has been challenging. We treated two AML cell lines, Nomo-1 and Kasumi-1, with increasing concentrations of bortezomib over 48 hours and measured changes in NKG2D ligand surface expression. Our results showed 24 hour treatment with 5 nM bortezomib significantly increased ULBP2/5/6 expression in Kasumi-1 cells, while 10 nM bortezomib significantly increased expression of ULBP2/5/6 in Nomo-1 cells (n = 3, p <0.05). Day 12 ex vivo expanded γδ T cells were incubated with target cells at the following effector to target (E:T) cell ratios: 0:1, 1:4, 1:2, 1:1, and 2.5:1. Target cells were treated with vehicle control or bortezomib 24 hours prior to the cytotoxicity assay, and cell death was determined via 7-AAD and Annexin V staining. Total target cell death of Kasumi-1 cells was significantly increased with bortezomib treatment compared to vehicle control from 16.8% to 52.1% at a 1:4 E:T ratio, 30.0 % to 64.0% at 1:2, and 48.0% to 77.8% at the 1:1 (n = 3, p <0.05). Nomo-1 cell death was significantly increased with bortezomib and γδ T cell combination treatment, compared to vehicle control, from 8.8% to 33.7%, 13.9% to 43.6%, 23.1% to 56.5%, and 45.8% to 71.3% at the 1:4, 1:2, 1:1, and 2.5:1 E:T ratios, respectively (n = 3, p<0.05). We further validated this combination approach in two T-ALL cell lines, Jurkat and MOLT4, which also showed significantly increased target cell death with the bortezomib and γδ T cell combination (n = 3, p <0.05). These results provides proof-of-concept for developing a platform for effective combination therapy of ex vivo expanded γδ T cells with stress ligand inducing drugs for high-risk leukemias.
Our goal is to develop a method to generate adrenal gland tissues from human pluripotent stem cells (hPSCs). Adrenal glands are located on top of the kidneys and are important for hormone production affecting metabolism, stress, immune response, and blood pressure. hPSCs are powerful because of their ability to self-renew, differentiate into any cell type of the body, and their potential for disease modeling, cell therapy, and drug discovery. They can also be used to create organoids which are 3D aggregates of progenitor cells that have the potential to interact and autonomously develop structure, cell types, and organ connections. Here, we aim to generate tissues of the adrenal gland in the form of a complex organoid. There are two major parts of the adrenal gland: the medulla and cortex. During development the medulla, comprised of chromaffin cells, develops from neural crest (NC), an ectodermal lineage. NC cells further specify into sympathoadrenal progenitor (SAP) cells, which give rise to sympathetic neurons and chromaffin cells. In our approach, we commence hPSC differentiation in a monolayer by TGFβ inhibition and activation of WNT and BMP4, resulting in SOX10-expressing NC cells. Cells are then pushed towards SAP differentiation using WNT, BMP4, SDF1, retinoic acid, and neuregulin. We compared these cells to cranial NC, which should not create SAP cells, as a control. The second region of the adrenal gland, the cortex, arises from intermediate mesoderm (IM), which is a known precursor to adrenal, gonadal, and kidney tissues. In our IM approach, we initiate hPSC differentiation in a monolayer with activation of WNT, fibroblast growth factor, and retinoic acid pathways inducing cells that express PAX2, LHX1, WT1 and GATA3. We generate SAP and IM progenitors individually, followed by their aggregation into organoids and allowing their self-organization over time. Then, we carefully study the localization, structure, and identity of each cell type within the organoid. Our adrenal gland organoids will be amenable to be made from patients hPSCs to study multiple diseases including neuroblastoma and congenital adrenal hyperplasia. In conclusion, we developed a preliminary protocol to study cells of the adrenal gland in vitro from hPSCs.
Clinical Utility of Targeted RNA-Sequencing for Primary Immune Deficiencies

Berger, Kiera; Arafat, Dalia; Chandrakasan, Shanmuganathan; and Gibson, Greg

Corresponding Author: Kiera Berger, Georgia Tech, kberger9@gatech.edu

Center: Center for Transplantation and Immune-mediated Disorders (CTID)

Type: Clinical or Translational

Keyword(s): RNAseq; Primary Immune Deficiencies

Related to Pilot Grant or Trainee Award: No

Poster Available: No

More than 200 primary immune deficiencies (PIDs) affect around 1:2000 people, many of whom exhibit chronic health issues early in life. Rare genetic disorders often take many years to diagnose and can be debilitating and costly for patients. Early, accurate, and timely diagnosis is critical for improving patient outcomes, but overlapping symptoms present challenges. Studies using DNA sequencing panels, WES, and WGS report less than 25% diagnostic rate for patients suspected of having a PID. We have previously used targeted RNAseq on a cohort of 50 patients suspected of having a dysferlinopathy and identified causative variants in over 80%. We believe that RNAseq could show similar results for other groups of rare genetic diseases with low diagnostic yield through DNA sequencing methods, like PIDs.

To apply this method to PIDs, we developed a panel of 260 genes associated with problems in immunity. Our 7 pilot cohort samples consisted of a mix of PID patients with unknown and previously known genetic variants, with all sample information withheld prior to and during RNA analysis. Preliminary analysis of the pilot cohort quickly identified causative variants in two patients. The first was a nonsense variant in CTLA4 with an observation of nonsense mediated decay acting on the transcript. Haploinsufficiency of CTLA4 causes autoimmune lymphoproliferative syndrome V (ALPS5) and a targeted therapy was given to the patient based on the genetic results that achieved good medical control. In the second patient, a LOF hemizygous skip of two exons in XIAP was identified during splicing analysis in RNA. Based on genetics, the patient’s therapy was altered for a better clinical outcome. Rare variants and/or aberrant splicing have also been found through RNAseq analysis in the remaining 5 pilot cohort patients, though none were previously classified as pathogenic or likely pathogenic. To aid in a more comprehensive analysis of the data, our next step is to integrate clinical information with the RNAseq results. The observation of possible splice defects in so many patients indicates that RNAseq for PIDs could help raise the diagnostic rate of these disorders and lead to earlier interventions and improved outcomes.
Potency Analysis of Mesenchymal Stem Cells in Patients With Crohn’s Disease

Chinnadurai, Raghavan; Gibson, Greg; and Kugathasan, Subra

Corresponding Author: Raghavan Chinnadurai, PhD, Mercer University School of Medicine-Savannah, chinnadurai_r@mercer.edu
Center: Center for Transplantation and Immune-mediated Disorders (CTID)
Type: Clinical or Translational
Keyword(s): Mesenchymal Stem cells; Crohn’s disease
Related to Pilot Grant or Trainee Award: No
Poster Available: No

Autologous and allogeneic bone marrow-derived mesenchymal stromal cells (MSCs) for adoptive cell therapy of luminal Crohn's disease (CD) are being tested in clinical trials. In Europe, adipose derived MSCs are approved to treat perianal fistulas in adult patients with CD. It is likely in the future, MSC based cell therapy will be the standard care of therapy for CD in United States. Strategies are needed to define and characterize MSCs that inform their functionality and fitness to use as cell therapy candidate. Here we have proposed a combination method to define MSCs derived from colonic and small bowel biopsies of patients with CD. (1) Selective RNA genomics: FluidigimTM nano scale chip PCR approach interrogate selective transcriptome of MSCs upon sensing inflammatory cues. MSC’s ability to respond to inflammatory cues are considered as the surrogate measure of their *in vivo* functionality. (2) Selective Secretomics: Luminex XmapTM technology approach to investigate the selective secretome of MSCs. MSCs intrinsic secretome fitness and their ability to modulate the secretome of immune responders are considered as the measure of their functionality. (3) Selective Phosphomics: Flow cytometry based PhosflowTM technology approach defining the phosphorylation status of MSCs. MSC’s ability to activate the phosphorylation of signaling proteins upon interaction with the immune responders predict their immunomodulatory properties and thus can serve as the surrogate measure of their *in vivo* fitness. All of these combinatory assays utilize patient/donor specific MSC populations as an internal ruler and thus external ruler references are not needed to measure MSC fitness. Altogether, this approach not only inform MSCs’ immunobiology in patients with CD, but also the best to inform cellular therapeutics to treat CD. Detailed data on CD patient driven (from intestinal) MSCs will be presented during the meeting.
Healthcare Access and End-Stage Renal Disease Detection in Children

Gandrakota, Nikhila; Wang, Chia-shi; Kamel, Margret; and Greenbaum, Laurence A.

Corresponding Author: Nikhila Gandrakota, MD, MPH, Emory University, nikhila.gandrakota@emory.edu
Center: Center for Transplantation and Immune-mediated Disorders (CTID)
Type: Clinical or Translational
Keyword(s): healthcare access, endstage renal disease
Related to Pilot Grant or Trainee Award: No
Poster Available: Yes - P15

BACKGROUND: Chronic kidney disease (CKD) refers to a state of irreversible loss of kidney function. There is little published information on factors that influence the detection and management of CKD in children prior to the development of end stage renal disease, the final stage of CKD.

METHODS: We are performing a retrospective review of all children who underwent transplantation at Children’s Healthcare of Atlanta between 1/1/2010 and 12/31/2018. We will collect patient demographics, insurance status, ESRD cause, and whether CKD was known prior to ESRD diagnosis. We will examine the proportion of patients with access to medical care, including general and nephrology care prior to ESRD diagnosis and the proportion of patients with unknown CKD prior to ESRD diagnosis. We will assess whether access to care is associated with unknown CKD prior to ESRD diagnosis via logistic regression, controlling for clinical factors.

PRELIMINARY RESULTS: 239 pediatric patients were transplanted between 1/1/2010 and 12/31/2019 at the Children’s Healthcare of Atlanta. Currently, we have completed a chart review on 20 of the patients. We found that 8 / 20 patients were not under the care of a nephrologist prior to ESRD diagnosis and 2 / 20 did not have an identified primary care physician. We will assess the associations between patient characteristics and access to medical care with known CKD status prior to ESRD diagnosis for all transplanted patients. We anticipate that all 239 patient charts will be reviewed, and analysis completed by May 2020.

DISCUSSION: CKD requires careful management and treatment to prevent and prepare for ESRD. Determination of the factors impacting CKD/ESRD diagnosis is important in caring for children with kidney disease.
Urinary Biomarkers - CXCL9 and CXCL10 for Diagnosing Rejection in Pediatric Transplantation: UTOPIA (Urinary Biomarkers to Produce Improved Outcomes After Renal Transplantation) Project

George, Roshan; Hanberry, Bradley; Dave, Ishaan; Kang, Christy; Winterberg, Pam; McCracken, Courtney; Garro, Rouba; and Warshaw, Barry

INTRODUCTION: Renal transplantation is the treatment of choice for End Stage Kidney 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 and also human leukocyte antigen testing, donor specific antibody formation and epitope matching, to stratify patients into immune-quiescent or immune-active groups to predict outcomes and either lower medication burden or escalate monitoring/therapy.
CD3 Downregulation on T Cells is Concomitant With Arginase Upregulation on Myeloid Cells in the Synovial Fluid of Patients With Juvenile Idiopathic Arthritis

Giacalone, Vincent; Cammarata-Mouchtouris, Alexandre; Moncada, Diego; Shenoy, Sreekala; Ponder, Lori; Gergely, Talia; Vega-Fernandez, Patricia; Manos, Cynthia; Flanagan, Elaine; Prahalad, Sampath; and Tirouvanziam, Rabindra

Corresponding Author: Vincent Giacalone, Emory University, vincent.giacalone@emory.edu
Center: Center for Transplantation and Immune-mediated Disorders (CTID)
Type: Clinical or Translational
Keyword(s): Juvenile Arthritis
Related to Pilot Grant or Trainee Award: 2019, CCTR, Emory Multi-Omics Juvenile Arthritis Immunology (EMOJI) Study: Bringing Deep Phenotyping to a Vexing Pediatric Problem (MPI: Sampath Prahalad, MD, MSc and Rabin Tirouvanziam, PhD)
Poster Available: No

RATIONALE: Juvenile idiopathic arthritis (JIA) is an inflammatory autoimmune disorder driven by dysfunction of the joint tissue and abnormal immune responses. As deep phenotyping of synovial fluid (SF) might yield new targetable mechanisms of inflammation in JIA patients, we initiated the Emory Multi Omics JIA Immunology (EMOJI) project.

METHODS: Patients with JIA between the ages of 6 and 18 were consented and enrolled for collection of SF from the knee and venous blood. Blood and SF leukocytes were analyzed by flow cytometry. Platelet-free plasma and debris-free SF supernatant were obtained by dual centrifugation and a 20-plex chemiluminescent assay (Meso Scale Discovery) was used to quantify cytokines.

RESULTS: We report data on blood and SF collected at the same visit from 7 JIA patients. JIA subtypes represented were psoriatic (N=1), oligoarticular extended (N=1), oligoarticular persistent (N=2) and polyarticular (N=3) arthritis; all rheumatoid factor-negative. CD3 expression was decreased on SF compared to blood T-cells. Concomitantly, expression of the CD3-inhibitory enzyme arginase was increased on SF compared to blood neutrophils and macrophages. However, arginase-rich primary granules were not significantly exocytosed by SF neutrophils. Finally, we measured high levels of neutrophil chemoattractants (IL-8, G-CSF, and IL-1β) in SF compared to plasma.

CONCLUSIONS: Neutrophils are recruited to the joints of JIA patients and accumulate arginase on their surface, presumably released from tertiary, but not primary, granules. Arginase activity in the SF may lead to CD3 downregulation and T-cell inhibition, potentially in an effort to limit autoimmune complications.
Naturally History of Pediatric Crohn’s Disease

Hercules, David; Prince, Jarod; Venkateswaran, Suresh; and Kugathasan, Subra

Corresponding Author: David Hercules, BS, Emory University, dhercul@emory.edu
Center: Center for Transplantation and Immune-mediated Disorders (CTID)
Type: Outcomes
Keyword(s): Crohn's Survival
Related to Pilot Grant or Trainee Award: No
Poster Available: No

BACKGROUND: Crohn’s Disease (CD) is an incurable chronic inflammatory disease that affects patients variably, ranging from no disease activity to severe disease activity in different parts of the gastrointestinal tract. The natural history of CD in pediatric populations has been sporadically studied in the Western world, but has not been well-studied in the more heterogeneous American pediatric population. In this study, we aimed to describe the natural history of Crohn’s Disease in a North American pediatric cohort by estimating the probability of remaining free of: (1) CD-related surgery, and (2) stricturing and penetrating complications in ileal, colonic, and ileo-colonic locations.

METHODS: Data for 1075 patients was obtained from the pediatric RISK stratification study, a North American-based study of children diagnosed with CD, enrolled between November 2008 and June 2012. Survival curves were created from date of diagnosis to time to event, or end of follow-up, whichever came first. Probability of survival from complicated disease behavior, and surgery were estimated using the Kaplan-Meier analysis.

RESULTS: Median follow-up time was 59 months (range 5 to 89). Complicated disease behavior was observed in 5% of patients at baseline and 17% of patients during follow up. The survival probability for remaining free of stricturing behavior was 91% at 3 years and 88% at 5yrs. The most frequent disease location was ileo-colonic at both diagnosis (51%) and maximal follow up (63%). Survival curves showed ileal and ileo-colonic complications were more frequent at baseline, whereas colonic disease can remain uncomplicated or inflammatory for many years. The survival probability for remaining free of surgeries were 91% at 3 years and 86% at 5 years.

CONCLUSION: Pediatric Crohn’s disease in the RISK cohort was characterized by frequent ileo-colonic location, more frequent stricturing disease behavior, as well as surgical intervention in about 12% of patients. In future studies, we plan to stratify our survival curves by early or late biologic therapy received.
Post-Transplant Diabetes Mellitus in a Single Pediatric Kidney Transplant Center

Khanna, Anjali; Ham, JeeYoung N.; McCracken, Courtney; Liverman, Rochelle; and Garro, Rouba

Corresponding Author: Anjali Khanna, MD, Emory University, anjali.khanna@emory.edu
Center: Center for Transplantation and Immune-mediated Disorders (CTID)
Type: Clinical or Translational
Keyword(s): post transplant diabetes, pediatrics, kidney transplant
Related to Pilot Grant or Trainee Award: No
Poster Available: No

BACKGROUND: The prevalence of post-transplant diabetes mellitus (PTDM) in pediatric kidney transplant recipients varies among studies due to the lack of a consistent definition. In addition, the risk factors and pathogenesis associated with its onset are poorly understood.

OBJECTIVE: Evaluate the prevalence of and risk factors for PTDM at a high volume, tertiary care pediatric hospital, and characterize the disease course in PTDM patients.

METHODS: We performed a retrospective review of pediatric kidney transplant recipients transplanted between 2006 and 2016 at our center. PTDM was defined as persistent hyperglycemia with serum glucose >200 mg/dl, HbA1C >6.5%, and requiring antihyperglycemic medication for ≥ 30 days. We compared demographic and transplant characteristics between those with PTDM (n=15) and a convenience sample of non-diabetic controls (n=57) using Chi-square tests and Wilcoxon rank sum tests. PTDM patients were further evaluated for various risk factors and outcomes.

RESULTS: Over the 10 years period, 312 patients were transplanted at our center. Five patients had pre-existing diabetes and were excluded. Fifteen developed PTDM with a prevalence estimate of 4.89% (95% CI: 2.98% - 7.90%) and median time from transplant to development of DM of 17.6 months (25th-75th: 2.8 – 82.8). The majority of patients who developed PTDM had a family history of diabetes in first degree relatives (81%), maintained on Tacrolimus at the time of diagnosis (67%) and had significant change in BMI from time of transplant to DM diagnosis (P=0.001). PTDM diagnosis and Insulin initiation occurred in the context of active rejection episode in only two patients. Despite a more stringent definition of PTDM, 3/15 (20%) patients came off Insulin therapy and were normoglycemic at 1, 5, and 8 years post DM diagnosis. Comparisons of patients with and without diabetes revealed PTDM patients were more likely to be Asian (p=0.025), and had higher BMI-Z scores pre-transplant (p=0.015).

CONCLUSION: A more consistent definition of PTDM and larger studies are warranted to better understand the prevalence, risk factors, and pathogenesis of hyperglycemia and diabetes post-transplant. Further evaluation of risk factors and effect on outcomes in pediatric patients using this more rigorous definition of PTDM is underway.
Loss to Follow-Up in Adolescent and Young Adult Renal Transplant Recipients

Melanson, Taylor; Mersha, Karie; Patzer, Rachel; and George, Roshan

INTRODUCTION: Patients’ lost to follow-up (LFU) has a significant impact on outcomes and is a barrier to improving care, especially in adolescent and young adult (AYA) renal transplant recipients. There is limited information regarding the relationship between transfer of care from pediatric to adult settings, age and LFU among AYA renal transplant recipients.

METHODS: 16,386 individuals who received kidney transplant between January 1, 2005 and December 31, 2015 from the Scientific Registry of Transplant Recipients were studied. The primary outcome variable was LFU, which was defined as greater than 1 year without return to care. Death or graft failure within a year of the last follow-up was not classified as LFU, whereas death or graft failure that occurs greater than 1 year after the last follow-up was classified as LFU. We performed a retrospective cohort study describing LFU using Pearson’s chi-square tests. Multivariable logistic regression was used to estimate the change in likelihood of LFU associated with recipient characteristics and institution transfer.

RESULTS: 3,647 individuals (22.26% of our study population) met criteria for LFU. 1,830 patients (11.17% of our study population) transferred institutions (largely from pediatric to adult settings) during the study period. LFU occurred in 50.18% of recipients who transferred institutions. The odds of LFU among renal transplant recipients who transferred institutions was 3.36 times greater (95% confidence interval: 3.1, 3.6) than the odds of LFU among those who did not transfer institutions. Age at transplant was not associated with odds of LFU. Current age was associated with LFU (p<0.01) and LFU peaked among recipients aged 17-22.

CONCLUSIONS: LFU is a critical problem facing AYA renal transplant recipients, and institution transfer is a significant risk factor for LFU. Current age is also a significant risk factor for LFU and AYA patients have the greatest odds of being LFU (compared to other age groups). Additional studies investigating the interplay between age, institution transfer, and LFU in the AYA population are still needed.
Colectomy Risk Stratification in Pediatric Ulcerative Colitis With Measured and Predicted Gene Expression

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

Corresponding Author: Angela Mo, Georgia Tech, amo3@gatech.edu
Center: Center for Transplantation and Immune-mediated Disorders (CTID)
Type: Basic
Keyword(s): IBD Genomics
Related to Pilot Grant or Trainee Award: No
Poster Available: No

An important goal of clinical genomics is stratifying risk of disease progression. Although 10% to 20% of ulcerative colitis (UC) patients eventually require colectomy, polygenic risk scores (PRS) are unable to provide meaningful prediction of this adverse status. Here we demonstrate that both measured and predicted gene expression identify UC patients at 5-fold elevated risk of colectomy with data from both a pediatric UC clinical trial, and UK Biobank population cohort studies. Patients in the top percentile of predicted risk are more likely than not to require colectomy.

To model progression to colectomy observed in 6% (25 of 400) of patients in the PROTECT trial, we performed differential expression analysis between colectomy and non-colectomy cases. The first principal component of the top 150 genes significantly distinguishes colectomy. Gene set enrichment analysis implicates multiple pathways including TNF and interferon signaling, and inflammation and immune response. We then generated a transcriptional risk score (TRS), analogous to the one we recently developed for prediction of risk of progression to complicated Crohn’s disease, and a 20 gene signature also discriminates colectomy from non-progressors at p<10-22.

This score requires gene expression profiling from biopsies, so we next asked whether it is possible to predict progression by first predicting gene expression using genotypes alone. We performed a transcriptome-wide association study capturing the effects of all polymorphisms within 1Mb of each transcript expressed in the PROTECT rectal biopsies, then used the weights to predict gene expression in the White British subset of the UK Biobank. A predicted polygenic transcriptional risk score (PPTRSUC) was then derived, which discriminates UC cases from UKBiobank non-IBD controls (p<10-210), as well as UC cases from colectomy in both UKBiobank and PROTECT (p<0.009 and p<0.006 respectively). Neither result is achieved using genome-wise association study data. Our results thus show for the first time the potential of transcriptional profiling to enable prediction of a severe adverse pediatric outcome, namely colectomy in ulcerative colitis.
Pediatric Crohn’s Disease Patient Derived Organoids Show Epithelial Cell Defects

Niklinska-Schirtz, Joanna; Matthews, Jason; Dodd, Anne; Prince, Jarod; Cutler, David; Venkateswaran, Suresh; and Kugathasan, Subra

BACKGROUND: Suppression of inflammation by anti-TNF is currently the primary treatment for Crohn’s disease (CD). However, some patients do not respond and thus a need exists for therapies that target different pathways/cell types. We hypothesize that the intestinal epithelium is contributing fundamentally to CD. We used patient derived organoids (PDOs), strict epithelial cell cultures, to test this hypothesis in the absence of immune cells.

METHODS: Biopsies from the ileum were obtained from pediatric subjects (CD = 16, healthy controls = 13) by colonoscopy, and processed to isolate intestinal crypts (ICs) and grow organoids. PDOs were classified based on donor phenotype; inflammatory (B1; n=8), stricturing (B2; n=8), grossly inflamed (n=7), or non-inflamed (n=9). The ICs and PDOs were subjected to bulk RNA-seq on an Illumina HiSeq. Read counts were alignment with Hg38 using STAR. DEseq2 was used for the differential expression analysis.

RESULTS: Of 19,900 genes tested, we found 15,554 expressed in either ICs or PDOs, with 14,149 of those expressed in both (1091 were expressed only in IC, and 314 expressed only in PDO), indicating they share approximately 90% similarity at the transcript level (r2 = 90.6; p<2.2e-16). Using a panel of 200 epithelial specific genes involved in the innate immune response, we found 5 genes to be differentially expressed between CD and control groups (p<0.05), while different CD phenotypes (B1 versus B2) showed 470 genes that were nominally different (p<0.05), of which, 156 genes have fold changes >2, but none with FDR <0.05. Analysis of PDO transcriptional signatures between inflamed vs non-inflamed CD groups revealed 6 genes that were significantly different (FDR < 0.05).

CONCLUSION: We demonstrate that PDOs are an excellent patient specific model system for the intestinal epithelium, and maintain a similar transcriptional profile of the donor patient. The inherent transcriptional changes in the CD PDOs suggest that defects in epithelial cell behavior are likely to be playing an important role in the early onset and/or progression of CD, and potentially exacerbate and/or sustain the immune cell response. Thus, therapeutic targeting of the epithelium could be an important advance in the treatment of CD.
Modifier Effects of Non-Coding RNA Levels During the Disease Progression in Crohn’s Disease

Pelia, Ranjit; Venkateswaran, Suresh; Matthews, Jason; Somineni, Hari K.; Cutler, David J.; and Kugathasan, Subra

Corresponding Author: Ranjit Pelia, Emory University, rpelia@emory.edu
Center: Center for Transplantation and Immune-mediated Disorders (CTID)
Type: Clinical or Translational
Keyword(s): ncRNAs, Crohn's disease
Related to Pilot Grant or Trainee Award: No
Poster Available: No

BACKGROUND: Crohn’s disease (CD), a form of inflammatory bowel disease (IBD), primarily manifests in the distal portion of the small-intestine (ileum), and to some extent the rectum. There are three characteristic stages of CD progression defined as B1 (inflammatory), B2 (stricturing) and B3 (penetrating). While many genetic loci have been mapped for IBD, epigenetic changes and factors in IBD are not well defined. We hypothesized that non-coding RNAs (ncRNA) could play a role in CD development/progression and serve as biomarkers during disease. Here in, we sought to compare ncRNA levels in diseased vs healthy tissue from different mucosal sites and disease severities to determine if biochemical profiles of ncRNA can serve as an index to phenotypic outcomes.

METHODS: We obtained 345 ileal mucosal biopsies from CD patients and 71 ileal biopsies from controls, along with rectal biopsies from 329 CD patients and 61 controls (RISK cohort). Whole biopsy total RNA was sequenced in one batch on an Illumina Hiseq X10. Reads were aligned, quantified, and analyzed with hg38 reference panel by STAR package, and DE performed with EdgeR using FDR < 0.05 and Fold change (FC) >2.

RESULTS: Analysis of ncRNA levels between CD and controls revealed significant differences in 89 ileal and 41 rectal ncRNAs, whereas ncRNA levels between stages (B1 vs B2, etc.) revealed no differences in rectum but significant changes between B1 and B2 disease (n=35) in the ileum. Differences were also found in the levels of ncRNA between inflamed and non-inflamed groups, ileum (n=41) and rectum (n=8). Finally, analysis of ncRNA levels across mucosal locations from the distal ileum to the rectum revealed differences in ncRNA levels specific to CD. Regardless of disease behavior, location, or inflammatory status, mir1244-2, mir1244-3, mir1244-4, and RN7SL2 levels were increased.

CONCLUSIONS: ncRNA levels change appears to be location specific during the progression of CD, although it is not clear if these epigenetic changes are corrective cellular measures or are causing further sustained mucosal injury. By mapping these changes in ncRNA levels over the course of disease, we have shown that ncRNA profiling has clinical utility in aiding/predicting disease.
Interferon Regulatory Factor 3 Activation in Juvenile Idiopathic Arthritis

Shenoy, Sreekala; Ponder, Lori; Giacalone, Vincent; Cammarata-Mouchtouris, Alexandre; Moncada, Diego; Manos, Cynthia; Flanagan, Elaine; Tirouvanziam, Rabindra; and Prahalad, Sampath

Corresponding Author: Sreekala Shenoy, MS, Emory University, svenugo@emory.edu
Center: Center for Transplantation and Immune-mediated Disorders (CTID)
Type: Clinical or Translational
Keyword(s): Interferon Regulatory Factor 3 Activation in Juvenile Idiopathic Arthritis
Related to Pilot Grant or Trainee Award: No
Poster Available: No

BACKGROUND: Juvenile Idiopathic Arthritis (JIA) refers to a set of autoimmune arthropathies occurring in children below the age of 16. In some JIA subtypes only the joints are inflamed, whereas in other subtypes inflammation occurs in other organs or even systemically. Inflammation in JIA patients occurs as a result of multiple dysregulated pathways, among which is the IRF3 pathway, previously implicated in autoimmune diseases and viral infections. Cytosolic double stranded DNA (dsDNA) presumably originating from endocytosed cell-free DNA (cfDNA) released upon cell death is a major stimulus of IRF3 pathway. The involvement of IRF3 has not been exhaustively studied in JIA.

AIM: The aim of this study was to characterize JIA subtypes based on phosphorylation of IRF3, an obligatory step in the activation of IRF3 and downstream inflammatory cascade.

METHODS: After informed consent, synovial fluid samples were collected from 10 children (ages 6-18) with oligoarticular or polyarticular JIA who underwent therapeutic arthrocentesis at Children’s Healthcare of Atlanta. Cells were separated from synovial fluid by centrifugation and subjected to H&E staining, immunocytochemistry / immunofluorescence analysis, and Western blotting.

RESULTS: All 10 subjects were negative for rheumatoid factor and cyclic citrullinated peptide, which are biomarkers of seropositive rheumatoid arthritis observed in adults. Total IRF3 was detected in 10 out of 10 samples, a few oligo and poly patient samples showed positivity for phosphorylated IRF3 suggesting its role in this subtype. cfDNA was detected in synovial fluid indicating cell death and release of DNA. The type of cell death is unclear; however, Netosis was seen in one sample.

CONCLUSIONS: Thus, based on these differences in the markers these samples can be categorized into subtypes and will be useful in treatment strategies. In the presence of pathogens or immune stimuli, IRF3 is phosphorylated and translocated to nucleus to function as transcription factor and thereupon activate other genes. The phosphorylation of IRF3 occurs at time points depending on intensity of inflammation, medication and other factors. Further, experiments will be performed with more pediatric synovial samples and controls to study inflammation at time points by induction and expression of certain genes.
African American Females With Pediatric Autoimmune Hepatitis Present With More Severe Disease and Have Worse Clinical Outcomes

Stevens, James; McCracken, Courtney; Palle, Sirish; Naik, Kushal; Kolachala, Vasantha; and Gupta, Nitika

Corresponding Author: James Stevens, MD, Emory University, japsteve@emory.edu
Center: Center for Transplantation and Immune-mediated Disorders (CTID)
Type: Clinical or Translational
Keyword(s): Autoimmune Hepatitis, Pediatric Liver Transplant
Related to Pilot Grant or Trainee Award: 2018, CTID, Correlation of Clinical Outcome and T Cell Repertoire and Homing in Overlapping Pediatric Autoimmune Diseases (PI: Nitika Gupta, MD, DCH, DNB, MRCPCH)
Poster Available: Yes - P16

BACKGROUND AND AIMS: Autoimmune hepatitis (AIH) has a bimodal age distribution, with a large peak occurring during childhood. Like many immune-mediated processes it more commonly presents in females, though is also seen in males. There are few studies analyzing the complex relationships between biological sex, race, and AIH in the pediatric population. The goal of our study was to assess the role a patient’s sex and race may have in pediatric AIH presentation, response to treatment, and outcomes.

METHODS: This was a retrospective cohort study of pediatric patients diagnosed with AIH and treated within a single, tertiary health system with a large pediatric liver transplantation program. Patients who met diagnostic criteria for AIH seen by our health system between January 1, 2000 and April 30, 2016 were included. We compared females versus males, African Americans (AA) versus non-AA, and finally AA females (AAF) versus all other groups in regards to: severity of liver disease at presentation; remission, relapse, and need for liver transplantation; recurrence after transplantation; and death.

RESULTS: Our cohort was comprised of 109 individuals with AIH. Compared to other patients, AAF had higher rates of End-Stage Liver Disease (ESLD, 33% vs. 18%) and Acute Liver Failure (ALF, 8% vs. 1%) at time of diagnosis, versus acute hepatitis without liver failure (59% vs. 81%) (p=0.018). Non-AA females had the second highest rates of ESLD (26%, p=0.013 all females vs. males) and ALF (3%); ALF was only seen in females (5% vs. 0%). AA patients of both sexes had higher Immunoglobulin G levels at diagnosis versus non-AA’s (median 2,855 vs. 1,790 mg/dL, p=0.05).

AAF had higher rates of liver transplantation for AIH than other patients (38% vs. 19%, p=0.037), with lower overall transplant-free survival (59% vs. 79%, p=0.029). Recurrence after transplantation was higher in AA than non-AA patients (47% vs. 8%, p=0.023). Death was only seen in female patients (14% AAF, 7% non-AA females, 0% males).

CONCLUSIONS: Female sex and AA race are separately correlated with more severe presentation and worse outcomes in AIH, with AAF females having the worst prognosis of all groups both at diagnosis and long-term.
Rectal Tissue DNA Methylation in Ulcerative Colitis Shows Disease-Specific Associations: Insights From Longitudinal Analysis of PROTECT Study Participants

Venkateswaran, Suresh; Somineni, Hari; Matthews, Jason D.; PROTECT Investigators; Cutler, David J.; Smith, Alicia K.; Conneely, Karen; and Kugathasan, Subra

Corresponding Author: Suresh Venkateswaran, MD, Emory University, bioinfovs@gmail.com

Center: Center for Transplantation and Immune-mediated Disorders (CTID)
Type: Clinical or Translational
Keyword(s): Ulcerative colitis, DNA methylation
Related to Pilot Grant or Trainee Award: No
Poster Available: No

BACKGROUND: Although inflammatory bowel disease (IBD) is heritable, the heritability is still largely unexplained despite substantial progress through GWAS and WGS. We recently showed that IBD-associated blood DNAm changes strongly correlate with inflammation, and such changes are more representative of the systemic inflammatory status (Somineni et al., Gastroenterology 2019). Here, we set out to examine DNAm patterns in disease-relevant tissue, rectal mucosa from pediatric ulcerative colitis (UC) patients, hypothesizing that DNAm in rectal biopsies will reflect disease status rather than systemic inflammatory status.

METHODS: Genome-wide DNAm was measured using the Illumina MethylationEPIC array on rectal mucosal DNA obtained from 85 non-inflammatory, non-IBD controls and 215 children newly diagnosed with UC prior to therapy, along with 49 one-year follow-up from the same UC cohort to identify disease-specific methylation patterns at diagnosis and following therapy. Epigenome-wide association analyses using linear regression models adjusted for age, gender, five genotype-based principal components and other unknown confounders from CATE package.

RESULTS: At diagnosis, differential methylation at 2446 CpG sites (CpGs) was found to be associated with UC (FDR<0.05). At diagnosis-DNAm signature in rectal tissue was distinct from our previous findings, where only 15 differentially methylated CpGs were common between the rectal mucosa and blood. In contrast to what was observed in blood where the initial DNAm signature reverted back to control levels upon the treatment, rectal tissue DNAm signature did not show signs of reversion during follow-up (Figure 1). In addition, the majority of the disease-associated CpGs identified in rectal mucosal samples showed a strong correlation (p<2.2e-16) with CRP (n=1754; R2=0.82), serum albumin (n=1668; R2=0.79) and hemoglobin (n=1778; R2=0.86) at diagnosis.

CONCLUSION: Our comparative analyses suggest that UC-specific DNA methylation signatures in rectal mucosa are distinct from blood. In contrast to blood DNAm which normalizes after therapy, rectal tissue disease-associated DNAm changes are not reverted back to normal after treatment. This data suggests that the currently available therapies aid in modifying systemic inflammation effectively, but have less direct effect on the disease-specific molecular signatures in the relevant tissue. Future therapies targeting disease-specific DNA methylation may be more effective in disease management and long-term remission.
Assessment of Pre-Transplant Memory T Cell Phenotypes in Children Associated With Costimulation Blockade Resistant Rejection in Adults

Duneton, Charlotte; George, Roshan; Ford, Mandy; and Winterberg, Pamela

Corresponding Author: Pamela Winterberg, MD, Emory University, pdwinte@emory.edu
Center: Center for Transplantation and Immune-mediated Disorders (CTID)
Type: Clinical or Translational
Keyword(s): immunology, memory T cell, kidney transplant
Related to Pilot Grant or Trainee Award: 2017, CCTR & CTID, UTOPIA-Urinary Biomarkers to Produce Improved Outcomes after Renal Transplant (PI: Roshan George, MD)
Poster Available: Yes - P17

BACKGROUND: Belatacept, which targets the CD28-CD80/86 T-cell costimulation pathway, has superior long-term graft outcomes than standard-of-care calcineurin inhibitors used in the majority of children and adults following kidney transplant. However, clinical adoption of Belatacept has been limited due to increased early acute rejection rates. Our group recently reported that pre-transplant frequencies of CD28+ effector memory (TEM, CCR7-CD45RA-) CD4+ T-cells in adults experiencing early-onset rejection on Belatacept were comparable to healthy controls. Conversely, adults that remained rejection-free post-transplant had lower frequencies of these cells, decreased cytokine production, and increased expression of T-cell co-inhibitory pathways. We aimed to determine if similar T-cell populations are detectable in children awaiting kidney transplant.

METHODS: We analyzed existing flow cytometry data studying memory T-cell populations in 30 children on dialysis (pre-transplant) and 18 healthy children, and examined expression of CD28, CD57, and PD-1 on CD4+ memory T-cells. Population frequencies were compared via Mann-Whitney test.

RESULTS: Regardless of kidney disease status, children in our study had a wide range of CD4+ TEM frequencies (median 26.6%, range 14.0-66.7). All children also retained high frequencies of CD28+CD4+ T-cells (median 99.6%, IQR 98.8-99.9), however, children on dialysis had lower frequency of CD28+ cells within the CD4+TEM population (99.4%, 97.7-99.7) compared to healthy children (99.8%, 99.5-99.9; p=0.007). None of the children on dialysis had CD28+CD4+TEM frequencies as low as those observed in adults that were rejection-free on Belatacept. However, 20% of those on dialysis (6/30) had CD28+CD4+TEM frequencies (91.4%, 87.8-93.9) below the minimum value (96%) we observed in healthy children (99.8%, 99.3-99.9, p<0.0001). These patients with the lowest CD28+CD4+TEM frequencies ("stable-like" phenotype), also had higher frequencies of CD57+ (p=0.001) and CD57+PD-1+ (p=0.01) CD4+TEM T-cells compared to the rest of the dialysis patients.

CONCLUSIONS: A subset of children on dialysis awaiting kidney transplant have lower frequency of CD28+ CD4+ effector memory cells compared to healthy children. These same patients also have increased expression of senescence-associated markers on their CD4+ TEM which is reminiscent of the phenotype in adults without rejection on Belatacept. The function of this T-cell population needs further study.
Effect of Chronic Kidney Disease on Alloimmune Response in Mice

Winterberg, Pamela D.; Li, Haiyan; Wagener, Maylene; and Ford, Mandy

Corresponding Author: Pamela Winterberg, MD, Emory University, pdwinte@emory.edu
Center: Center for Transplantation and Immune-mediated Disorders (CTID)
Type: Basic
Keyword(s): transplant, immunology
Related to Pilot Grant or Trainee Award: 2016, CTID, Impact of Chronic Kidney Disease on Alloimmunity (PI: Mandy Ford, PhD)
Poster Available: No

BACKGROUND: We have previously reported that children with chronic kidney disease (CKD) have alterations in memory T cell subsets with increased cytokine potential. The impact of these CKD-induced immune alterations has not been tested in animal models of transplantation. We aimed to determine how CKD alters the alloimmune response in a mouse model of transplantation to identify novel therapeutic strategies to prevent rejection.

METHODS: CKD was induced in male 129X1/SvJ mice through five-sixths nephrectomy (5/6Nx) in a two-stage surgery. Age-matched mice served as controls. Flow cytometry was performed on peripheral blood at 8 weeks following CKD to evaluate expression of coinhibitory pathways PD-1, KLRG1, DNAM and TIGIT, and the integrin, CD11a, on CD44hi memory T cells. Graft survival following minor-antigen mismatch skin transplant (C57BL/6J donor) was compared using Mantel Cox log-rank test between mice with and without CKD and with and without treatment.

RESULTS: Mice with CKD had higher pre-transplant frequencies of CD44hi T cells compared to normal mice (14.7% vs 8.9% of CD4+, p=0.001; 12.1% vs 9.2% of CD8+, p=0.001). Circulating CD44hi CD8+ T cells in CKD mice had higher mean fluorescence intensity (MFI) for CD11a (8532 ± 950 vs 7001 ± 474, p<0.001), DNAM (1278 ± 94 vs 1155 ± 45, p=0.003), and TIGIT (1092 ± 202 vs 745 ± 224, p=0.001) compared to normal mice. CD44hi CD4+ T cells from CKD mice displayed increased expression of TIGIT (MFI 1422 ± 164 vs 558 ± 230, p<0.001) and PD-1 (MFI 2330 ± 202 vs 745 ± 224, p<0.001) compared to controls. Untreated mice with CKD had longer graft survival (median 18 days) compared to mice with normal kidney function (15 days; p=0.01, Figure 2). Monotherapy with agonistic TIGIT antibody prolonged graft survival only for CKD mice (p=0.008), but not normal mice (p=0.40).

CONCLUSION: Mice with CKD accumulate CD44hi T cells with increased expression of the co-inhibitory pathways TIGIT and PD-1 and experienced delayed graft loss compared to normal mice. Targeting the inhibitory TIGIT signaling pathway appears promising as an adjunct therapy to prevent rejection that would not have been anticipated in standard transplant models.
BACKGROUND: Abusive head trauma (AHT) is a serious cause of head injury in young children. Identification is challenging but critical, as children who are misdiagnosed risk returning to a violent environment. Current clinical practices identified several characteristics in AHT, including subdural hematoma (SDH), retinal hemorrhage (RH), and positive skeletal survey (SS). These characteristics are not unique to this population. To date, there are no biomarkers for AHT. Osteopontin (OPN), an inflammatory cytokine indicative of microglia activation, has been shown to predict severity in TBI and may be useful in this population. Aim 1 is to compare the frequency of abuse characteristics in confirmed abusive head trauma (cAHT), suspected abusive head trauma (sAHT), and non-abusive TBI (TBI). Aim 2 is to examine levels of OPN and abuse characteristics in the three TBI groups.

METHODS: Seventy-seven patients between 0-4 years admitted to CHOA between 2014 and 2019 were identified, including 24 cAHT, 12 sAHT, and 41 TBI (e.g., falls, motor vehicle collisions). Retrospective chart review was completed. Serial blood samples were collected at admission, 24, 48, and 72 hours; plasma OPN levels were obtained using these samples.

RESULTS: No significant differences exist in Glasgow Coma Score (GCS) between cAHT, sAHT and TBI groups (mean GCS 6.3 vs. 5.3 vs. 4.3). Rates of characteristics in the cAHT group were as follows, SDH = 91.7%, RH = 87.5%, and SS = 73.9%. Rates in the sAHT were as follows, SDH = 75%, RH = 33.3%, and SS = 40%, compared to the TBI group, SDH = 40%, RH = 11.1% and SS = 33.3%. cAHT were more likely to have multiple abuse characteristics (58.3%) compared to 8.3% and 0% in the other groups (χ² = 73.45, p <.01). OPN levels were significantly higher in the cAHT versus other groups, but did not differ based on abuse characteristics.

CONCLUSIONS: To our knowledge, this is the first study to investigate plasma OPN and abuse characteristics. Results suggest these characteristics may not be sufficiently accurate in identifying children with AHT. OPN levels may be a useful measure in determining severity of injury as it relates to AHT.
Differential Structural Brain Development of Healthy Adolescents With Lower and Higher IQ

Bajaj, Sahil; Zhang, Ru; Bashford, Johannah; Lukoff, Jennie; Blair, Karina; and Blair, James

Corresponding Author: Sahil Bajaj, PhD, Boys Town National Research Hospital, sahil.bajaj@boystown.org

Center: Children's Center for Neurosciences Research (CCNR)

Type: Basic

Keyword(s): General Intelligence, Adolescents, Cortical Structure

Related to Pilot Grant or Trainee Award: No

Poster Available: No

Previous work has reported associations of cortical structure (CS) either with age or general intelligence (IQ). However, little work has examined the extent to which there are differences in age-related CS development in adolescents as a function of IQ. On the assumption that lower IQ might partly reflect slower cortical development, we hypothesized that there would be a significant association between age and the measurements of CS particularly for lower IQ adolescents.

Neuroanatomical data and IQ scores (vocabulary, matrix and total using WASI-II) were collected from 113 healthy adolescents (mean age = 13.95 ± 2.52 years, 52 F). The entire sample was categorized into two groups: G1 (median of total IQ > 108, n = 55, mean age = 13.66 ± 2.55 y, 20 F); and G2 (median of total IQ ≤ 108, n = 58, mean age = 14.22 ± 2.48 y, 32 F). The mean cortical thickness (MCT) and mean cortical surface area (MCSA) from seven different networks (N1: Visual, N2: Somatomotor, N3: Dorsal Attention, N4: Ventral Attention, N5: Limbic, N6: Frontoparietal and N7: Default-Mode) were analyzed via two 2 (G1 and G2) x 2 (Sex) x 2 (Hemisphere: left/right) x 7 (N1 to N7) analysis of covariance (ANCOVAs) with age included as covariate – one for the MCT and one for the MCSA data.

There was a significant Group x Network x Age interaction for the MCSA data ([F (6,648) = 2.82, p = 0.01]) but not for the MCT data [F (6,648) = 0.336, p = 0.92]). The correlation analyses reflected that the networks N2 and N7 for G2 showed significantly greater increases in MCSA as a function of age than individuals in G1 (N2: z = 2.70, p < 0.01 and N7: z = 2.61, p < 0.01).

Our findings indicate that the cortical development for higher IQ adolescents stabilizes with age. However, it continues to develop for lower IQ individuals. The development rate differs across brain networks. Lower IQ may reflect in part a slower cortical maturation process that does not reach the same level of neuronal efficiency as that shown by higher IQ individuals.
New Advanced Genomics: Merging Clinical and Research Genetics to Enhance Molecular Diagnostics and Resolve Genotype-Phenotype Correlations of >400 Neuromuscular Disease Patients

Chakravorty, Samya; Gloster, Logan; Berger, Kiera; Rufibach, Laura; Emmons, Sarah; Wicklund, Matthew; Harms, Matthew; Mozaffar, Tahseen; Hartzell, Criss; Choo, Hyojung; Gibson, Greg; and Hegde, Madhuri

INTRODUCTION: Currently, at least ~50-70% of both adult and pediatric Neuromuscular Disease (NMD)-patients never receives a definite molecular-diagnosis, among which at least ~20-25% of pediatric NMD-patients specifically, remain undiagnosed limiting their clinical-trial-enrolment or therapeutic-options creating both medical/financial burden. Moreover, definitive genetic-diagnoses do not always provide the reason for variability in disease-presentations/progressions needed to be functionally resolved for better trial-readiness. The major hurdles are: a) lack of genotype-phenotype-correlation knowledge in heterogeneous-NMDs with multiple monogenic-subtypes, b) high-prevalence (72%) of variants of uncertain significance (VUSs), c) >30% of all NMD-patients with pathogenic variant(s) or VUSs in ≥2 genes (multigenic), and d) lack of less-invasive biomarker-driven approaches. METHODS: We functionally resolved VUSs and multi-genic cases, by combining clinical and genetic data with different functional-omics platforms using minimally-invasive biomarker approach or target muscle-biopsies, to enhance diagnostic-yields and better trial-readiness of patients by resolving genotype-phenotype correlations. RESULTS: We show in a cohort-study of 394 Dysferlinopathy-suspected genetically-unconfirmed NMD patients, a significant increase in diagnostic yield from 25% to 82% by using a combinatorial blood biomarker-driven CD14+ monocyte-assay and whole-blood targeted-RNA-seq along with clinical-correlation through VUSs reclassification, identification of novel causal-variants and patho-mechanisms. Importantly, in multiple pediatric-NMD patients including Duchenne/Becker’s muscular dystrophy, we resolved the molecular cause for differential disease presentation and progression allowing better precision medicine and trial-readiness. For example, using functional genomics on an 8-year-old-boy’s muscle-biopsy, we identified the c.957-11C>G variant in SGCA gene to be a “leaky” splice-variant causing only subtle splicing abnormalities resulting in milder disease. This level of resolution facilitates better trial-readiness and disease management. We also have discovered a new gene, DRGX associated with peripheral neuropathy in a 16-year-old-boy at CHOA using whole genome sequencing, which we are following up to functionally characterize disease-association further. CONCLUSIONS: Using clinically-driven-functional-omics platforms, we are reclassifying VUSs for diagnostics, connecting dots from gene-variant-to-phenotype for better precision-medicine and trial-readiness, and resolving nature of defects in different pathways that lead to multi-genic contribution in NMDs. Our results show the importance of using a multi-tiered diagnostic approach that includes biomarkers, omics-platforms and genotype-phenotype correlations not only for precision-medicine-diagnostics but also for testing clinical-trial efficacy.
Autism Spectrum Disorder (ASD) and Attention Deficit Disorder/Attention Deficit Hyperactivity Disorder (ADD/ADHD) in Children Exposed to Polybrominated Biphenyls (PBB)

Christensen, Grace; Terrell, Metrecia; Pearce, Brad; and Marcus, Michele

Corresponding Author: Grace Christensen, MPH, Emory University, gmchris@emory.edu
Center: Children's Center for Neurosciences Research (CCNR)
Type: Outcomes
Keyword(s): Neurodevelopment, chemical exposure
Related to Pilot Grant or Trainee Award: No
Poster Available: No

BACKGROUND: In 1973 PBB, a brominated flame retardant chemical was accidently mixed into livestock feed, leading to widespread distribution of contaminated farm products across the state of Michigan [1]. Endocrine disrupting chemicals (EDCs), like PBB, mimic hormones like estrogen and can disrupt hormone balances leading to atypical neurodevelopment [2]. In utero and early life exposure to neurotoxicants are important because this is a critical period for neurodevelopment. This pilot study examines exposure to PBB in utero and early childhood in relation to diagnosis of ADD/ADHD or ASD.

METHODS: Data from the 2017-2018 health questionnaires administered to Michigan PBB registry participants were analyzed. In this questionnaire, mothers were asked about their child’s history of ADD/ADHD and ASD diagnosis, and gave a blood sample to be tested for PBB. Children born after 1962 were included in the ADD/ADHD analyses, and children born after 1968 were included in the ASD analyses. The prevalence of ADD/ADHD or ASD in this exposed population was assessed. In addition, preliminary univariate logistic regression models were run to assess the effect of mother’s PBB level and odds of child’s ADD/ADHD or ASD diagnosis. PBB levels below the limit of detection (LOD) (0.02 ng/mL) were imputed as LOD/sqrt(2). PBB levels were log transformed for analysis.

RESULTS: 139 children were included in the analyses of ADD/ADHD. Of these children, 21 (15.11%) had a diagnosis of ADD/ADHD. The univariate logistic model showed a protective effect for log-PBB level on odds of diagnosis of ADD/ADHD (OR: 0.705; 95% CI: 0.536, 0.928). For ASD analyses, 124 children were included 5 (4.03%) of whom were diagnosed with ASD. The univariate logistic model showed null results for log-PBB level on odds of diagnosis with ASD (OR: 0.956; 95% CI: 0.560, 1.631).

DISCUSSION: The prevalence of ADD/ADHD is higher in this study sample than in the United States general population (15.11% vs 9.4%)[4]. No significant association was seen for ASD. The small number of cases among mother’s with PBB serum measurements is a limitation. Future analyses will be able to examine a larger sample and adjust for confounders and covariates.
Over the last two decades, rates of neonatal opioid withdrawal syndrome (NOWS) have quadrupled in the United States to 5.8 per 1000 hospital births. Symptoms of NOWS include hypersensitivity, autonomic dysfunction, and gastrointestinal distress. After the initial withdrawal behaviors have resolved, children born with NOWS have an elevated risk of developmental and cognitive delays, but other deficits are mostly unknown in the clinic. Use of preclinical animal models will facilitate our understanding of the long-term developmental consequences of gestational opioid exposure, and provide insight into the underlying neural mechanisms. Previous animal models of NOWS often utilized steady-state truncated dosing paradigms, rather than intermittent and prolonged use typical of human opioid users. As such, we have developed a model of NOWS that utilizes intermittent morphine exposure before, during, and after pregnancy. Specifically, female rats are implanted with osmotic minipumps for pulsatile delivery of morphine prior to and throughout breeding, gestation and parturition. Morphine delivery is terminated 3 days following birth. With this model, pups are indirectly exposed to morphine throughout gestation in a clinically relevant model of NOWS. Our preliminary data suggests that this model recapitulates many of the clinical features observed in children born with NOWS, including low body weight. Additional analysis examining social behavior, response to anxiety- and stress-provoking stimuli, and immune signaling are underway. This preclinical model provides an opportunity to study the long-term effects of perigestational opioid exposure in a tractable way, and will identify potential therapeutic targets for children born with NOWS.
Exploring the Potential of Bio-Engineered PC12 Cells as a Prospective Treatment for Infantile Parkinsonism Dystonia

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

Corresponding Author: Devyani Joshi, Mercer University, devyani.jaideep.joshi@live.mercer.edu
Center: Children’s Center for Neurosciences Research (CCNR)
Type: Technology
Keyword(s): Cell microencapsulation
Related to Pilot Grant or Trainee Award: No
Poster Available: No

Infantile Parkinsonism Dystonia is the neurological disorder that is a result of decreased dopamine release from dopaminergic neurons of brain. Goal of our study is to use regenerative medicine-based cell therapy to increase dopamine level in brain. Rat adrenal Pheochromocytoma (PC12) cells produce, store and secrete dopamine. In a proof of concept study, we are interested in using PC12 cells to demonstrate reversal of Parkinson’s like symptoms in pre-clinical studies. Microencapsulation of the PC12 cells in alginate-chitosan polymeric membrane can be used to immune-isolate these cells. If the proof- of concept studies are successful, neuron differentiated stem cells can be later substituted for PC12 cells.

For fabrication of microcapsules, cells were suspended in the trehalose-sodium alginate solution and sprayed through 1.40mm Buchi spray dryer nozzle into calcium chloride solution. The microcapsules were reacted with chitosan glutamate and stored in media. FTIR spectroscopy was performed to confirm crosslinking of alginate microcapsules with calcium chloride to form calcium alginate in microcapsule membrane. Short- and long-term stability study was performed to evaluate the strength of the microcapsule membrane and mechanical stability of the microcapsules. The viability of cells inside microcapsules was determined. Griess’s nitrite assay was performed to confirm non-immunogenicity of microcapsules.

The microcapsules with average diameter of 52µm were formulated using air flow rate of 350L/Hr and pump speed of 9RPM. FTIR spectra confirmed formation calcium alginate in the microcapsule membrane. The cells were viable for a period of 30 days. More than 85% of the microcapsules were intact in short- and long-term stability studies. Griess’s assay showed that microcapsules encapsulating PC12 cells were non-immunogenic. The microencapsulated PC12 cells were found to release dopamine over a period of 30 days. The successful formulation of microcapsules encapsulating viable PC12 cells demonstrate the potential of using cells-based therapy. The potential of this therapy to reverse Parkinsonism symptoms will be tested in on-going pre-clinical studies.
Surface Functionalized Nanoparticles of Neuropeptide Y: Fabrication and Characterization

Kale, Akanksha; Uz Zaman, Rokon; Murnane, Kevin; and D'Souza, Martin

Corresponding Author: Akanksha Kale, Mercer University, Akanksha.Madhav.Kale@live.mercer.edu
Center: Children's Center for Neurosciences Research (CCNR)
Type: Technology
Keyword(s): Nanoparticles, Blood-Brain Barrier
Related to Pilot Grant or Trainee Award: No
Poster Available: No

INTRODUCTION: Neuropeptide Y is a neurotransmitter with a significant role in the pathophysiology of anxiety and depression. It also has antiseizure activity. However, it is a small hydrophilic molecule that cannot cross the Blood-Brain Barrier (BBB) when administered exogenously. Endogenous biological mechanisms can be exploited to cross the BBB to deliver proteins and therapeutic peptides to the brain. To enable Neuropeptide Y to cross BBB, we encapsulated it in nanoparticles and conjugated these particles to brain targeting ligands such as Transferrin (Tf), Rabies Virus Glycoprotein (RVG).

METHODS: NPY loaded PLGA nanoparticles were formulated by multiple emulsion-solvent evaporation method while NPY loaded BSA nanoparticles were formulated by the nanoprecipitation method. These nanoparticles were conjugated with the ligands followed by lyophilization. Particles were characterized for size, zeta potential, surface morphology, encapsulation efficiency, and release profile. The total amount of Neuropeptide Y released was detected by ELISA. Immunogenicity and cytotoxicity were assessed by nitric oxide and MTT assay respectively.

RESULTS: The size of PLGA and BSA nanoparticles ranged between 190-200 nm. Both types of nanoparticles were negatively charged with zeta potential between -16 to -20 mV. No particles were immunogenic or cytotoxic. The NPY content of PLGA nanoparticles determined using ELISA was found to be 78 ± 2.5 % w/w. The NPY content of BSA nanoparticles was found to be 71 ± 2.5 % w/w. PLGA-based nanoparticles released NPY for 23 days while BSA-based nanoparticles released NPY for 20 days in a sustained manner.

CONCLUSION: Neuropeptide Y loaded Tf or RVG conjugated PLGA and BSA nanoparticles were non-immunogenic, non-cytotoxic and showed sustained release of Neuropeptide Y \textit{in vitro}. 
Does the NEOS Score Predict Outcomes in Pediatric Anti-NMDA Receptor Encephalitis Patients?

Loerinc, Leah; Blackwell, Laura; Howarth, Robyn; and Gombolay, Grace

OBJECTIVE: Pediatric anti-N-methyl-D-aspartate receptor encephalitis (NMDARE) can cause long-term disability with up to 10% mortality. Accurate prediction of poor outcomes could help to guide treatment. The NMDARE One-Year Functional Status (NEOS) score was developed to predict poor functional status at one-year in adult NMDARE. This study is to validate the NEOS score in pediatric patients.

METHODS: Retrospective chart review was conducted on pediatric NMDARE patients at Children’s Healthcare of Atlanta from 2010-2019. Data included demographics, symptoms, laboratory testing, imaging, and treatments. The Modified Rankin Scale (mRS) for children was used for one-year functional status with scores > 2 considered poor outcomes. NEOS scores range from 0-5 and were calculated with one point for each criterion: (1) intensive care admission, (2) treatment initiated > 4 weeks from symptom onset, (3) abnormal imaging, (4) no improvement within 4 weeks of treatment, and (5) CSF white blood cell count > 20 cells/L.

RESULTS: Twenty-one NMDARE patients with one-year follow up were included. Mean age was 10.45 years (range 2-18 years) with 71.4% female and 28.6% male. 28.6% were white, 66.7% were African American, and 4.7% were Hispanic. 57.1% had good functional outcomes (mRS 0-2) and 42.9% had poor functional outcomes at one-year. For good functional outcome patients, two had a NEOS score of 0, three with score 1, four with score 2, and three with score 3. For poor functional outcome patients, three had a NEOS score 1, two with score 2, three with score 3, and one with score 4. No patients had a NEOS score 5. Probability of good outcomes were: 100% for NEOS 0, 50% for NEOS 1, 67% for NEOS 2, 50% for NEOS 3 and 0% for NEOS 4.

CONCLUSIONS: NEOS scores may identify which NMDARE patients are at risk for worse outcomes at one-year, with 100% good outcomes in NEOS score 0 and 100% bad outcomes in NEOS score 4. Good outcomes were observed in 50-67% of patients with NEOS scores ranging from 1-3. Additional patients and longer-term data are needed to determine viability of NEOS scores for pediatrics and whether other factors may predict pediatrics outcomes.
Identifying *Staphylococcus aureus* as a Novel Stimulator for Chronic Itch

Nho, Yeseul; Hilley, Henry; Lawson, Katy; Banovic, Frane; and Han, Liang

**Corresponding Author:** Yeseul Nho, BA, Georgia Tech, ynho3@gatech.edu
**Center:** Children’s Center for Neurosciences Research (CCNR)
**Type:** Basic
**Keyword(s):** Staphylococcus aureus, itch
**Related to Pilot Grant or Trainee Award:** No
**Poster Available:** No

**BACKGROUND:** Chronic itch is a debilitating symptom associated with skin disorders including atopic dermatitis (AD), which affects 8-30% of children (Eyerich, Eyerich, and Biedermann 2015). Current anti-itch medications are typically ineffective with undesirable side effects (Lee et al. 2015).

*Staphylococcus aureus* (*S. aureus*) is a gram-positive bacterium that has been found on the skin of greater than 90% of pediatric AD patients, and plays a critical role in triggering eczema flares of pediatric AD patients (Hoeger et al. 2017). The bacteria has been found to induce pain behavior (Chiu et al. 2013), and to trigger mast cell degranulation, thought to play an important role in pathogenesis of AD (Nakamura et al. 2013). However, the *S. AUREUS* and itch relationship is largely unexplored. Here, we investigated the bacteria’s influence on stimulating itch using murine model behavioral tests and calcium imaging.

**METHODS:** We performed murine behavioral tests consisting of cheek injections to identify the roles of *S. aureus* virulence factors (δ-Toxin and PSMα2) in inducing acute itch behavior. The control group was injected with saline, and the experimental group with one of the aforementioned virulence factors in three concentrations. The bacteria was also injected. Itch behavior was observed measured in scratching bouts. To test the direct effects of the bacterium’s virulence factors on sensory neurons, calcium imaging was performed with sensory neurons from Pirt-GCaMP3 mice. Fluorescence intensity activity was measured.

**RESULTS:** 0.5mM and 0.8mM δ-Toxin injections showed significantly increased scratching bouts (P = 0.018 and 0.003 respectively, two-tailed t-test) in comparison to the control group. A 0.5mM PSMα2 cheek injection induced significant scratching bouts (P = 0.036, two-tailed t-test). 1.00 x 10^9 C.F.U. *S. aureus* cheek injections induced significant scratching (P = 0.016). δ-Toxin alone induced a calcium flux response in sensory neurons (responsive cells = 41 of 1,992). Chloroquine treatment before and after δ-Toxin application induced neuron sensitization in 131 out of 1003 cells.

**CONCLUSION:** *S. aureus* is significantly associated with the itch, and is likely involved in triggering an itch response on cellular and behavioral levels. Further investigation appears relevant for better understanding the pathogenesis and clinical significance of *S. aureus*. 
MrgprC11+ Neurons Mediate Glabrous Skin Itch

Steele, Haley; Xing, Yanyan; Hilley, Henry; Lawson, Katy; and Han, Liang

Chronic itch is a debilitating disease that arises from a multitude of etiologies, is the most common reason for visiting a dermatologist, and has few effectual treatments. In children, skin diseases such as atopic dermatitis, which is estimated to effects approximately 17% of the global population, are the most common cause of itching. Additionally, they are the primary cause (accounting for greater than 90%) of all skin lesions in children. However, almost all previous itch research has focused almost exclusively on hairy skin itch, despite glabrous skin itch (located on the palms of hands and soles of feet) being considered particularly disabling as it can impact a child’s ability to hold objects, write, run, or play. Although anatomical and clinical differences that exist between hairy and glabrous skin, our research for the first time demonstrates the potential differences that exist in itch circuitry between hairy and glabrous skin. Our data reveals that the previously identified itch-specific population of MrgprA3+ neurons preferentially innervates hairy skin. Then, using a novel glabrous itch behavior assay, as all formerly established itch behavior tests are applicable only to hairy skin, we identify MrgprC11+ neurons as the best candidate for mediating itch response. As MrgprC11+ neurons have not been extensively studied or characterized, we generated a BAC transgenic MrgprC11CreER mouse line that expresses an inducible Cre recombinase controlled by the MrgprC11 promoter. We then validated that, unlike MrgprA3+ neurons, MrgprC11+ neurons densely innervate both hairy and glabrous skin. Furthermore, chemogenetic activation of MrgprC11+ neurons using the DREADD system evoked itch-behaviors in both hairy and glabrous skin. In contrast, chemogenetic activation of both MrgprA3+ and MrgprD+ neurons using the DREADD system evoked an itch response only in hairy skin. These findings suggest that MrgprC11+ neurons are the primary mediators for glabrous skin itch. Along with our newly developed glabrous skin models, this data opens up new avenues for future glabrous skin itch research and the development of glabrous-skin specific anti-itch therapies.
History of Obstetric Complications and Neonatal Hypoxia in Patients with 22q11 Deletion Syndrome

Tenorio Martinez, Sofia; Coleman, Karlene; Ousley, Opal; Kobynski, Lisa, Cubells, Joseph; Oster, Matthew; and Pearce, Brad

Corresponding Author: Sofia Tenorio, MD, MPH, Emory University, s.tenorio91@gmail.com

Center: Children's Center for Neurosciences Research (CCNR)

Type: Basic

Keyword(s): SGA, 22q11, C-section

Related to Pilot Grant or Trainee Award: No

Poster Available: No

BACKGROUND: 22q11.2 Deletion Syndrome (22q11.2DS) has a prevalence of 1/4000 live births and is the second most common cause of developmental delay and Congenital Heart Disease (CHD) in the US. Preterm birth and low birthweight (BW) can influence brain development and lead to adverse outcomes. However, there is a lack of prevalence data on preterm births and BW, delivery methods, neonatal hypoxia and poor respiration at birth for this population.

HYPOTHESIS: As part of our ongoing study of patients with 22q11DS we determined the distribution of gestational age (GA), BW, delivery method, neonatal hypoxia and poor respiration.

METHODS: We analyzed a sample of 158 participants, all with genetically confirmed 22q11DS. Data were abstracted for BW, GA, small for gestational age (SGA), delivery method, neonatal hypoxia, poor respiration at birth and CHD presence—classified into three levels based on the severity and association to hypoxia. Chi-square tests were used to analyze the relationships.

RESULTS: Of the participants, 29.75% (47) were delivered by C-Section, with the main reason being “repeat C-section.” Hypoxia was reported in 11.39% (18) and there were 26.58% (42) with poor respiration at birth. Most of the participants had at least one congenital heart defect (79.11%, 125). Of those labeled as Level 2 (Hypoxic CHD), the majority had Tetralogy of Fallot (18.99%, 30). Of those considered as level 1 CHD (lowest risk of hypoxia) the majority had Ventricular Septal Defects (39.24%, 62). Mean GA was 38.06 weeks (sd 1.83), BW of 2,920 grams (sd 560) and birthweight percentile of 32.07% (sd 28.61), with 33.33% meeting established criteria for SGA. Neonatal hypoxia was associated with an increased likelihood of C-Section (Prevalence Odds Ratio 3.10, p .03).

CONCLUSION: Compared to the general population, patients with 22q11.2DS have similar GA and BW, but higher prevalence of SGA. Even though the main established reason for C-Section was repeat C-Section, there was an increased likelihood of C-Section when having neonatal hypoxia. Additional investigations are underway to determine the relationship between these obstetric complications and adverse neurodevelopmental conditions, including autism spectrum disorders.
Maternal and Child Depression: The Role of Genetic Sensitivity and Telomere Length

Thompson, Amanda and Henrich, Chris

Corresponding Author: Amanda Thompson, MA, Georgia State University, athompson119@student.gsu.edu
Center: Children's Center for Neurosciences Research (CCNR)
Type: Basic
Keyword(s): Child depression, genetic sensitivity, and telomeres
Related to Pilot Grant or Trainee Award: No
Poster Available: Yes - P18

Current findings suggest telomere length could serve as a biomarker of health and wellness. The dynamic relation between psychological health and telomere length is unclear, but research finds that people who have suffered from depression typically have shorter telomeres. The ubiquitous health disparities among those experiencing depression could be explained by underlying mechanisms of stress which are thought to accelerate telomere attrition. Importantly, genetic makeup may determine who is at greater risk for depression in the context of stress. Those who carry one or more short alleles of the serotonin transporter gene (5HTT) are believed to be at greater risk for depression in the context of life stress. This genetic sensitivity toward depression is especially salient to children of a mother who experienced depression. These children are at increased risk for negative socioemotional outcomes through heritability and environmental factors. The 5HTT genotype has been found to moderate the effects of maternal depression on various child socioemotional outcomes, but the moderating effects of child 5HTT genotype on maternal depression and child telomere length remain unexplored. Further, the relation between child telomere length and depressive symptoms is largely unexplored as most research has only considered the length of telomeres after the experience of depression. Using Mplus V8, this research used structural equation modeling to test the mediating effect of child telomere length at age nine on maternal depression and adolescent depressive symptoms at age 15. Using multigroup modeling, this research also tested for differences in child sex and genotype. From a subset (N=2,884) of the large and diverse Fragile Families and Child Wellbeing dataset, we did not find support for the mediation hypothesis by child telomere length. Further, we found no evidence of moderation by child genotype or sex. More longitudinal research is needed to understand telomere variability within children as they experience depression over the life course. While our findings did not support the hypotheses, interesting findings emerged that warrant future investigation into the dynamic nature of depression and telomere attrition over time.
INTRODUCTION: Delayed presentation of young infants with a skull fracture often signals healthcare providers to screen for non-accidental trauma (NAT). The purpose of this study was to explore crying behavior in young infants presenting to the emergency department (ED) with skull fracture. We investigated whether absence of crying after an inciting event would be associated with delayed presentation in young infants; as well as whether infants presenting in a delayed fashion would have a higher frequency of social work consults for NAT workup.

METHODS: Children < 6 months old admitted to the neurosurgery service with simple skull fractures were enrolled in this study. Variables included time of presentation (immediate < 6 hours, acute 6-24 hours, delayed > 24 hours); presence of crying after initial injury; and whether social work and/or Division of Family and Children Services (DFCS) were involved.

RESULTS: Forty-five infants were included. The vast majority of infants infants cried at initial injury (n=38, 84%). Three were unwitnessed. Four infants (8.9%) did not cry, but they all presented to the ED immediately. Most children presented immediately (n=37) or acutely (n=6). Only 2 children had delayed presentations. DFCS was consulted in 37.8% of immediate presentations, 17% of acute presentations, and 50% of delayed presentations.

CONCLUSIONS: Lack of crying after a trauma event in young infants did not lead to delayed presentation. Rates of social work or DFCS involvement were frequent, and highest in patients with delayed presentations. Future work will include evaluating additional social factors that may lead to delayed presentation and increase social work involvement in larger sample sizes.
Clinical Outcomes Research and Public Health (CORPH)

An Exploratory Gonorrhea Vaccine: Determination of Immunostimulation in Dendritic Cells

Bajaj, Lotika; Bagwe, Priyal; Gala, Rikhav; Zughaier, Susu; and D’Souza, Martin

Corresponding Author: Lotika Bajaj, Mercer University, lotika.bajaj@live.mercer.edu
Center: Clinical Outcomes Research and Public Health (CORPH)
Type: Clinical or Translational
Keyword(s): Formulation
Related to Pilot Grant or Trainee Award: No
Poster Available: No

INTRODUCTION: The CDC has listed gonorrhea as one of the urgent threats for which a vaccine approach is immediately required. We have developed a sustained release novel particulate vaccine using formalin fixed whole-cell of N. gonorrhoeae as the vaccine antigen. This helps in preserving all the surface epitopes in their native form, and adding adjuvants to this antigen can offer better immune response. The particulate vaccine allows better uptake of antigen by antigen presenting cells, APCs (dendritic cells and macrophages), causing improved antigen presentation and subsequent activation of T cells.

METHODS: N. gonorrhoeae was grown on GC agar and piledated colonies were used for bulk production in GC broth with defined supplements. The culture was formalin-fixed, gonococcal (Gc) pellets were harvested, washed and saved as dense suspension at -80°C. Whole-cell formalin fixed N. gonorrhoeae was quantified for protein content prior to encapsulation, using Micro BCA™ Protein Assay Reagent Kit. The matrix for vaccine particles contained pre-crosslinked bovine serum albumin (BSA) and microparticles were prepared using Buchi Mini Spray Dryer B-290. Adjuvants (Alum and MF59®) particles were prepared with the same method. Particle size and zeta potential of these microparticles were measured and scanning electron microscopy (SEM) was done for the morphology. The cytotoxicity of vaccine microparticles at different concentrations was assessed by the MTT assay. The invitro immunogenicity was assessed by measuring the amount of nitrite released by dendritic cells (DC2.4) in presence of microparticulate formulations. The Dendritic cells exposed to particulate vaccine were evaluated for expression of MHC I and MHC II molecules and costimulatory molecules, CD40 and CD86.

RESULTS: Formalin-fixed gonococci were not lysed prior to processing into particulate vaccine, as observed by scanning electron microscopy. Cytotoxicity assay showed that Vaccine microparticles loaded with fixed gonorrhea were non-cytotoxic within the tested concentration range. Nitric oxide assay demonstrated that vaccine microparticles produced significantly higher nitric oxide release compared to blank microparticles and control groups. Expression of MHC I, MHC II, CD40 and CD86 was significantly higher in the adjuvanted vaccine (fixed Gc and adjuvant) microparticles group as compared to untreated control group (p <0.001).
The Accuracy of Non-Invasive Blood Pressure Measurement in Obese Children

Berry, Christopher; Fundora, Michael; Beshish, Asaad; Rao, Nikita; Figueroa, Janet; McCracken, Courtney; and Maher, Kevin

Corresponding Author: Christopher Berry, Auburn University, mberry6799@gmail.com
Center: Clinical Outcomes Research and Public Health (CORPH)
Type: Clinical or Translational
Keyword(s): Pressure Accuracy
Related to Pilot Grant or Trainee Award: No
Poster Available: Yes - P20

BACKGROUND: Obesity and hypertension are important public health priorities in the United States, and often occur simultaneously. Accurate blood pressure (BP) measurement is critical to both diagnosis and management of hypertension in children, with automated devices being typically used to measure BP. The accuracy of these devices is unknown in obese children and may erroneously be labeling patients as hypertensive. We sought to compare the intra-arterial blood pressure to non-invasive (NI) cuff blood pressure measurements in obese and non-obese children.

METHODS: We performed a retrospective matched case-controlled study of 100 obese (97-99th %tile body-mass index) and 100 non-obese children after cardiac surgery with simultaneous intra-arterial (IA) and cuff BP measurements. Obese children were matched 1:1 for age, sex, race and Risk Adjustment in Congenital Heart Surgery score (RACHS-1). Systolic, diastolic and mean pressures were recorded with simultaneous intra-arterial and NI cuff measurements. Intraclass correlation coefficients and Bland-Altman plots were used to determine arterial vs cuff agreement with ICC 0.75 as threshold for agreement. Approximately 4,000 individual BP measurements were utilized.

RESULTS: Median age 12.9 years, (10-15, interquartile). Body mass index 97-99% tile for the obese cohort, 25-69% tile for controls. Systolic and diastolic BP interclass correlation coefficient was <0.75. Bland Altman plots demonstrate that NI cuff systolic BP was underestimated vs invasive arterial measurement, especially during hypertension for both obese and non-obese patients and overestimated systolic hypotension at the lower range of measurements. The diastolic and mean BP measurements by NI cuff correlated well with intra-arterial measurements for both obese and non-obese children.

CONCLUSIONS: NI cuff blood pressure measurements do not appear to be affected by body habitus in children. However, for both obese and non-obese children, NI cuff BP measurements underestimate the true arterial systolic pressure during hypertension and overestimate the degree of hypotension when present. Mean and diastolic BP by NI cuff were accurate when compared to intra-arterial measurements. NI cuff BP devices can be used to diagnose and manage hypertension in the obese and non-obese pediatric patient, with recognition that the true systolic BP may be underestimated.
BACKGROUND: Hypertension (HTN) is underdiagnosed in the pediatric outpatient setting. Children can be especially challenging to obtain an accurate BP reading, which is affected by environmental stimulation, user training, and cuff size variation. In many instances, when retaken manually, with the correct equipment, BP falls in the normal range. Current literature reports the prevalence of HTN decreases with repeat measurements. For those patients who have true abnormal BP, repeat readings increase the odds of the patient receiving a correct diagnosis.

OBJECTIVE: We aimed to enhance and assess nursing staff and resident physician knowledge of proper technique to obtain accurate BP readings, special populations of patients in the outpatient setting that need their BP taken, and additionally for residents, correct classification of BP using the 2017 AAP guidelines.

METHODS: A clinical guideline for attainment and recognition of abnormal BP was developed by a multidisciplinary team, based on the AAP guidelines. Intervention consisted of two content lectures reviewing the new clinical guideline for residents and video training presentation of technique and guidelines for nurses. Pediatric and Medicine-Pediatric resident knowledge of the guidelines was assessed pre- and post-intervention using an anonymous online survey, which included multiple choice and free response questions. Nurses’ competency was assessed pre- and post-intervention using a comprehensive multiple choice quiz.

RESULTS: Surveys were completed by 32 and 29 residents pre- and post-intervention, respectively. Knowledge increased pre- to post- intervention 32% to 65% for the process of verification of abnormal BP, 44% to 79% for categories of BP classification, and 32% to 86% for recognition of special populations, respectively. Quizzes were completed by 12 nurses pre- and post- intervention, and competency increased from 92% to 100%.

CONCLUSION: Nurses and resident education along with a specific protocol for BP measurement in the outpatient clinic led to an improved understanding of the AAP best practice guidelines and increased competency in performing BP measurements by nurses. Our next step is to utilize chart review to evaluate if these knowledge and performance increases correlate to clinical change in HTN diagnosis on repeat BP measurements.
Trends in Adolescent Chlamydia Rates by Region, Race, and Sex: A Secondary Data Analysis

Corcoran, Jessica; Li, Peng; Davies, Susan; Knight, Candace; Lanzi, Robin; and Ladores, Sigrid

Corresponding Author: Jessica Corcoran, University of Alabama at Birmingham, jlp1992@uab.edu
Center: Clinical Outcomes Research and Public Health (CORPH)
Type: Outcomes
Keyword(s): STI, adolescent sexual health
Related to Pilot Grant or Trainee Award: No
Poster Available: No

In the United States, adolescents ages 15 to 19 have the highest chlamydia rates second only to the young adult population (20 through 24). Rising chlamydia rates in adolescents are a significant public health concern as untreated chlamydia in women can cause cervicitis, pelvic inflammatory disease, and ultimately infertility. In men, untreated chlamydia can cause urethritis and epididymitis. The purpose of this secondary analysis is to describe the five-year trends in adolescent chlamydia rates by region, race, and sex in order to reveal the regions where targeted outreach and intervention research could be most beneficial.

Quantitative data for chlamydia rates (2013-2017) were obtained from the CDC National Center for HIV/AIDS, Viral Hepatitis, STD, and TB Prevention (NCHHSTP) database. Chlamydia cases were imported from the NCHHSTP data set over the five-year period from all 50 states, seven ethnicities, and two sexes. Each case was paired with the population and a proportion of cases per population was created. The 50 states were aggregated to their respective regions (e.g., Northeast, South, Midwest, or West) and the race/ethnicity categories were collapsed, subsequently, creating the dataset and final variables used for analysis.

When dividing the data by region, the Midwest and South have the highest total chlamydia cases per 1000 with 2017 rates of 17.47 and 17.32, respectively. The Northeast and West have the lowest regional rates reporting 10.67 and 10.66 cases per 1000 adolescents, respectively. Data from this secondary analysis show significant racial differences in adolescent chlamydia rates. African American adolescents’ chlamydia rates for 2017 were 47.02, whereas Hispanic, White, and “Other” races were 11.57, 9.09, and 5.84, respectively. There are substantial sex differences in chlamydia rates. Overall, rates in women are 3.5 to 5.5 times the rates in men. Examining the trends in adolescent chlamydia rates by region, race, and sex elucidates the areas where targeted programs and future research can be focused to improve outcomes.
Association Between Safety Attitudes and Medical Error Reporting Among Neonatal Intensive Care Unit Staff

Culbreth, Rachel; Spratling, Regena; Scates, Lauranne; Frederick, Laryssa; Kenney, Jordan; and Gardenhire, Douglas

INTRODUCTION: Critically ill neonates are particularly susceptible to medical errors; however, few studies have evaluated NICU safety climate in the context of medical error reporting. This study aims to identify the association between perceptions of safety and culture among NICU staff with medical error reporting behaviors.

METHODS: This study utilized a convenience sample of 79 NICU staff members (38 Nurses and 41 Respiratory Therapists). Questionnaires consisted of demographic factors (years of experience, sex, education), the Safety Attitudes Questionnaire (SAQ), and hypothetical medical error reporting scenarios (categorized into minor harm or major harm). The SAQ consists of six domains: job satisfaction, teamwork climate, safety climate, perceptions of management, working conditions, and stress recognition. Scores ranged from 0-5, with a 5 indicating a more positive perception. Safety attitudes and demographic factors were analyzed using structural equation modeling to identify the statistically significant domains associated with reporting medical errors by harm level. Additionally, linear regression was used to determine statistically significant predictors for each individual harm scenario.

RESULTS: Among those who completed the study, approximately 84.8% were female. For the six domains for the SAQ, the highest mean scores were job satisfaction (M= 4.54; SD= 0.54) followed by teamwork climate (M=4.43; SD= 0.55), perceptions of management (M= 4.13; SD=0.76), working conditions (M= 3.83; SD=0.74), and stress recognition (M= 3.49; SD= 1.01). There were no statistically significant domains associated with reporting hypothetical medical errors. However, there was a slight trend toward statistical significance for the association between working conditions and reporting a medical error of major harm (Est: 0.39, SE: 0.20, p=0.054). Additionally, a higher number of years of experience corresponded with a lower likelihood of reporting a medical error of minor harm (Est: -0.02, SE: 0.01, p=0.049).

CONCLUSIONS: This study suggests that safety climate may not play a significant role in promoting medical error reporting in the NICU setting. However, individuals with more experience were less likely to report a minor medical error. Interventions aimed at increasing medical error reporting should also incorporate established employees rather than targeting new employees only.
Bronchiolitis is a leading cause of hospitalization in young children, costing the healthcare system 1.7 billion dollars annually, with approximately one-third of patients hospitalized. The most recent American Academy of Pediatrics (AAP) guidelines conclude on supportive measures for treatment of bronchiolitis. Further, evidence shows no difference in hospital length of stay (LOS) from interventions such as bronchodilators, corticosteroids, or hypertonic saline nebulizers in infants with bronchiolitis. These interventions have not been shown to hasten symptom duration in the general ward or ambulatory setting, and may potentially increase cost. The role of therapeutics and correlation to outcomes and cost has not been well defined in the pediatric intensive care unit (PICU). Across a range of settings, ‘value’ is defined as: quality divided by cost. This study is a post hoc analysis of children ages 2 to 24 months admitted to a 36-bed tertiary care PICU over the years of 2016-2019 with an admission diagnosis of bronchiolitis. Patients requiring invasive mechanical ventilation will be excluded. The operational variable of “value” defined in the first 48 hours of PICU course, will compare quality and cost. Quality will be a composite assessment based on time to enteral nutrition after de-escalation of respiratory support, lab utilization, and exposure to radiation (as per chest film orders). Cost parameters will be focused on commonly employed diagnostics (eg. respiratory viral panels) and therapeutics (eg. beta-agonists, corticosteroids, hypertonic saline, etc.). Our primary outcome is hospital LOS. Our value analysis includes a daily score card which spans for the first 48 hrs of PICU care (T0 to T48 hr). Our two groups we will isolate in our analysis are “low-utilizers” and “high-utilizers.” Low utilizers are defined as those who have high quality of care and low cost, whereas high utilizers incur more cost and potentially less quality. We theorize that “high-utilizers” have reduced value of care and no difference in LOS compared to “low-utilizers.” We expect that the creation of a daily value-based score card and subsequent analyses will allow us to create a value-based model in bronchiolitis, which moreover may be applicable to other PICU diagnoses.
The Effect of Functional Electrical Stimulation Cycling Followed by Over Ground Dynamic Body Weight Support on Gross Motor Skills and Quality of Life in Children With Cerebral Palsy

Eggebrecht, Erin; Moore, Kelly; Van Den Eynde, Els; and Vova, Joshua

**Corresponding Author:** Erin Eggebrecht, DPT, Children's Healthcare of Atlanta, erin.eggebrecht@choa.org

**Center:** Clinical Outcomes Research and Public Health (CORPH)

**Type:** Clinical or Translational

**Keyword(s):** pediatric rehabilitation and technology, cerebral palsy, advanced technology, functional electrical stimulation, body weight support

**Related to Pilot Grant or Trainee Award:** 2015, Dudley Moore Nursing and Allied Health Research Fund, The Effect of Functional Electrical Stimulation Cycling Followed by Over Ground Dynamic Body Weight Support on Gross Motor Skills and Quality of Life in Children with Cerebral Palsy (PI: Erin Egg

**Poster Available:** Yes - P23

**PURPOSE:** This study evaluated feasibility and preliminary efficacy of intensive therapy using functional electrical stimulation and over-ground body weight support on gross motor function in children with mild to moderate cerebral palsy (CP).

**METHODS:** A convenience sample of 12 children (5.57 to 17.93 years) with CP participated in therapy two times per week, one hour per session over twelve weeks followed by a one month and three month follow up. Outcomes were measured using the Gross Motor Functional Measure Form 88 dimensions D/E (GMFM-88, D/E), Pediatric Berg Balance Scale (PBBS), 10 meter walk test (10MWT) and the Patient Reported Outcomes Measurement Information System (PROMIS).

**RESULTS:** Wilcoxin rank-sum tests showed a significant (p-value < 0.05) improvement in both the GMFM-88, D/E average and PBBS from pre to post testing in the comparison and experimental group. Results indicate improvements in both groups in functional and balance skills. Conclusions: An intensive episode of physical therapy using functional electrical stimulation biking followed by over-ground or body weight support may be effective in improving functional gross motor skills, balance and quality of life in children with CP.
PURPOSE: This study (1) examined the prevalence of anxiety and depressive symptoms among adolescents and young adults (AYA) with epilepsy and (2) examined demographic and medical characteristics, illness beliefs, and social factors associated with anxiety and depressive symptoms to guide intervention development.

METHODS: A community-based sample of AYA with epilepsy (n = 179, ages 13 to 24 years, 39% male) completed online questionnaires measuring anxiety (GAD-7), depression (PHQ-9), illness beliefs (helplessness; acceptance; perceived benefits), and social factors (family functioning; social stigma; connectedness). Participants also reported medical information (epilepsy type; years since diagnosis; time since last seizure; current medications).

RESULTS: Prevalence of clinically significant anxiety and depression scores, 36% and 35%, respectively, was high compared to population prevalence. In multivariable regression models, demographic and medical factors explained only 2% of the variance in depressive symptoms and 6% in anxiety symptoms. Illness beliefs and social factors accounted for a majority of the explanatory power of both models (partial R2 = 0.37 for anxiety; 0.44 for depression). Acceptance, family functioning, and social stigma accounted for the greatest variance (p’s < .01).

CONCLUSIONS: This study found a high prevalence of anxiety and depressive symptoms among AYA with epilepsy. The majority of variance in symptoms was accounted for by potentially modifiable beliefs and social factors. Consistent with broader literature, AYA with epilepsy may experience significant psychological distress even in the context of mild epilepsy symptoms. Interventions that promote illness acceptance, enhance family functioning, and reduce social stigma may ameliorate psychological distress among AYA with epilepsy.
Heterogeneity of Mild-to-Moderate Persistent Asthma in Children: Confirmation by Latent Class Analysis and Association With One-Year Outcomes

Fitzpatrick, Anne M. and Mauger, David T.

Corresponding Author: Anne Fitzpatrick, PhD, Emory University, anne.fitzpatrick@emory.edu
Center: Clinical Outcomes Research and Public Health (CORPH)
Type: Outcomes
Keyword(s): asthma, asthma outcomes
Related to Pilot Grant or Trainee Award: 2018, CORPH, Childhood Asthma Phenotypes: Identification and Utility in Outcome Prediction (PI: Anne Fitzpatrick, PhD, RN, CPNP, MSCR)
Poster Available: No

BACKGROUND: Compared to adults, phenotypic characterization of children with asthma is still limited and it remains difficult to predict which children with asthma are at highest risk for poor outcomes.

OBJECTIVE: We sought to: 1) identify latent classes in a large population of treatment-adherent children with mild-to-moderate asthma enrolled in NIH/NHLBI Phase III clinical trials, and 2) determine whether latent class assignment predicts lung function abnormalities and exacerbation rate at one-year of follow-up.

METHODS: Latent class analysis (LCA) was performed on 2,593 children with mild-to-moderate asthma 5 up to 18 years, with 19 variables encompassing demographics, medical history, symptoms, lung function, allergic sensitization and Type-2 inflammation. Outcomes included lung function and the annualized exacerbation rate at 12 months of follow-up.

RESULTS: Five latent classes were identified with differing demographic features, asthma control, sensitization and Type-2 inflammatory markers, and lung function. Exacerbation rates were 1.30 +/- 0.12 for class 1 ("multiple sensitization with partially reversible airflow limitation"), 0.90 +/- 0.05 for class 2 ("multiple sensitization with reversible airflow limitation"), 0.87 +/- 0.08 for class 3 ("lesser sensitization with reversible airflow limitation"), 0.87 +/- 0.05 for class 4 ("multiple sensitization with normal lung function"), and 0.71 +/- 0.06 for class 5 ("lesser sensitization with normal lung function"). Lung function abnormalities persisted in class 1 at 12 months.

CONCLUSIONS: Children with mild-to-moderate asthma are a heterogeneous group. Allergic sensitization and lung function may be particularly useful in identifying children at greatest risk for future exacerbation. Additional studies are needed to determine whether latent classes correspond to meaningful phenotypes for the purpose of personalized treatment.
Procedural Support Leads to Decreased Pain, Distress, and Long-Term Impacts on Healthcare Consumership

Fraser, Camille and Woodburn, Ashlie

Painful procedures are an unfortunate reality in pediatric healthcare. According to a recent survey, hospitalized children experience an average of 6.3 painful procedures each day (Harrison, et al., 2014). When these procedures occur without the proper pain management and support, there are long term impacts including increased stress during future procedures and the potential to develop needle phobia (Kennedy, Juhman, & Zempsky, 2008). Children who experience painful procedures during hospitalization feel less in control of their health and can develop medical fear and post-traumatic stress disorder (Rennick, Johnston, Dougherty, Platt, & Ritchie, 2002), thus impacting long term healthcare consumership. There is suggested correlation between painful pediatric procedures and pain sensitivity in adulthood, compounded with escalated fear and even avoidance of healthcare (Kennedy et al., 2008; Pate, Blount, Cohen, & Smith, 1996).

When a child is appropriately supported throughout medical procedures, the negative outcomes associated are minimized. Through the provision of procedural support, both perceived and measured pain, as well as distress are mitigated (Burns-Nader, Joe, & Pinion, 2017; Cristal et al., 2018; Gursky, Kestler, & Lewis, 2010; McCarthy et al., 2014; Ortiz et al., 2017; Piskorz & Czub, 2018). Studies have further demonstrated procedural support to be equal to or more effective than Midazolam in aiding children through the completion of procedures (Dastgheyb, Flishlock, Daskalakis, Kessel, & Rosen, 2018; Marechal et al., 2017; Seiden, 2014; Sola et al., 2017). Moreover, patient engagement in this support allows for more thorough wound care and has been connected to accelerated healing in burn patients (Brown et al., 2015).

Although medical team members frequently provide patients with procedural support, their ability and desire to provide this care varies across individuals and teams. Team members report a lack of training and time to devote to adequate support and pain management (Cramton, & Gruchala, 2012; Katende, & Mugabi, 2015). Child life specialists are trained team members who are experts in providing developmentally appropriate procedural support and advocating for appropriate pain management; they are uniquely positioned to provide this necessary support in an effective manner.
OBJECTIVES: Adolescence is a critical time for the preservation or loss of cardiovascular health. We aimed to describe trajectories of cardiovascular health in adolescent girls and identify early adolescent factors associated with cardiovascular health in young adulthood.

METHODS: We used data from the National Growth and Health Study, a longitudinal cohort of 2,379 girls followed annually from ages 9-19 years. We classified participants as having ideal, intermediate, or poor levels of seven cardiovascular health metrics at four developmental stages: early (ages 9-11), middle (ages 12-14), and late (ages 15-17) adolescence, and early young adulthood (ages ≥18). We calculated total cardiovascular health scores (range 0-14) at each stage and empirically identified patterns of cardiovascular health trajectories. We examined associations between trajectory group membership and a range of demographic, behavioral, and physiological factors.

RESULTS: Mean cardiovascular health scores declined with age from 10.8 to 9.4 in white girls and 10.3 to 8.9 in black girls; 17% of white girls and 23% of black girls had low cardiovascular health (score <8) by early young adulthood. We identified five cardiovascular health trajectories: high-stable (14% of participants), high-to-moderate (48%), high-to-low (20%), moderate-stable (10%), and moderate-to-low (8%). Exceeding 14 hours per week of television in early adolescence and teen pregnancy were associated with higher odds of being in several less healthy trajectory groups.

CONCLUSIONS: Cardiovascular health declines during adolescence and black-white disparities begin before early adolescence. Key targets for improving cardiovascular health in adolescent girls may include reductions in sedentary behavior and prevention of teen pregnancy.
Using 3D Imaging Technology to Improve Post-Mortem Anthropometric Measurements in a Pediatric Hospital Setting

Gupta, Priya; Klein, Jamie; Alexander, Eugene; Akelo, Victor; Addo, OYaw; Sivalogan, Kasthuri; Oliech, Richard; Gethi, Dickson; Tippett Barr, Beth; Blau, Dianna; and Suchdev, Parminder

Corresponding Author: Priya Gupta, MPH, Emory University, priya.mehta.gupta@gmail.com
Center: Clinical Outcomes Research and Public Health (CORPH)
Type: Technology
Keyword(s): Malnutrition, Anthropometry
Related to Pilot Grant or Trainee Award: No
Poster Available: No

The Child Health and Mortality Prevention Surveillance Network (CHAMPS) aims to identify causes of under-5 mortality in sub-Saharan African and South Asian surveillance sites. To address challenges in postmortem nutritional assessment, we evaluated the feasibility and accuracy of 3D imaging compared to high quality standard anthropometry in the CHAMPS Kenya site and within the pediatric morgue at Egleston Hospital, Children’s Healthcare of Atlanta.

Staff were trained using World Health Organization (WHO) recommended standard anthropometry equipment as well as 3D imaging to collect postmortem measurements (length, weight, head circumference, mid-upper arm circumference (MUAC)). Following the training, 76 cases from the Kenya site were measured in duplicate using standard anthropometry and 3D imaging, and 3 cases have been measured in Atlanta to date. Outcomes included data quality metrics (digit preference), measurement reliability (technical errors of measurement, TEM), and accuracy (correlation coefficients and Bland Altman plots of standard vs. 3D scan measurements). Malnutrition was defined as weight-for-length z score <-2 or MUAC < 12.5 cm.

In both sites, standard anthropometry showed high data quality as indicated by no digit preference, Reliability of length measurements in both sites was high as indicated by low relative TEM of 0.53% in Kenya and 0% in Atlanta. Accuracy of 3D imaging was high (R=0.99 in Kenya; R=1.00 in Atlanta) comparing standard anthropometry vs. 3D imaging for length; however, examination of data from Kenya using Bland Altman plots revealed that on average 3D scans overestimated length by 3.87 centimeters. Additional results from the Atlanta site are still pending, however preliminary results from both sites suggest malnutrition is prevalent.

With proper training, standard anthropometry can provide high quality assessment of post-mortem nutritional status. 3D imaging may be an accurate alternative to standard anthropometry, but adjustment of the technology is needed to avoid overestimation of length. Future research on the appropriate use of reference standards to define malnutrition in clinically ill populations is warranted.
An Environmental Scan of Human Papillomavirus Vaccination in the State of Georgia

King, Adrian; Gullatte, Maryl; and Bednarczyk, Robert

Corresponding Author: Adrian King, MPH, Emory University, adrian.king@emory.edu
Center: Clinical Outcomes Research and Public Health (CORPH)
Type: Basic
Keyword(s): HPV Vaccine, Environmental Scan
Related to Pilot Grant or Trainee Award: No
Poster Available: Yes - P25

BACKGROUND: Low Human Papillomavirus (HPV) vaccine uptake was identified as a priority in the 2014-2019 Georgia Cancer Plan. In the 2018 National Immunization Survey-Teen (NIS-Teen), it was estimated that only 68.1% of Georgia adolescents initiated the HPV vaccine series with only 49.6% of adolescents up-to-date for the vaccination series. Georgia’s diverse population illuminates a great need for state-specific research to identify effective strategies to improve HPV vaccination uptake in Georgia.

METHODS: We conducted an environmental scan of the factors influencing HPV vaccination uptake and completion across the state of Georgia using a variety of qualitative and quantitative methods. Main activities included: (1) Conducting a systematic review of uptake, promotion, and coordination of HPV vaccination specific to the state of Georgia; (2) Assessing adolescent vaccine coverage through analysis of Georgia Registry of Immunization Transactions and Services (GRITS) immunization records; (3) Conducting 23 focus group discussions with stakeholders involved in vaccination promotion and decision making (e.g., caregivers of adolescents, healthcare providers, community/religious leaders, Georgia HPV Working Group); and (4) Surveying 700 parents of adolescents across the state of Georgia on adolescent vaccine knowledge, attitudes, and practices.

RESULTS: Analysis and dissemination of results from this Environmental Scan are ongoing. To date, we have published findings from our systematic review of HPV-related research in Georgia and our assessment of the Georgia Cancer Control Consortium (GC3) HPV Working Group. Four additional manuscripts from the focus group discussions are under review, with four in development, examining the facilitators and barriers to HPV vaccination among caregivers of adolescents, healthcare providers, and religious communities. Analysis of GRITS data, on over 2 million unique individuals, is ongoing, and two manuscripts based on the parental survey are in development. Our findings on the need for more Georgia-specific information led to the development of the hpvcancerfreega.org website.

DISCUSSION: Addressing subnational-level vaccine uptake data, alongside analysis of facilitator and barriers to vaccine uptake, is essential to improving HPV vaccination rates. Our findings, and ongoing analyses, will continue to support research on, and implementation of, best practices to improve HPV vaccine coverage in Georgia.
Healthcare Providers’ Strategies to Promote HPV Vaccination in the State of Georgia

King, Adrian; Vu, Milkie; and Bednarczyk, Robert

**BACKGROUND:** Limited literature has examined strategies used by providers in Georgia to promote HPV vaccination. We explore current HPV vaccine discussion and recommendation practices among healthcare providers as well as providers’ perceptions of how different strategies may encourage or dissuade parents’ HPV vaccine uptake for their children. Such an understanding is critical for identifying effective recommendation and promotion strategies for HPV vaccination.

**METHODS:** We conducted six focus group discussions (FGDs) with healthcare providers throughout Georgia. We sought to develop an understanding of healthcare provider recommendation and promotion strategies for HPV vaccination and to understand how these strategies may motivate or dissuade vaccine uptake within Georgia. All FGDs were audio-recorded and verbatim transcribed. We created a codebook of both inductive and deductive codes, which the research team used to organize and analyze data. We conducted a thematic analysis to uncover common issues and themes across the data. Data analysis focused on understanding vaccination promotion strategies from providers’ perspectives.

**RESULTS:** Findings fell within three domains: strategies and best practices, sharing HPV vaccine-related knowledge, and adolescent engagement. Providers emphasized that their most effective HPV vaccine recommendation strategy was to emphasize the protective value of vaccination and the potential consequences of non-vaccination. Providers felt it was critical to recognize the views and opinions of caregivers and patients and to work alongside these in a partnership to provide resources and information to achieve vaccination uptake. Providers also believed that it was critical to involve adolescents in parts of the decision-making process related to HPV vaccination uptake as, oftentimes, adolescents were found to understand the importance of the vaccination more than their parents or caregivers.

**DISCUSSION:** Increasing HPV vaccination uptake in Georgia requires specific and targeted strategies which are attentive to the needs, views, and opinions of caregivers and patients. Persistence and repetition of HPV vaccination recommendations and provision of information as well as engagement of adolescents could be effective methods to increase vaccine uptake. The collaboration of healthcare staff and community-based organizations in developing interventions and educational tools can ensure the creation of culturally competent and comprehensive tools and interventions.
Implementation of a How-to Guide for Updating Pediatric Practice Websites With Vaccine Content: A Georgia Based Pilot Project

Meza, Cristina; King, Adrian; Orenstein, Walter; Bednarczyk, Robert; and Chamberlain, Allison

Corresponding Author: Adrian King, MPH, Emory University, adrian.king@emory.edu
Center: Clinical Outcomes Research and Public Health (CORPH)
Type: Basic
Keyword(s): Pediatrics, Website, How to Guide
Related to Pilot Grant or Trainee Award: No
Poster Available: Yes - P27

BACKGROUND: Vaccine hesitancy has been increasing in the U.S in recent years. While pediatricians are generally a parent’s most trusted source of vaccine-related information, little is known about the usefulness of pediatric practice websites in providing vaccine-specific information to parents. The aim of this pilot study was to assess the general acceptability and utility of a “how-to guide” developed to assist pediatric practices with thoughtfully incorporating evidence-based vaccine-related content into their existing practice websites.

METHODS: We conducted six qualitative focus group discussions (FGDs) and six in-depth interviews (IDIs) with 35 total healthcare providers (e.g. nurse, pediatrician, practice manager) along with observations of participating practice websites. Participating practices were located in metro-Atlanta and varied by practice size (e.g. number of physicians, patient population). Thematic analyses of FGDs, IDIs, and practice website updates were conducted to identify themes related to the usability and usefulness of the how-to-guide, frequently encountered vaccine-related questions and concerns, and providers’ perceptions of vaccine-related messages they most want to convey to their patient populations.

RESULTS: Healthcare providers believed that presenting vaccine-specific information on their practice website would be well-received by parents. The parental concerns most frequently encountered by providers related to the number of vaccinations given at once (and related questions about alternate schedules), vaccine ingredients, specific concerns about human papillomavirus and influenza vaccines, and vaccine side effects. Participants felt the guide was easy to use and that the section devoted to appealing to patients’ core values when crafting answers to commonly asked questions would be most useful. Bi-weekly check-ins with participating practices are ongoing and all website updates are expected to be complete by May 16, 2020.

DISCUSSION: Preliminary findings support the acceptability and usability of the vaccine-related how-to guide for pediatric practices interested in more effectively using their websites to communicate vaccine-related information to their specific patient populations. Broader evaluation of the guide’s implementation including its impact on parent vaccine-related attitudes and decisions is needed.
Improving Inpatient Pediatric Influenza Vaccination Using a Clinical Decision Support Intervention

Elsayed-Ali, Omar; Kandaswamy, Swaminathan; Shane, Andrea; Jernigan, Stephanie; Lantis, Patricia; Masterson, Erin; Shah, Pareen; Blanco, Reena; Iyer, Srikant; and Orenstein, Evan

Corresponding Author: Erin Masterson, MPH, Children’s Healthcare of Atlanta, erin.masterson@choa.org
Center: Clinical Outcomes Research and Public Health (CORPH)
Type: Outcomes
Keyword(s): Immunization delivery, Implementation science
Related to Pilot Grant or Trainee Award: No
Poster Available: No

BACKGROUND: The pediatric population is at high risk of morbidity and mortality from influenza, yet pediatric influenza vaccination rates remain <50% in the US. Additionally, a majority of children experience missed outpatient opportunities for immunization. Inpatient settings represent an additional opportunity for providing seasonal influenza immunizations to eligible children. Four prior studies have shown promise in improving inpatient pediatric influenza vaccination. However, these studies had limited effect sizes and included interventions requiring ongoing maintenance with dedicated staff. OBJECTIVE: We hypothesized that a clinical decision support (CDS) intervention designed with user-centered design principles would improve inpatient influenza vaccine administration rates at a metropolitan children’s healthcare system.

DESIGN/METHODS: Through formative usability testing with front-line clinicians, we developed an order set module containing a default influenza vaccine order. This module was dynamically incorporated into admission order sets for pediatric patients meeting the following eligibility criteria: ≥6 months old, no prior documented influenza vaccine in the current season in our health system or the state immunization registry, and no anaphylaxis to a prior influenza vaccine. We implemented the CDS into select order sets, sequentially expanding based on operational leader support. Our intervention group is defined as patients for whom a clinician used an order set including our CDS at the time of admission. We compared the proportion of eligible patients to which influenza vaccine was administered during our 2019-20 intervention period (9/19/2019 – 12/6/2019) and the 2018-2019 season (historical controls). To account for secular trends, we also compared the vaccine administration rates for our intervention group to those whose admission order sets did not incorporate the CDS during the 2019-20 season (concurrent controls).

RESULTS: During the intervention period, influenza vaccine was administered to 1402/4815 (27%) of eligible patients, compared to 596/5302 (11%) among historical controls admitted during the same time period ($\chi^2(1) = 507$, $p< 0.00001$). Among the intervention group, vaccination rates were 37% compared to 18% for concurrent controls ($\chi^2(1) = 195$, $p< 0.00001$). CONCLUSION: A CDS intervention targeting influenza vaccine eligible patients incorporated into a pediatric admission order set, doubled seasonal influenza vaccine uptake during inpatient healthcare encounters in a metropolitan children’s healthcare system.
Does Race Moderate the Association Between Parent Strategies for Managing Children’s Violent Media Exposure and Child Anxiety?

Ortiz, Hillary; Ronkin, Emily; Murphy, Katie; McQuarrie, Susanna; and Tone, Erin B.

Corresponding Author: Hillary Ortiz, BS, Georgia State University, hillaryortiz20@yahoo.com
Center: Clinical Outcomes Research and Public Health (CORPH)
Type: Basic
Keyword(s): Race, parenting
Related to Pilot Grant or Trainee Award: No
Poster Available: Yes - P28

Children often report anxiety after exposure to violent media. Parents can modulate their child’s anxiety through behavioral strategies (e.g., restricting violent media, supervising/watching together, parent/child discussion) (Padilla-Walker & Coyne, 2011; Valkenburg et al., 1999), but effective strategies may vary for members of different demographic groups. In this study, we examined whether race moderates the association between child anxiety and parent use of common strategies for responding to children’s violent media exposure (McQuarrie & Caporino, 2017).

We present findings regarding associations between self-reported parent behavior and child anxiety from two studies. In study one, 516 white and 56 black primary caregivers of children aged 6-17 recruited through the Amazon Mechanical Turk website completed the Caregiver Responses to Youth Media Exposure questionnaire (CRYME; McQuarrie & Caporino, 2018) assessing their behavior in response to their child’s violent media exposure and the Screen for Child Anxiety Related Disorders (SCARED; Birmaher et al., 1997; Birmaher et al., 1999) reporting their child’s anxiety. Study two focused on 66 black mothers (with children aged 8-13 years) with histories of exposure to traumatic events. These mothers completed the CRYME (McQuarrie & Caporino, 2018) and the Behavior Assessment System for Children (BASC; REF).

We found that Black caregivers used Scaring for Safety more often than White caregivers, and that use of this behavior correlated with lower levels of anxiety in their children \( t(568) = 2.69, p < .05 \). For White caregivers, Scaring for Safety showed a quadratic association with child anxiety (both low and high levels of Scaring for Safety were associated with higher levels of anxiety), which may suggest that a moderate use of Scaring for Safety is adaptive. These findings support the idea that parenting strategies/behaviors relate to child anxiety following exposure to violent media and that this association might vary by race. Future studies should investigate diverse participants and examine how culture, in combination with race, might relate to parenting strategies.
Evaluation of Health Literacy and Quality of Life in Pediatric-Onset Systemic Lupus Erythematosus Patients and the Effects on Healthcare Utilization

INTRODUCTION: Systemic Lupus Erythematosus (SLE) affects people of all ages and races, but minority and ethnic groups are disproportionately affected. An adequate functional health literacy level (HLL) is crucial to comprehend information and develop lifelong behaviors ultimately needed for favorable long-term outcomes. In SLE, health literacy may be an important underlying factor in further widening the gap in healthcare disparities. Additionally, achieving favorable long-term outcomes in a disease process that has a severe impact on quality of life can prove to be challenging. To date, there are limited studies in pediatric-onset SLE (p-SLE) patients assessing health literacy and quality of life as well as the effects from both on clinical and patient perceived outcomes.

METHOD: Currently we have enrolled 56 p-SLE patients (8-19 years old) at Children's Healthcare of Atlanta in Atlanta, GA. Demographic information and medical history were collected at clinic visits and/or hospitalizations. Health and numeracy literacy were assessed using the validated Short Test of Functional Health Literacy (S-TOFHLA) and the Newest Vital Signs (NVS). Validated Pediatric Quality of Life survey (PedsQL) and Simple Measure of Impact of Lupus Erythematosus in Youngsters (SMILEY) were used to assess quality of life. Fisher's exact and Wilcoxon rank sum were used to analyze data.

RESULTS: In the completed cohort - 86% were female, 45% were Black, and 32% identified as Hispanic-White. Median age at diagnosis was 13.5 years. 100% scored adequate on S-TOFHLA while 64% scored in the limited/inadequate range on NVS. There was a significant difference in hospital utilization rates between adequate and limited/inadequate groups.

DISCUSSION: Although 100% scored adequate on S-TOFHLA, the majority scored in the limited/inadequate range for numeracy skills. This could be problematic given that SLE patients, particularly ones that have renal involvement, are responsible for taking multiple prescriptions and comprehending food labels. There is a need for non-standard methods, such as visual aides, videos, or resources available on various social media avenues, to educate patients based on their HLL. Of equal importance, a patient can be clinically doing well, but perception of quality of life can affect patient perceived outcomes.
Examining the Association Between Neighborhood Safety, Crime, and Obesity in Adolescents

Patterson, Sierra and Suglia, Shakira

Corresponding Author: Sierra Patterson, MPH, Emory University, slpatt2@emory.edu
Center: Clinical Outcomes Research and Public Health (CORPH)
Type: Outcomes
Keyword(s): Obesity, Neighborhood Violence
Related to Pilot Grant or Trainee Award: No
Poster Available: No

BACKGROUND: Childhood obesity is highly prevalent in the United States (US) and is a growing public health concern. While studies have investigated several features of the neighborhood social environment and their relation to obesity, few have explored neighborhood violence including safety and crime, particularly in adolescents.

OBJECTIVE: To examine the relationship between perceived neighborhood safety and crime and obesity among adolescents.

METHODS: We used data from the Fragile Families and Child Wellbeing Study, a prospective cohort study following 4898 children and parents from 20 large US cities. Using logistic regression, we examined overall associations between perceived neighborhood safety and crime and obesity, while controlling for race and ethnicity, poverty category, physical activity, and gender. We conducted separate logistic regression analyses among boys and girls to examine gender as a potential modifier of these associations.

RESULTS: Adolescent participants were more likely to report feeling unsafe in their neighborhoods at night (53%) rather than during the day (25%). Additionally, 34% of participants reported that they had witnessed or known about a crime in their neighborhood. Perceived neighborhood safety and crime was not significantly associated with the overall prevalence of obesity. However, in girls there was a significant association between crime and obesity (PR: 1.28, 95% CI: 1.03-1.59), but the same association was not seen in boys.

CONCLUSION: These results suggest that gender modifies the relationship between neighborhood crime and obesity. Future studies investigating potential mediators such as sleep and dietary patterns may better explain the relationship between neighborhood violence and obesity.
OBJECTIVE: Adolescent and young adult women are rarely the target population of cardiovascular disease (CVD) prevention campaigns. The purpose of this study is to evaluate how this population sources cardiovascular health information.

METHODS: We surveyed 331 females between 15-24 years of age to determine 1) exposure to sources of CVD information over the past 12 months and 2) whether participants felt informed regarding CVD or stroke. Secondary outcomes of interest included information source preferences and motivations to seek information on CVD risk. We assessed for associations between exposure to sources of CVD information and being informed about CVD or stroke, while adjusting for participant demographics (age, race, ethnicity, caregiver education).

RESULTS: Participants reported being exposed to CVD information through various sources including TV (38%), social media (30%), and healthcare professionals (19%); however, 28% of participants reported that they had not been exposed to any sources of information. Approximately 49% and 59% of patients reported that they were not at all informed about CVD and stroke, respectively. There was a positive association between being exposed to information sources and feeling informed about CVD and stroke. Participants who reported that they had not been exposed to any sources of CVD information were less likely to feel informed about CVD (OR: 0.10, 95% CI: 0.05-0.22) and stroke (OR: 0.37, 95% CI: 0.19-0.72). Half of the participants chose assorted media (social media, ads, etc.) as their preferred method of receiving CVD information, while 42% chose healthcare providers as their preferred method. Many participants reported family (64%) as a motivating factor to become more informed about CVD, followed by healthcare professionals (61%) and friends (48%).

CONCLUSIONS: Less than half of adolescent and young women feel informed about CVD and stroke, which remains the greatest lifetime health risk for women in the United States. Public health campaigns directed at youth should focus on promoting cardiovascular health and wellness and use sources frequently accessed by adolescents and young adults. Health care providers should be encouraged to discuss cardiovascular disease risk factor modification from a young age in order to promote cardiovascular health across the life course.
Impact of Acute Pancreatitis Order Set Implementation on Pediatric Clinical Outcomes

Shah, Meera; Leong, Traci; and Freeman, A. Jay

Corresponding Author: Meera Shah, MD, MPH, Emory University, meera.shah@emory.edu
Center: Clinical Outcomes Research and Public Health (CORPH)
Type: Outcomes
Keyword(s): Pediatric Pancreatitis
Related to Pilot Grant or Trainee Award: No
Poster Available: Yes - P29

BACKGROUND: The management of acute pancreatitis (AP) in children was previously derived from adult practice. Pediatric-specific guidelines for treatment of AP were recently developed but their impact on clinical outcomes has yet to be evaluated. We developed an AP order set based on these guidelines to assess the impact of these new recommendations on clinical outcomes.

METHODS: Patients admitted to Children’s Healthcare of Atlanta for isolated AP were included in a retrospective review. Patient demographic data, order set utilization, treatment variables (e.g., fluid type and rate, type and timing of initiation of a diet, and narcotic use), and outcome variables (e.g., length of stay (LOS), PICU admission and 30-day readmission) were collected. Mixed effects modeling was used to determine the impact of the implementation of the order set on clinical outcomes.

RESULTS: Between 2017-2018, 175 pancreatitis encounters met inclusion criteria. After implementation of the order set on Jan. 1, 2018, there was a 20.7% decrease in median LOS between 2017 and 2018, p=0.02. Although there was no difference in the number of patients who received at least one dose of narcotics, among the 119 patients who received some narcotic, there were significantly more doses prescribed in 2017 than in 2018 (p=0.03). Hospital readmission rates in the subsequent 30 days (p=0.13) and PICU admission rates (p=0.68) were not different after order set implementation.

CONCLUSIONS: The implementation of a pancreatitis order set demonstrated decreased LOS and total narcotic use in pediatric patients with AP without increasing readmission rates or PICU admissions. Order sets based on clinical guidelines may positively impact clinical outcomes in other pediatric disorders.
Impact of Computer-Based Augmentative and Alternative Communication Education for Pediatric Acute Care Nurses

Simmons, Amanda; McCarthy, Jillian; and Koszalinski, Rebecca

| Corresponding Author: | Amanda Simmons, MS, University of Tennessee Health Science Center, amcroycn@uthsc.edu |
| Center: | Clinical Outcomes Research and Public Health (CORPH) |
| Type: | Clinical or Translational |
| Keyword(s): | Pediatric nurse education, augmentative and alternative communication |
| Related to Pilot Grant or Trainee Award: | No |
| Poster Available: | No |

Nurses, the primary point of contact during hospitalization, are common communication partners. Nurses report limited ability to troubleshoot communication breakdowns during communication interactions to meet patients’ needs. We enlisted a four-part computer-based learning program for nurses, nursing students, and speech-language pathology students (control group). Outcomes include increased knowledge on basic aspects of communication and AAC as well as perceived relevance of the content and effectiveness of the education method.

Data collection was completed on May 15, 2019, with data indicating knowledge gains, minimally increased comfort using AAC, and but positively perceived relevance for nurses following their participation in the four-part CBL series. Nurses demonstrated gains in education pre- to post-test in knowledge gains following the short education modules. In addition, measures of perceived relevance of AAC in general, and specifically low-tech, mid-tech and high-tech AAC were found to improve from pre- to post-test. Finally, nurses reported that they were more likely to attempt to use AAC following completion of education modules. There are clear clinical implications for the implementation of education on AAC and communication supports for pediatric acute care nurses to improve healthcare provision and quality of life for patients.

Current ongoing research includes focus group discussions with pediatric acute care nurses to create succinct CBL content. These education modules will be piloted to determine if new CBLs will provide more efficient education.
Qualitative Analysis of the Usability of Teen Well-Check: A Tablet-Based Program for Substance Use, Sexual Assault, and Sexual Risk Behaviors

Umo, Idara; Mekonnen, Mahider; Leone, Ruschelle M.; Wallis, Elizabeth; Patterson, Sierra; Gooding, Holly; Danielson, Carla; Self-Brown, Shannon; and Gilmore, Amanda K.

Corresponding Author: Idara Umo, MPH, Georgia State University, iumo1@student.gsu.edu
Center: Clinical Outcomes Research and Public Health (CORPH)
Type: Clinical or Translational
Keyword(s): Technology-based program
Related to Pilot Grant or Trainee Award: No
Poster Available: No

Previous research has shown that adolescent drug and alcohol use is common and associated with sexual risk behaviors, including sexual assault. Primary care centers are promising sites for preventing substance use and sexual risk. In order to prevent adolescent teen drug and alcohol use, researchers have suggested targeting common settings for adolescents to seek and receive preventative healthcare, such as primary care centers. Thus, this study assesses the usability of an intervention program, Teen Well-Check, that targets adolescents in primary care settings to prevent drug and alcohol use, sexual assault, and/or sexual risk behavior. We recruited nine adolescents (and ongoing) ages 14-18 from the Medical University of South Carolina, a pediatric primary care center. Participants were first screened, asked to complete a survey that evaluated their risk of sexual behavior and drug use, then viewed Teen Well Check and provided feedback in an interview that was audio-recorded, transcribed, and coded for themes using NVivo software. Preliminary analyses found that 80% of participants had positive reactions to content aimed to educate adolescents on effects of drugs on the brain, 72% found the content aimed at educating adolescents on sexual risk prevention to be effective, and 60% of participants found the portion containing information on the effects of substance use and sexual consent to be helpful. In regards to future improvements, 92% suggested increased visual components, and 92% suggested the addition of multiple sexual assault scenarios using comics. These findings suggest that teens find Teen Well Check usable. After further testing of this intervention in future trials, the goal is to have an effective, highly disseminable prevention program for teens.
Attention-Deficit Hyperactivity Disorder (ADHD) affects approximately 7% of children in the United States. Children with ADHD exhibit inattention, hyperactivity, and impulsivity features that can impact their academic, personal, and occupational lives through adulthood. While several pharmacologic treatments are currently available, there are known long-term health risks to prolonged use of methylphenidate and amphetamines and additional research is needed to identify other therapeutic and preventative strategies. Several epidemiologic studies of ADHD identify environmental exposures that could increase the susceptibility of certain groups to ADHD. In the National Health and Nutrition Examination Survey (NHANES), our group and others have shown, for example, that exposure to currently used pyrethroid insecticides, measured via urinary pyrethroid metabolite 3-phenoxybenzoic acid (3-PBA), increases ADHD prevalence. Pyrethroid insecticides are considered safe alternatives to phased-out insecticides including organophosphates, such as DDT and chlorpyrifos, though a large body of epidemiologic, animal model, and in vitro work illustrates that they have neurotoxic effects. Early life stress and allostatic load, a measure of how chronic stress affects the body, are associated with additional neurodevelopmental disorders as well. In rodent models, chronic stress leads to diverse neurodevelopmental alterations, including ADHD-like behaviors such as hyperactivity and inattention. Thus, complex and cumulative chemical and psychosocial factors have been shown to increase the risk of ADHD in children. Utilizing NHANES, we examined whether pyrethroid insecticide and increased stress exposure would increase prevalence of ADHD in children ages 6-18 years living in the United States. To more comprehensively model stress exposure, we developed a novel Allostatic Load Index that encompasses both biological markers of stress reactivity and sociodemographic factors known to increase psychosocial stress. We found that 3-phenoxybenzoic acid (3-PBA) pyrethroid metabolites in the urine were associated with increased prevalence of ADHD, as well as increased allostatic load. Additionally, the presence of 3-PBA above the limit of detection (LOD) multiplicatively interacts with allostatic load score to increase prevalence of ADHD. This is the first description of a pediatric allostatic load score in the NHANES dataset and provides a more inclusive understanding of the role of the exposome on children’s health.
BACKGROUND: Necrotizing enterocolitis (NEC) is a major driver of morbidity and mortality in the NICU and accounts for 10% of all deaths. While recent data suggest the incidence of NEC is decreasing in the United States (US), a national view of NEC-related infant mortality and its racial and geographic disparities is lacking.

OBJECTIVE: This study characterizes the infant mortality rates (IMR) from NEC in the US from 1999-2017 along with the racial disparities and the geographic differences in NEC-related infant mortality by state and by US census region. DESIGN/METHODS: We analyzed data from the CDC’s Multiple Cause of Death File from 1999-2017. IMR up to 1 year of age were calculated per 100,000 live births. Only deaths with the underlying cause of death listed as necrotizing enterocolitis of newborn (ICD-10 P77) were evaluated. Using Joinpoint regression & SPSS statistical software, we examined NEC-related IMR by year as well as racial (Black vs. White) & geographic differences in NEC-related infant mortality by state. Data based on fewer than 10 deaths were excluded.

RESULTS: From 1997-2017, 7,998 infants died from NEC among 75,537,486 live births. The NEC IMR per 100,000 live births was 8.6 (95% CI 7.7-9.5) in 2017 (n=338 deaths) and declined by 4.3% per year (95% CI 3.1-5.4) from a peak incidence of 13.6 (95% CI 12.5-14.8) in 2005. NEC IMR trends for Black infants differed from White infants, with no improvements in mortality since 2012 for Black infants. The South had the highest NEC IMR and the West the lowest, although similar trends in IMR over time were observed across regions. NEC-related IMR by US state demonstrated a different pattern than ratio of Black-to-White IMR, which ranged from 0.97-6.05. Conclusions: Infant mortality due to NEC has declined in the US since 2006. Despite this, significant geographic differences in deaths from NEC exist, with a wide range of Black-White disparity across states. Improvements in NEC IMR among Black infants plateaued after 2012. These data may support clinicians, patient-families & policy makers in understanding national trends in NEC to inform care practices and policies to address these disparities.
Demonstrating Our Worth: Utilizing a Value Proposition Statement as an Evidence-Based Platform for the Integration of Child Life Services

Woodburn, Ashlie; Fraser, Camille; and Hoskins, Katy

Positive outcomes associated with the work of Certified Child Life Specialists have been well documented across the literature (Diener, Lofgren, Isabella, Magana, Choi & Gourley, 2018; Mastro, Flynn, Millar, DiMartino, Ryan, & Stein, 2019; Sanchez-Cristal, Staab, Chatham, Ryan, McNair, & Grubenhoff, 2018; Wong et al., 2018). However, there has yet to be a systematic effort to strategically compile and mobilize these findings to empirically validate child life as a profession and a distinct field of research and practice. To address this need, presenters secured a multi-organizational grant to create a value proposition statement illustrating the evidence-based argument for child life services for youth, families, and organizations. Combining research evidence with persuasive, buyer-driven rhetoric, a value proposition statement offers a comprehensive platform for advocacy based on scientific results, grounded in the realities of clinical practice, and demonstrative of the multifold impacts of the profession for individuals and institutions. More than 5,000 articles were reviewed utilizing the Johns Hopkins Evidence-Based Practice Rating Scale (Dang & Dearholt, 2017) to standardize appraisal; team members tracked content and summarized the information found within each topic to include key findings and high-impact sources. Following the exhaustive literature review, 408 high-quality articles were selected to demonstrate the five key outcomes of child life services: 1) Driving positive and effective outcomes for healthcare organizations by optimizing the use of resources and limiting waste 2) Generating positive behavioral, psychosocial, and physiological outcomes through individualized interventions with pediatric patients 3) Empowering children and families to become informed and active participants in their healthcare experiences 4) Promoting and sustaining development and psychosocial growth from infancy through emerging adulthood and 5) Improving population health by fostering long-term patterns of healthcare consumership that reduce the risk of preventable conditions (Boles et al., 2020). This value proposition statement has been released internationally in multiple formats. It is oriented towards hospital administration, thus child life specialists are mobilizing the statement to advocate for pay and staffing increases, as well as service expansion. It is the hope of presenters that this document will increase the integration of evidence-based patient- and family-centered care practices across institutions.
Knowledge Is Power: Preparation as a Means of Optimizing Procedural Outcomes for Children, Families, and Institutions

Woodburn, Ashlie; Fraser, Camille; and Hoskins, Katy

Corresponding Author: Ashlie Woodburn, Vanderbilt University Medical Center, ashlie.woodburn@vumc.org
Center: Clinical Outcomes Research and Public Health (CORPH)
Type: Outcomes
Keyword(s): preparation, communication
Related to Pilot Grant or Trainee Award: No
Poster Available: No

It is well documented that hospitalization and medical procedures can have a significant negative impact on the psychosocial wellbeing of children and their caregivers by increasing experience of anxiety and distress. Children, regardless of previous medical experience, indicate wanting information about upcoming medical procedures (Fortier et al., 2009). Preparation is meant to inform children and families about upcoming medical experiences; this is done via play and modelling, coping skills training, provision of procedural and sensory information, and fostering emotional expression and trust (MacLaren & Kain, 2007). Children who received developmentally-appropriate preparation demonstrated lower anxiety scores in the pre- and post-operative periods, even when compared with children who received oral Versed or parental presence at induction (Cuzzocrea et al., 2013; Kain et al., 2007; Li & Lopez, 2008). Furthermore, parents report decreased anxiety and greater satisfaction with care when their child is prepared for medical procedures (Bartik & Tourner, 2017; Cuzzocera et al., 2013; Kain et al., 2007; Li & Lopez, 2008).

Fear and anxiety regarding medical procedures may increase the need for the use of sedation medications, thus heightening risk for medical complications, length of stay and staffing requirements for hospitals. Significantly lower procedural sedation rates occur following preparation by a child life specialist, even among young children who are typically automatically scheduled with sedation, thereby increasing patient safety (Carter, Greer, Gray, & Ware, 2010; Grissom et al., 2016; Khan et al., 2007; Scott et al., 2016). This research demonstrates a clear return on investment when considering reductions in anesthesia use as one MRI program reports savings of $117,870 following the implementation of a mock scanner preparation intervention and radiation-oncology savings have been reported up to $775,000 annually in one program with a child life specialist (McGuirt, 2016; Scott et al., 2016). Reductions in emergence delirium, pain medication consumption and faster PACU discharge were found to be the result of child life preparation interventions (Kain et al., 2007).

Attendees will gain knowledge of the benefits of preparation for children and families and explore basic principles of how to best communicate preparatory information with patients and families to optimize outcomes.
Bone loss in children is a difficult surgical problem for which there is no viable regenerative approach. Our group is focused on developing bone regenerative therapies and we recently described the requirement of TGFβR3, a receptor involved in TGFβ pathway signaling, during osteoblast lineage commitment. TGFβR3 human mutations are associated with reduced bone mineral density, making TGFβR3 a unique target for bone regenerative therapy. The hypothesis is that delivery of TGFβR3 will induce localized bone growth. Human bone derived osteoblast cells from pediatric fibular bones treated with soluble TGFβR3 (sR3) for 17 days demonstrated significative mineralization using Alizarin Red staining. In addition, osteogenic induction was recognized by induction of osteogenic genes like RUNX2, osteocalcin, osteopontin and osterix. Activation of AKT, ERK and p38 MAP kinases, non-canonical targets of BMP2 signaling, indicated that sR3 was able to induce bone formation in human osteoblast cells despite no observed effect on classical canonical targets like SMAD 1, 5, 9. Our results demonstrate an important role of sTGFβR3 in osteoblast induction of mineralization in pediatric bone cells. We propose that sR3 represents a potential therapeutic target for pediatric bone regeneration, occurring through a non-canonical bone pathway.
Bioprinted 3D Models for In Vitro Analysis and Treatment Planning in Pulmonary Artery Atresia

Bauser-Heaton, Holly; Tomov, Martin L; Do, Katherine; Cetnar, Alex; and Serpooshan, Vahid

Corresponding Author: Holly Bauser-Heaton, MD, PhD, Emory University, bauserh@kidsheart.com
Center: Heart Research and Outcomes Center (HeRO)
Type: Basic
Keyword(s): 3D Bioprinting, Cardiovascular Disease
Related to Pilot Grant or Trainee Award: No
Poster Available: No

Tetralogy of Fallot (TOF) with major aortopulmonary collateral arteries (MAPCAs) is a heterogeneous form of pulmonary artery (PA) stenosis which requires multiple surgical interventions. Here, we present an in vitro platform, capable of sustained homeostatic flow, that can be used to train proceduralists and surgical teams in current interventions, as well as in developing novel therapeutic approaches to treat various vascular anomalies. Further, our model can be cellularized, which allows for high-throughput data collection regarding flow dynamics, cell viability, and metabolite expression. Briefly, we developed a model of pulmonary artery stenosis, based on patient data, which can be used as an in vitro phantom to model cardiovascular disease and explore potential anastomosis interventions. Using patient scans obtained via computer tomography (CT) or 3D rotational angiography to produce high resolution digital models of the pathology, we generated in vitro models of TOF with MAPCAs, which were 3D printed using biocompatible resins, and then bioprinted using gelatin methacrylate (gelMA) hydrogel, to simulate either neonatal vasculature or second order branches in adolescent TOF patients. Printed models consisted of an occluded (atretic MAPCA) and an open (pulmonary artery) vessel with 2.2 mm lumen for both. The lumen diameter and the length of target vessels were kept within the range that was deemed suitable for clinical intervention. These hybrid phantoms of PAA pathology were subsequently employed to test a model vascular anastomosis (unifocalization) procedure to recanalize the atretic artery. While models like this have been done in synthetic materials, resin and thermoplastics could not adequately support phantom cellularization, specifically cellular remodeling as they lack the physiomechanical properties of native tissue microenvironment. Further, the demonstrated interventional procedure would not be possible in a synthetic model. GelMA, in contrast, has been repeatedly shown to mimic soft tissue stiffness and support cell attachment, function, and remodeling in cellularized constructs. Such vascular phantoms, based on clinical data and additive biomanufacturing, would allow for in vitro investigation of disease processes otherwise not possible due to patient and animal model variability, leading to improved patient outcomes and more faithful disease models for novel drug and/or procedure development.
INTRODUCTION: Cardiac arrest (CA) is a devastating event with high mortality. Of survivors, neurologic morbidity is associated with cerebral hypoxic-ischemic injury, highlighting the importance of monitoring "cerebral vital signs” post-CA, namely: cerebral blood flow (CBF), oxygen extraction fraction (OEF), and cerebral oxygen metabolism (CMRO2). We hypothesize that these cerebral vital signs are acute biomarkers predictive of survival in the first week post-CA. METHODS: This a pilot prospective observational study. Children <2 y who suffered an in- or out-of-hospital CA and received chest compressions for >2 min are eligible for participation. To quantify cerebral vital signs, we employ a novel non-invasive optical device that combines frequency domain near-infrared spectroscopy (fdNIRS) and diffuse correlation spectroscopy (DCS). Longitudinal measurements of CBF, OEF, and CMRO2 in the frontal cortex are made bilaterally once daily for 7 days after enrollment and mortality was assessed at 28 days post-CA. RESULTS: We report preliminary results from 5 subjects (4 male; mean age: 3.2mo) who experienced CA (1 out-of-hospital arrest; OHCA) with average CPR time of ~20 min and 40% survival (N=2). Subjects presented with highly variable prior medical histories associated with congenital malformations including atrioventricular septal defects, Tetralogy of Fallot, tracheomalacia with RSV bronchiolitis, truncus arteriosus, and a previously healthy OHCA patient. Analysis of cerebral vital time courses revealed the following qualitative insights: In all patients, CBF trended lower than literature-reported normal values post-CA and either low CBF immediately post-CA or non-recovering CBF was weakly associated with mortality. Elevated OEF (>60%) was strongly associated with mortality and may indicate the most prognostically meaningful cerebral vital sign, an insight consistent in other disease-states. CMRO2 exhibited variable tends among subjects suggesting there is no clear association between CMRO2 and mortality. CONCLUSIONS: Herein we employ two novel, non-invasive, bedside optical techniques to longitudinally monitor CBF, OEF, and CMRO2 after cardiac arrest. Although an exploratory pilot cohort, initial results suggest CBF is reduced post-CA and mortality may be strongly associated with higher OEF or weakly associated with limited recovery of CBF. Recruitment is ongoing to confirm these findings and further quantitatively determine the prognostic potential of these cerebral hemodynamic biomarkers post-CA.
N-terminal-pro-B-type Natriuretic Peptide as a Screening Tool for Pulmonary Hypertension in the Pediatric Population

Dasgupta, Soham; Bettermann, Erika; Kelleman, Michael; Kanaan, Usama; Sachdeva, Ritu; and Bauser-Heaton, Holly

Background: Although cardiac catheterization (cath) is the gold standard for the diagnosis of pulmonary hypertension (PH), echocardiogram (echo) is a routinely used non-invasive tool. Prior studies have also suggested that PH leads to increased N-terminal-pro-B-type natriuretic peptide (NTproBNP). With the risks of an invasive procedure, there is a need for a reliable PH screening tool in the pediatric population. We hypothesized that NTproBNP may be such a tool.

Methods: In this prospective study funded by Pediatric Research Alliance, patients (0-18 years) were divided into PH (PH by echo) and Control group (ASD < 10 mm, PDA < 2 mm, Qp:Qs <2 by cath and patients s/p heart transplant undergoing routine biopsy). Patients with hemodynamically significant ASD/PDA were excluded. Echo and NTproBNP were obtained during cath under the same conditions.

Results: Thirty-one patients met inclusion criteria (10 PH, 21 Control). Median NTproBNP was significantly higher in the PH group (462.5 pg/ml vs 87 pg/ml; p=0.02). Echo parameters of right ventricular fractional area change, pulmonary artery acceleration time and tricuspid annular plane systolic excursion were significantly lower in the PH group. ROC analysis demonstrated an NTproBNP > 2458 pg/ml was 100% specific in predicting a mean pulmonary artery pressure > 25 mmHg during cath.

Conclusion: NTproBNP and certain echo parameters appear to be useful screening tools for PH in the pediatric population. This needs to be validated in studies with a larger patient population.
Anticancer therapies have significantly improved the outcomes of cancer treatment, however cardiac side effects of cancer therapies remain a big challenge. Different kinds of proteasome inhibitors (PIs) such as Carfilzomib (Cfz) have been approved for the treatment of blood-borne cancers. The potential cardiovascular toxicities following Cfz have been identified in several clinical settings. To find out if using human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) can recapitulate cardiotoxicity following Cfz treatment, we generated highly purified hiPSC-CMs. These cells were exposed to Cfz at various concentrations (0.001-10 µM) that overlapped the clinically relevant dose based on Cmax for Cfz (5.8 µM). We used fluorescence-based viability assay to measure the cell viability of hiPSC-CMs at 4-5th week post differentiation. The concentration-dependent increase in Cfz-mediated toxicity was correlated with morphological changes detected using phase-contrast microscopy upon 48 hours of Cfz treatment. Additionally, a similar trend of response was observed in three different hiPSC lines (SCVI 273, IMR-90, and 902) with a significant decrease in viability within the therapeutic range. Significant reduction in mitochondrial membrane potential and increased mitochondrial oxidative stress were detected following the exposure of the cells to Cfz at higher concentrations (1-10 µM) as measured by mean fluorescence index of TMRM (Tetramethylrhodamine Methyl Ester) and MitoSox using high-throughput Arrayscan. Additionally, a reduction in mitochondrial oxidative respiration rate (OCR) was characterized as an indicator of alteration in cellular energy following one-day exposure to Cfz treatment as detected using Seahorse XF analyzer. Our data suggest mitochondrial damage as the likely mechanism underlying the cardiotoxicity associated with Cfz therapy. Since chemotherapeutic drugs can also induce arrhythmias, we performed high-throughput assays to analyze alterations in cell contractility and Ca2+ signaling. Using video microscopy with motion vector analysis, we detected alterations in contractility properties post-Cfz treatment. In addition, we observed impaired calcium handling at the single-cell level following Cfz treatment, which possibly contributes to contractility dysfunction of the cells. Altogether these results show that hiPSC-CMs can be a tool for in vitro testing of cardiotoxicity induced by Cfz and potentially anticancer compounds progressing in drug development.
Beta-Blockers Reduce Exercise Induced Left Ventricular Outflow Tract Obstruction in Pediatric Hypertrophic Cardiomyopathy

Gaitonde, Mansi; Simpson, Megan; Wetzel, Martha; Border, William; Sachdeva, Ritu; Ferguson, Matthew E.; Stark, Megan; Simpson, Patricia; and Whitehill, Robert

Corresponding Author: Mansi Gaitonde, Emory University, gaitondem@kidsheart.com
Center: Heart Research and Outcomes Center (HeRO)
Type: Clinical or Translational
Keyword(s): beta-blocker, hypertrophic cardiomyopathy
Related to Pilot Grant or Trainee Award: No
Poster Available: Yes - P30

BACKGROUND: Inducible left ventricular outflow tract (LVOT) obstruction in patients with Hypertrophic Cardiomyopathy (HCM) can lead to functional and hemodynamic impairment during activity. In pediatric patients, beta-blockers have been used to improve heart failure symptoms. However, use of beta-blockers to reduce LVOT obstruction during exercise has not been studied. The aim of this study was to assess whether beta-blockers can lessen inducible LVOT obstruction during exercise in pediatric patients with HCM.

METHODS: We reviewed records of patients < 22 years with a diagnosis of HCM who underwent an exercise stress echocardiogram from Jan 2009 – Dec 2019 at our center. Patients were included if they were treated with beta blocker therapy and underwent a staged exercise stress echocardiogram both before and after initiation of beta-blocker therapy. Demographics, clinical status, and echocardiographic indices were assessed. LVOT gradients were evaluated at rest, stage 1 of exercise, and peak exercise both before and after beta-blocker therapy. Statistical significance was assessed using Wilcoxon signed rank tests with p-value set at <0.05.

RESULTS: Thirteen patients met inclusion criteria. Of these, 5 were gene positive (38.5%), and 1 had a history of aborted sudden cardiac arrest (7.7%). The median time between the exercise stress echocardiograms done before and after initiation of beta-blocker therapy was 237 days. At rest, the median LVOT gradient before beta-blocker therapy was 21 mmHg. At maximal exercise, the median LVOT gradient improved from 198 mmHg before beta-blocker therapy to 66 mmHg following beta-blocker therapy (p < 0.001). There was also a statistically significant decline in LVOT gradient at stage 1 of exercise from 55 mmHg before beta-blocker therapy to 22 mmHg following beta-blocker therapy (p=0.008).

CONCLUSION: Pediatric HCM patients who develop elevated LVOT gradients during exercise have a reduction in inducible LVOT gradients with beta blocker therapy. These improvements are seen in the submaximal and peak stages of exercise. Future studies should examine whether this reduction in exercise gradient translates into more favorable outcomes.
Mitochondrial Transcription Factor A (TFAM): New Insights Into Its Role in the Murine Adult Heart

Ghazal, Nasab; Peoples, Jessica N.; Mohuiddine, Daisy; Faundez, Victor and Kwong, Jennifer Q.

Mitochondria are the energy producing organelles that generate ATP through oxidative phosphorylation (OXPHOS) via the electron transport chain (ETC). Because mitochondria are under the regulation of both the nuclear and mitochondrial genomes, perturbations to any of them could cause mitochondrial dysfunction. Transcription Factor A mitochondrial (TFAM) is a nuclear encoded transcription factor that has two main functions in the mitochondria: transcription and maintenance of the mitochondrial genome. Previous studies have shown that while TFAM full body knockouts were embryonically lethal in mice, cardiomyocyte specific knockouts died a few weeks after birth due to mitochondrial dysfunction induced cardiomyopathy, suggesting that TFAM is indispensable for proper heart development and function. To further study the signaling mechanisms of mitochondrial dysfunction in the heart we generated an inducible cardiomyocyte-specific deletion of TFAM in the adult mouse heart. Surprisingly these mice developed late onset cardiomyopathy 8 months (32 weeks) following TFAM deletion unlike what was previously described in literature. In conjunction with cardiomyopathy, mitochondrial DNA (mtDNA) and mitochondrial transcripts were depleted and oxygen consumption—which is proportional to energy production—was reduced with long term deletion. Mitochondrial transcription was reduced and mitochondrial ultrastructure was severely disrupted with early TFAM deletion, however mtDNA content was still preserved, and energy production and heart function was unaffected. To further understand what causes this late onset mitochondrial dysfunction we looked at DNA and RNA binding proteins involved in mtDNA replication and transcription which were downregulated in response to prolonged TFAM deletion but no change was observed with acute TFAM loss early on. These results suggest that mtDNA turnover times might not be as fast as previously described. Additionally there may be a molecular switch that gets activated upon enough loss of TFAM causing mitochondria to initiate a feedback signal to the nucleus to downregulate mtDNA replication and transcription machinery proteins. Moving forward our goal is to identify the mechanisms causing this long term quiescent period of no functional loss of energy production, and to uncover the molecular switch that signals to the nucleus to halt production of proteins required biogenesis and transcription of mtDNA.
Transforming Cardiovascular Disease Risk to Cardiovascular Health: Opportunities to Educate Young Women About CVD

Liu, Jingyi; Goel, Shivani; Patterson, Sierra; Brown, Courtney; De Ferranti, Sarah; Stamoulis, Catherine; and Gooding, Holly

Corresponding Author: Shivani Goel, MD, Emory University, sgoel3@emory.edu
Center: Heart Research and Outcomes Center (HeRO)
Type: Outcomes
Keyword(s): Adolescent, Cardiovascular
Related to Pilot Grant or Trainee Award: No
Poster Available: Yes - P31

PURPOSE: Cardiovascular disease (CVD) remains the leading cause of death in the US. However, young women are rarely the target population of prevention campaigns. The purpose of this study is to evaluate how this population sources cardiovascular health information in order to transform risk to wellness.

METHODS: We surveyed 331 females 15-24 years of age to determine 1) exposure to sources of heart disease information over the past 12 months and 2) whether participants felt informed regarding heart disease or stroke (modeled as binary outcome). Secondary outcomes included source preferences and motivations to seek information. Using linear and logistic regression models, we assessed for associations among participant demographics (age, race, ethnicity, caregiver education) and these outcomes.

RESULTS AND OUTCOMES: Adolescent and young adult women reported a median of one source of exposure to CVD information over the past 12 months, although 25.7% of young women had none. Hispanic women were more likely to report zero exposures to any heart disease information compared to white women (OR 1.7, 95% CI: 1.0-2.9, p=0.05); no other significant demographic associations were identified. The most commonly accessed sources across all participants were television (38%), social media (30%), and web (26%). Approximately 48.5% and 59.0% of patients rated themselves as not at all informed about heart disease and stroke respectively, and 61.8% had never spoken to a healthcare professional about their risk. There was a positive association between number of exposures to cardiovascular health material and feeling informed about heart disease and stroke (p<0.001 for each). Additionally, respondents who had discussed their personal risk with a healthcare provider were more likely to be informed about heart disease (OR: 6.6, 95% CI: 2.2-19.6) and stroke (OR: 7.0, 95% CI: 1.5-33.2), with 42.6% stating this as their preferred method of information.

CONCLUSIONS: Less than half of young women feel informed about heart disease and stroke, the greatest lifetime health risk for women in the US. Public health campaigns should focus on promoting cardiovascular health using media channels frequently accessed by this demographic. It is also essential for healthcare providers to discuss risk factor modification from a young age.
The achievements of antineoplastic drug discovery have increased survival in patients with cancer, but treatment-related cardiotoxicity appears to be a leading noncancer-related cause of morbidity and mortality in long-term cancer survivors. Human induced pluripotent stem cell-derived cardiomyocyte (hiPSC-CM) platform is a cutting-edge tool to complement and replace animal cell equivalents in many applications, especially drug development and disease modeling. Melphalan is commonly used to treat cancers by breaking the double strands of DNA, but increasing clinic cases reported that it could induce cardiotoxicity including severe arrhythmias and heart failure. As the mechanism by which melphalan impairs cardiac cells remains poorly understood, we aimed to use hiPSC-CM platform to determine melphalan-induced cardiotoxicity and explore molecular mechanisms and potential targeted therapeutics. We found that melphalan had severe deleterious effects on hiPSC-CMs as indicated by significant cell death, early stage apoptosis, excessive reactive oxygen species, deranged Ca2+ handling, and dysfunctional contractility in a dose-dependent manner. We also found that N-acetyl-L-cysteine, a powerful antioxidant, reduced melphalan-induced cell death and reactive oxygen species production and attenuated the transcriptomic alterations and functional defects. These findings suggest that oxidative stress could possess the central position in the mechanism underlying melphalan-induced cardiotoxicity. In addition, proteomic and RNA-Seq analyses uncovered the melphalan-induced changes in several other signaling pathways including the p53 and TGF-β signaling pathways. In conclusion, melphalan induces cardiotoxicity through oxidative stress in hiPSC-CMs. We anticipate that this study can potentially open up new clinical mechanism-based targets to prevent and treat melphalan-induced cardiotoxicity.
Electrical Stimulation of Pediatric Cardiac-Derived c-kit+ Progenitor Cells Improves Retention and Cardiac Function in Right Ventricular Heart Failure

Maxwell, Joshua; Trac, David; Shen, Ming; Brown, Milton E.; Davis, Michael; Lopera, Maria; Chao, Myra; Supapannachart, Krittin; Zaladonis, Carly; Baker, Emily; Li, Martin; and Jacobs, Daniel

Corresponding Author: Josh Maxwell, PhD, Emory University, jmaxwe@emory.edu
Center: Heart Research and Outcomes Center (HeRO)
Type: Basic
Keyword(s): pediatric heart failure, stem cells
Related to Pilot Grant or Trainee Award: 2018, JFF, The Role of Paracrine Factors in Cardiac Progenitor Cell-mediated Cardiac Regeneration (PI: Joshua Maxwell, PhD)
Poster Available: No

Nearly 1 in every 120 children born has a congenital heart defect (CHD). While surgical therapy has improved survival, many of these children go on to develop right ventricular heart failure (RVHF). The emergence of cardiovascular regenerative medicine as a potential therapeutic strategy for pediatric HF has provided new avenues for treatment with a focus on repairing or regenerating the diseased myocardium to restore cardiac function. While primarily tried using adult cells and adult disease models, stem cell therapy is relatively untested in the pediatric population. Here, we investigate the ability of electrical stimulation to enhance the retention and therapeutic function of pediatric cardiac-derived c-kit+ progenitor cells (CPCs) in an animal model of RVHF. Human CPCs isolated from pediatric patients were exposed to chronic electrical stimulation and implanted into the RV myocardium of rats. Cardiac function and cellular retention analysis showed electrically stimulated CPCs (ES-CPCs) were retained in the heart at a significantly higher level and longer time than control CPCs and also significantly improved right ventricular functional parameters. ES also induced upregulation of extracellular matrix and adhesion genes and increased in vitro survival and adhesion of cells. Specifically, upregulation of β1 and β5 integrins contributed to the increased retention of ES-CPCs. Lastly, we show that ES induces CPCs to release higher levels of pro-reparative factors in vitro. These findings suggest electrical stimulation can be utilized to increase the retention, survival, and therapeutic effect of human c-kit+ progenitor cells and can have implications on a variety of cell-based therapies.
Long-Term Outcomes in Children With Williams Syndrome With Congenital Heart Disease Interventions

Montero, A. J.; Thomas, Amanda S.; and Kochilas, Lazaros K.

**BACKGROUND/OBJECTIVE:** Williams syndrome (WS) is a rare genetic condition frequently associated with severe cardiovascular anomalies causing multilevel aortic or pulmonary artery stenosis. Previous studies have examined the long-term outcomes of congenital heart diseases (CHD) in patients with WS, but the results remain ambiguous pertaining to those who had interventional procedures for CHD. In this study we aimed to describe children with WS after interventions for CHD (either surgical or interventional catheterization) and their long-term outcomes.

**METHODS:** We conducted a retrospective cohort study utilizing data collected from the Pediatric Cardiac Care Consortium (PCCC), a large US-based registry of interventions for CHD. We identified 203 WS patients with their first surgical or transcatheter intervention for CHD between 1982 and 2003 in the PCCC registry. Patient characteristics and long-term outcomes were examined from information available in the PCCC and long-term outcomes for those with adequate identifiers through linkage with the National Death Index and the Organ Procurement and Transplantation Network up until December 31, 2014.

**RESULTS:** Of the 203 WS patients identified, 177 (87%) had adequate identifiers for long-term follow-up and comprised our study cohort. A majority of patients undergoing CHD treatment received transcatheter intervention (159; 90%) in comparison to surgical (20; 10%) to alleviate cardiovascular complications resulting from CHD. The most common cardiovascular diagnosis was supravalvular aortic stenosis (SVAS), present in 102 (50%) patients followed by pulmonary artery stenosis in 11% patients. The median weight and age in patients at intervention was 11.1 (IQR= 6.5-19.5) kgs and 2.13 (0.7-6.6) years, respectively. Transplant-free survival after exclusion of in-hospital mortality was observed to be 92.8% in our cohort. We observed 21 total deaths in our cohort, 6 attributing to in-hospital mortality. Of those that died, the median age at death was 0.92 (IQR= 0.3-12.5) years or around 11 months.

**CONCLUSION:** With a 15-year transplant-free survival rate of 92.8%, long-term outcomes are favorable for WS patients undergoing surgical or transcatheter interventional procedures for CHD in this cohort. Further information about treatment pathways and lengthened follow-up time is essential in discovering late effects in this study population.
Identifying the Role of Post-Translational Acylations in Mitochondrial Energy Stress Response Signaling

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

Corresponding Author: Jessica Peoples, Emory University, jnpeopl@emory.edu
Center: Heart Research and Outcomes Center (HeRO)
Type: Basic
Keyword(s): Mitochondrial Dysfunction; Post-translational signaling
Related to Pilot Grant or Trainee Award: No
Poster Available: No

Among their many functions, including bioenergetics and calcium dynamics, mitochondria can act as signaling hubs using metabolites as messengers to convey stress. One group of messenger metabolites are reactive acyl-CoA’s. These intermediary metabolites are derived from mitochondrial metabolism, and can modify and regulate proteins through post-translational acylations. We hypothesized that reactive acyl CoAs are used as signaling effectors of the mitochondrial energy stress response. We modeled mitochondrial dysfunction in the heart by generating mice with inducible cardiomyocyte-specific deletion of the mitochondrial phosphate carrier (Slc25a3), a transporter critical for energy production. Inducible deletion of Slc25a3 results in cardiac hypertrophy, indicated by elevated heart to body weight ratios. Strikingly, PiC-deleted hearts have significantly elevated acetylated and malonylated proteins indicating reactive acyl CoAs involvement in mitochondrial stress signaling. Using liquid chromatography-mass spectrometry proteomics, we observed upregulation of acetylation and malonylation of mitochondrial proteins, including isocitrate dehydrogenase (IDH2) and sirtuin 5 (SIRT5). We also observed upregulated acetylation and malonylation of TCA cycle enzymes indicating targeting of mitochondrial metabolism. IDH2, a TCA cycle enzyme, contained the most modified sites with 9 malonylated and 5 acetylated lysine residues. Metabolomics-based data also showed that PiC-deleted hearts have significantly increased malonyl-CoA substrate, but unchanged acetyl CoA levels suggesting differential regulation of the two acylations. Since acylations as well as deacylations may occur either enzymatically via acyl-transferases/deacylases or non-enzymatically under permissive conditions (such as high substrate availability), we noted SIRT5, a mitochondrial deacylating enzyme known to target many mitochondrial enzymes, being hyperacetylated with PiC-deletion. In addition, acetylation of SIRT5 at lysine residue K203 had inhibitory effects on SIRT5 demalonylating activity suggesting a potential feedback mechanism contributing to hypermalonylation in PiC-deleted hearts. By combining cardiac physiology with systems-based proteomics, we have thus far demonstrated that post-translational modification signals, in response to cardiac mitochondrial dysfunction, possibly compromises mitochondrial function by targeting enzymes of mitochondrial metabolism.
Human induced pluripotent stem cells (hiPSCs) due to their potential capacity to differentiate in other cell types and virtually unlimited self-renewal ability could ideally be used for disease modeling, drug discovery and regenerative therapies. We have previously demonstrated the efficient generation of cardiomyocytes from hiPSCs (hiPSC-CMs) by exposing 3D cardiac progenitors to a simulated microgravity environment. We are currently investigating the effect of real microgravity during the differentiation process of hiPSC-CMs from 3D cardiac progenitors at the International Space Station (ISS). For this study, cardiospheres are to be sent to the ISS and cultured by the astronauts using the Multi-specimen Variable-gravity Platform (MVP) from Techshot, Inc. (Greenville, IN). At the end of the mission, we will then perform on the ground molecular, cellular and electrophysiological analyses, comparing the microgravity samples with a parallel culture under standard gravity. In order to facilitate the space experiments, we evaluated and optimized protocols for cryopreserving 3D cardiac progenitors. At day 6 of the differentiation, 3D progenitor cardiospheres were formed using Aggrewell plates and were cryopreserved at day 7. The cryopreservation allowed to pre-test aliquots of hiPSC-CMs before the mission to evaluate differentiation efficiency and cell purity. Cardiospheres were relivable after thawing into suspension culture at 37°C, and further differentiated into beating cardiomyocytes with morphology and expression of cardiac markers comparable to unfrozen parallel cultures. The cryopreservation facilitated the astronauts’ working schedule by directly thawing the cardiac progenitors onboard at the ISS. We evaluated also the use of CO2-independent medium with supplements for the differentiation of cardiac progenitors. The CO2-independent medium supports cell growth after thawing, without the need of a CO2 incubator, facilitating the cell maintenance at the ISS. Cardiac progenitors survived and differentiated into beating cardiomyocytes with morphology and expression of cardiac markers comparable to normal culture condition. The use of medium supplements provided additional support for cell survival in the culture condition without CO2. The feasibility of these protocols facilitates spaceflight experiments to evaluate the impact of microgravity on the expansion of cardiac progenitors cells and the production of CMs compared with traditional culture methods.
Arginine Dysregulation Correlates With Cardiac Remodeling in Mice With Chronic Kidney Disease

Reyes, Loretta; Winterberg, Pamela; George, Roshan; Kelleman, Michael; Harris, Frank; Brown, Lou Ann; and Morris, Claudia

Corresponding Author: Loretta Reyes, MD, Emory University, Ireyes7@emory.edu
Center: Heart Research and Outcomes Center (HeRO)
Type: Basic
Keyword(s): Arginine in CKD
Related to Pilot Grant or Trainee Award: 2016, HeRO, Role of Dysregulated Arginine Metabolism in Uremic Cardiomyopathy (PI: Pamela Winterberg, MD)
Poster Available: Yes - P34

BACKGROUND: Nitric Oxide (NO) is critical for cardiovascular homeostasis. Arginine, the sole nitrogen donor for NO synthesis, is the common substrate for NO synthase and arginase enzymes. Low global arginine bioavailability ratio (GABR), a potential endothelial dysfunction biomarker, has been associated with increased mortality in adults with heart failure. Arginine metabolism is dysregulated in chronic kidney disease (CKD) and is associated with changes in myocardial function. This study aimed to determine the relationship between metabolites/enzymes in arginine metabolic pathways with cardiovascular measures in mice with CKD and investigate the effect of arginine supplementation.

METHODS: CKD established in male 129X1/SvJ mice via 5/6th nephrectomy. Plasma collected at 8- and 16-weeks post-surgery was analyzed for arginine, citrulline, ornithine, asymmetric dimethylarginine (ADMA) via LC-MS/MS; GABR calculation=arginine/(ornithine+citrulline). Arginase activity was analyzed via colorimetric assay. Echocardiographic measures of left ventricular hypertrophy (LVH), diastolic dysfunction and ventricular strain were obtained at 8- and 16-weeks. In a separate experiment, CKD and control mice received chow supplemented with arginine or alanine (nitrogen control) for 12 weeks. Plasma and echocardiograms obtained at 8- and 12-weeks were examined as above. Blood pressure was measured noninvasively.

RESULTS: In mice with CKD, low GABR correlated with decreasing E/A ratio (measure of diastolic dysfunction) \([r=0.58; p=0.01]\) and increasing relative wall thickness (RWT) (measure of LVH) \([r=-0.49, p=0.03]\). Plasma arginase activity was significantly increased in CKD mice at 16-weeks \([\text{median (IQR)} 10.5 (8.4-11.7)]\) compared to controls \([5.5 (1.5-10.0); p\leq 0.05]\) and to CKD mice at 8-weeks \([7.0 (3.7-7.6); p=0.002]\). Increased arginase activity correlated with impaired ventricular strain \([r=-0.34; p=0.04]\). ADMA was significantly increased in CKD mice at 16-weeks \([7.2 (7.1-7.3)]\) compared to controls \([7.1 (7.1-7.3); p=0.036]\) and correlated with lower E/A ratio \([r=-0.34; p\leq 0.05]\). After 12-weeks of supplementation, arginase activity was significantly lower in arginine supplemented CKD-mice compared to arginine supplemented normal mice \([7.8 (3.1-8.5) \text{ vs } 14.3 (10.0-15.6); p=0.004]\) and compared to alanine supplemented CKD-mice \([18.8 (12.9-19.7); p=0.006]\). In arginine supplemented CKD-mice, blood pressure was significantly lower at 12 weeks compared to 8 weeks; \(p=0.03\).

CONCLUSIONS: In a mouse model of CKD, dysregulation in arginine metabolism correlates with myocardial dysfunction and hypertension is ameliorated with arginine supplementation.
Arginine Metabolism is Dysregulated in Children With Chronic Kidney Disease and Correlates With Left Ventricular Hypertrophy

Reyes, Loretta; Winterberg, Pamela; George, Roshan; Kelleman, Michael; Harris, Frank; Brown, Lou Ann; and Morris, Claudia

Corresponding Author: Loretta Reyes, MD, Emory University, lreyes7@emory.edu
Center: Heart Research and Outcomes Center (HeRO)
Type: Clinical or Translational
Keyword(s): Pediatric CKD and arginine
Related to Pilot Grant or Trainee Award: 2016, HeRO, Role of Dysregulated Arginine Metabolism in Uremic Cardiomyopathy (PI: Pamela Winterberg, MD)
Poster Available: Yes - P35

BACKGROUND: Cardiovascular disease is the leading cause of death in children and young adults with chronic kidney disease (CKD). Left ventricular hypertrophy (LVH) is common in pediatric CKD patients and is an independent risk factor for cardiovascular morbidity/ mortality. Nitric oxide (NO), a vasoactive substance critical for vascular homeostasis, is synthesized from arginine by NO synthase. Arginine can also be catabolized by arginase enzymes, thereby reducing NO bioavailability. Since the kidneys play a key role in endogenous arginine synthesis, we hypothesized that arginine bioavailability is altered during CKD and predicts cardiovascular complications.

METHODS: Banked plasma from children with (n=47) and without (n=11) CKD was analyzed for arginine, ornithine, citrulline and asymmetric dimethylarginine (ADMA) by LC-MS/MS; arginase concentration/activity by ELISA/colorimetric assay; and total nitrate/nitrite concentration (NOx) by Greiss reaction. Plasma data were correlated with echocardiographic LVH measures in CKD patients.

RESULTS: Arginase activity was significantly higher in children with CKD vs healthy controls [median (IQR) 3.65 (2.02-5.63) vs 1.52 (1.23-2.15); p=0.047]. Dialysis patients had a non-significantly lower arginase concentration compared to healthy controls (p=0.072) but arginase activity was significantly increased in dialysis patients compared to healthy controls, despite the lower concentration [2.92 (2.04-5.34) vs 1.52 (1.23-2.15); p=0.04]. Arginase activity trended higher in patients with LVH (p=0.08). ADMA was significantly higher in CKD and dialysis patients compared to healthy controls [7.5 (7.4-7.8) and 8.5 (7.6-8.7) vs 7.1 (7.1-7.1) respectively; p=<0.001]. ADMA was significantly higher in patients with LVH and correlated with left ventricle relative wall thickness on spearman correlation [r=0.54; p=0.003]. NOx was significantly higher in CKD and dialysis patients compared to healthy controls [174.8 (145.1-257.9) and 163.9 (124.5-220.2) vs 92.9 (83.2-128.7) respectively; p<0.001] but interestingly, higher NOx levels was observed in patients without LVH [r=−0.52; p=<0.001].

CONCLUSIONS: This pilot study demonstrated significant dysregulation in the arginine metabolism pathway and identified correlations between arginase activity and ADMA with LVH; NOx appeared to protect against LVH development. The finding of increased arginase activity despite lower concentration in dialysis patients is a novel observation that has not previously been reported. The findings of this pilot have the potential for therapeutic impact and further studies are underway.
3D Bioprinted In Vitro Platform to Study Cellular and Molecular Mechanisms of Pulmonary Vein Stenosis

Tomov, Martin L.; Jing, Bowen; Bhamidipati, Sai R.; Avazmohammadi, Reza; Lindsey, Brooks; Bauser-Heaton, Holly; and Serpooshan, Vahid

Patient-inspired 3D bioprinted tissue models can provide a unique platform to recapitulate and analyze the complex cardiovascular microenvironments impacted by pulmonary vein stenosis (PVS) and can help develop novel clinical interventions by engineering functional in vitro tissue phantoms. Here we present some of our current work that demonstrates the feasibility of bioprinting a variety of cardiovascular cells, suspended in bioinks, that can be coupled with complex in vivo-inspired geometries to create perfusable vascular tissue constructs that mimic perfused tissues. We have developed a perfused in vitro model of pulmonary vein stenosis (PVS) by using 3D reconstructed computer tomography (CT) and X-ray angiography (XA) data of healthy and stenotic human pulmonary veins and used them to print a vascular model of PVS within 3D hydrogel blocks. We were able to quantify the flow hemodynamics through our bioprinted pulmonary vein model vessels using computer fluid dynamics (CFD), 4-dimensional magnetic resonance imaging (4D MRI) and 3D ultrasound doppler readings (3D echo PIV). These 3D bioprinted models were connected to a perfusion bioreactor and cultured in vitro for 2 weeks under different flow conditions. Cell viability, proliferation, and endothelialization of the bioprinted channels were tracked and quantified. While models like this have been done in synthetic materials, resin and thermoplastics could not adequately support phantom cellularization, specifically cellular remodeling as they lack the physiomechanical properties of native tissue microenvironment. GelMA, in contrast, has been repeatedly shown to mimic soft tissue stiffness and support cell attachment, function, and remodeling in cellularized constructs. Such vascular phantoms, based on clinical data and additive biomanufacturing, would allow for in vitro investigation of disease processes otherwise not possible due to patient and animal model variability, leading to improved patient outcomes and more faithful disease models for novel drug and/or procedure development. In combination, our approach allowed us to build a detailed map of the flow patterns in our models and adjust the physically printed phantoms based on input from our in silico models, offering a substantial advance in in vitro modeling of vascular disease through a biomimetic, tunable microenvironment to study cellular and molecular mechanisms of PVS.
Nurses’ Perceptions of Rooming-In for Caregivers of Infants With Critical Congenital Heart Disease

Shackleford, Jenna; Chambers, Rebecca; Nelson, Jennifer; Scott, Megan; and Brasher, Susan

Corresponding Author: Jenna Shackleford, PhD, MSN, RN, Kennesaw State University, jshackle@kennesaw.edu
Center: Heart Research and Outcomes Center (HeRO)
Type: Clinical or Translational
Keyword(s): Congenital Heart Disease, Rooming-In Practices
Related to Pilot Grant or Trainee Award: No
Poster Available: No

PROBLEM STATEMENT: Transitioning from the hospital to home for infants who require complex care can be an overwhelming experience for caregivers. Infants with critical congenital heart disease (CCHD) have complex medical needs. To adequately and safely care for their child, caregivers require extensive training prior to being discharged to home. The use of a rooming-in process has been found to be successful during the post-partum period to improve the transition of mothers and newborn infants. However, little research has been done to examine the rooming-in process in a pediatric cardiac setting, particularly from the nurses’ perspective.

PURPOSE: The purpose of this study was to describe nurses’ perceptions of the rooming-in process in a pediatric Cardiac Acute Care Unit.

METHODS: A qualitative research design was used to describe the nurses’ experiences and perceptions of the rooming-in process for caregivers of infants with CCHD. A total of three focus groups were conducted with 14 registered nurses trained who care for infants with CCHD. Qualitative content analysis was performed to analyze data.

RESULTS: Four themes were identified during data analysis: Improved Nursing Outcomes, Improved Patient and Family Outcomes, Leading the Way, and Room for Improvement.

IMPLICATIONS AND CONCLUSION: Infants with CCHD have complex needs and thus caregivers must be adequately prepared to care for these infants. This study is the first to explore the nurses’ perspective of the rooming-in process for caregivers of infants with CCHD. Nurses work directly with caregivers during the rooming-in process and thus can provide critical insight as to ways to better support caregivers of infants with CCHD and improve the transition to home. An understanding of the nurses’ perspectives and experiences has the potential to improve the rooming-in process, which can translate to better patient and family outcomes.
Pacemaker Implantation Is Associated With Decreased Long Term Survival in Repaired Two Ventricle Congenital Heart Disease

Sorensen, Matt; Claxton, J’Neka; McCracken, Courtney; Cortez, Daniel; Vinocur, Jeffrey; Kochilas, Lazaros; and Whitehill, Robert

BACKGROUND: Heart block (HB) can complicate congenital heart disease (CHD) surgery and require pacemaker (PM). Adverse consequences of chronic ventricular pacing are increasingly recognized, yet little is known about the long-term outcomes of CHD patients who require PM.

OBJECTIVE: To evaluate the effect of PM implantation on the long-term transplant-free survival of patients with two-ventricle (2V) CHD.

METHODS: Among pts aged ≤ 21 yrs undergoing initial surgery associated with HB for 2V CHD in the Pediatric Cardiac Care Consortium, we identified pts having a PM implanted within 90 days. We then matched controls 2:1 by gender, operation, age at surgery (±18 months) and surgical era. Primary outcome was transplant-free survival via linkage to the National Death Index and Organ Procurement and Transplantation Network.

RESULTS: We identified 333 pts and 662 controls who survived to hospital discharge. At time of surgery, median age/weight were 7.7 months (34% infants) and 5.9 kg (11% <2.5kg) and 50.2% were male. The most common diagnosis associated with PM implantation was AV Canal (31%) followed by VSD (27%). Over a mean follow-up of 16.9 years follow-up, 156 deaths occurred. Patients surviving to hospital discharge demonstrated decreased long-term transplant-free survival with PM (p = <0.05) at 20 yrs follow up (p = <0.0001). Subgroup analysis, including hospital deaths, revealed that VSD pts with PM had significantly lower survival at 20 yrs (85% vs 95%). Analyzing by surgical era, survival was 73% vs 81% during 1982-1992 compared to 77% vs 82% during 1993-1997.

CONCLUSION: PM insertion for HB after initial surgical procedure for 2V CHD is associated with overall diminished long-term transplant free survival in our overall cohort. We noticed a trend of decreased 20 year survival among patients with Tetralogy of Fallot, AV Canal defects, primary mitral valve pathology as well as coarctation of the aorta associated with VSD.
Neonatal reflexes function in cognitive development as predispositions that provide opportunities for babies to interact with the world. Between 4-6 months of age many reflexes begin to disappear as volitional actions emerge. A longstanding model posits that this transition is mediated by a shift from subcortical to cortical control, with subcortically-mediated reflexes declining as cortical control develops. This project tests this hypothesis by mapping developmental trajectories of infant reflexes and white matter (WM) microstructure from 0-6 months in the same infants. Diffusion MRI and reflex data (assessed using the NICU Network Neurobehavioral Scale (NNNS)) were collected at up to 14 and 3 time points, respectively, before 6 months in N=32 infants. Fractional anisotropy (FA) values were used to measure tract maturity in 7 WM tracts: inferior fronto-occipital fasciculus, uncinate fasciculus, inferior longitudinal fasciculus, pyramidal tract, and body, genu and splenium of the corpus callosum. Trajectories were fit using functional principal components analysis. The presence of infant reflexes decreased across all measures across the 6 months. However, the Babinski and plantar grasp reflexes while decreasing in strength did not disappear. The WM tracts had increasing FA values with their greatest rates of change corresponding to the timing of the decreasing reflexive behaviors. This project provides the first test of hypothesized brain-behavior transitions using densely-sampled prospective, longitudinal measures of brain and behavior collected in the same infants. Preliminary results show that many infant reflexes begin to decline in the first month of life, paralleled by rapid WM tract maturation.
Elementary school is an important developmental and educational milestone for children, and Active Engagement (AE) has long been identified as a key component of effectively educating children with autism. Social and Emotional Engagement-Knowledge and Skills (SEE-KS) is a professional development program developed to increase classroom active engagement in general education classrooms. This research was completed in 15 K-3 General Education Classrooms within three elementary schools affiliated with one small, urban school district. Classroom teachers participated in SEE-KS for one school year. Classroom observations were recorded at the beginning and end of the school year, and coded for indicators of student AE (i.e., initiating communication, social attention) and teacher fidelity of SEE-KS implementation (i.e., individualised attention, providing multiple means of expression, representation and engagement). In addition, we collected classroom-level measures of student’s academic outcomes (MAP assessment) for the 15 SEE-KS classrooms, as well as matched comparison classrooms from the same schools. We hypothesised that in SEE-KS classrooms, (1) higher teaching fidelity scores are associated with higher levels of AE, and (2) that average classroom scores of teaching fidelity and AE will increase over the course of the school year. Finally, we predicted that SEE-KS classrooms show larger academic gains than matched control classrooms. The importance of this research lies in informing classroom practices to improve student engagement and academic outcomes, serving as the foundation of our long-term goal to implement community-viable educational innovations for children with autism in Georgia and beyond.

(Note: Data analysis for this project is in progress, and will be available before May 2020)
Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder characterized by deficits in social communication and interactions. Although social functioning is recognized as existing along a spectrum (Social Spectrum), little is known about the early development of social functioning across the full spectrum from typically developing children to children with ASD. Understanding when skills are developing and when developmental differences first emerge is crucial for informing early intervention services.

Developmental progressions of three social skills were evaluated from 9 to 24 months. Additionally, this study examined if and how social development varied across the Social Spectrum and across gender.

High-risk (N=108) and low-risk (N=125) infants were assessed at 9, 12, 18, and 24 months. Weighted raw scores for three skill categories (Emotion/Eye Gaze, Communication, and Gestures), from the Social domain of the Communication and Symbolic Behavior Scales, were analyzed to assess for skill development. The sum of parent-ratings (Not Yet/Rarely, Sometimes, or Often) on 22 items related to Social Interacting & Communicating at 9 months was used as a measure of Social Spectrum, with higher scores depicting greater social functioning impairment. LME models were conducted separately for each skill with Visit, Gender, and Social Spectrum as predictors. Non-significant interactions and main effects were removed from models when the AIC was lower.

Results indicated a significant Visit by Social Spectrum interaction on Emotion/Eye Gaze and Communication development. Significant main effects for these variables were indicated for Gesture development. There was also a significant effect of Gender for both Communication and Gestures.

Social skills related to emotion and eye gaze, communication, and gestures are developing from 9 to 24 months of age. Additionally, the development of these social skills varies depending on infants’ social functioning at 9 months of age. Results related to gender differences are also consistent with extant literature regarding females typically demonstrating better social abilities. Early identification of trajectories and factors impacting development are imperative to better understand developmental pathways across the Social Spectrum as it relates to ASD. Such an understanding will help in the provision of individualized early invention services for children with varying degrees of social impairment.
Children diagnosed with Autism Spectrum Disorder (ASD) are more likely to be admitted to the emergency department (ED) for behavioral or psychiatric reasons than their typically developing peers (Lytle, Hunt, Moratschek, Hall-Mennes, & Sajatovic, 2018; Straus, Coburn, Maskell, Pappagianopoulos, & Cantrell, 2019). These children have higher rates of inpatient psychiatric admissions and often require longer lengths of stay, ultimately incurring significantly larger medical costs (Carbone, Young, Stoddard, Wilkes, & Trasande, 2015). Additionally, they experience higher rates of sedation, restraint, and inpatient hospitalization (Lunsky, et al., 2014). The current study seeks to further extend the research on children with ASD in the ED by identifying common themes to better understand why higher rates of ED visits occur in this population. We examined the charts of 56 children (M = 14; range, 8-20) diagnosed with ASD and admitted to the ED presenting with a chief complaint of behavioral health concerns. Data were collected on a number of variables including demographics, diagnoses, admission length, and interventions from the ED admission encounter. A preliminary analysis of these data found various themes. The majority of the children admitted to the ED were male, Non-Hispanic, and insured through Medicaid or Medicaid managed care plans. In terms of diagnoses, 68% of children had a comorbid behavioral or psychiatric disorder and 70% were admitted for aggressive behavior. Prior to the ED admission, 98% of the sample were prescribed behavioral medications, with 80% prescribed two or more. The average length of admission was 27 hours with 55% transferred to another medical or psychiatric facility at discharge. Approximately 70% of families traveled 0-25 miles to the ED location of admission and 30% traveled 26-100 miles. Interventions in the ED included one-to-one monitoring (88%) and administration of a chemical or physical restraint (20-25%). Overall, these findings suggest that this population of children require an intensive level of intervention and care from the ED to manage presenting concerns. Further analysis of the data are required to examine if a unique profile exists which when indicated, could help medical providers better prepare and treat children in the ED with behavioral health concerns.
Designing for Kids by Kids

Foster, Amanda and Denham, Megan

Patient-centered design is increasingly becoming a priority to the healthcare industry. GTRI takes this approach by designing solutions with kids who would benefit from these solutions in pediatric care.

Quick Wins MRI Project with Marcus Autism Center

MRI-associated anxieties are magnified for children with Autism Spectrum Disorders (ASD) because of increased sensitivity to stimulation. When a child with ASD requires an MRI, the child is commonly sedated to eliminate agitated movement and guarantee the acquisition of high-quality data creating inherent risks associated with sedation. The Marcus Autism Center uses an MRI simulator coupled with an Adaptive Behavior Approach (ABA) to successfully scan children with ASD while awake. Children with ASD often lack the ability to generalize experiences from one situation to the next, translating to a jarring experience shifts when moving to the actual scanner.

The goal of this project was to create consistent, less intimidating simulated and actual MRI experiences for children with ASD, resulting in increased success rates for scanning without sedation. Modifications were made to the MRI simulator to resolve differences between the simulator and the actual MRI. The feeling of control was the most insightful finding during interviews with children and their families, so an ambient lighting system was installed to allow the children to choose the color of the room, giving them some control over their environment.

Passport App with Aflac Cancer and Blood Disorders Center

The program "Designing Cancer Care for Kids By Kids" engages patients as partners in care. The passport collects quantitative data about the patient visit, and qualitative data about how the kids feel at each step in the process. The results have revealed where kids spend their time waiting, and where they are unhappy or scared. The Aflac team has begun focused efforts to improve inefficient processes, such as adding another registration desk, and identifying Aflac teams who are more efficient with their care so other teams can learn from them. The Aflac team has continued to use the passport tool for additional service lines to better understand those processes.
Early identification and treatment of ASD is crucial during the first three years of life to maximize developmental potential when neuroplasticity is optimal. The Early Social Interaction Model (ESI) is a parent-implemented intervention teaching family members how to support their child’s active engagement in everyday activities. The effectiveness of the ESI model has been documented in a multisite randomized controlled trial (RCT) that resulted in improved child outcomes on standardized measures of social communication, developmental functioning, and adaptive behavior (Wetherby, Guthrie, Woods, Schatschneider, Holland, Morgan, & Lord, 2014).

The purpose of this study is to expand the results of the initial RCT by training Part C Early Intervention Providers (EIPs) to coach families of toddlers with early signs of ASD. EIPs will have access to an autism specific screening tool that can identify children at risk for ASD. Our goal is to identify children under the age of 24 months, then provide intervention with the ESI model. 18 EIPs will be recruited in Georgia and 22 in Florida by our collaborator, FSU. Participants will receive online training and randomize into two study arms: Business-as-Usual (BAU): monthly group support meetings or Early Social Interaction (ESI): weekly individual and group supervision meetings. Providers will offer study participation to families with children demonstrating red flags for ASD. We expect EIPs to work with an average of three toddlers for a collaborative total of 120 participating families. Currently, we are reviewing the grant’s first year of EIPs recruiting and implementing the intervention with families.

CURRENT DATA: Of the 18 EIPs planned for Georgia, 13 are active (6 in Cohort 1 started in May 2019, 7 in Cohort 2 started in March 2020=72%). Of the active EIPs, 5 have active families (38%). Of the 54 families planned for Georgia, 7 families have received services (13%). Cohort 2 has identified 3 families so far. Conclusions will be discussed on the poster when data becomes more available in the next two months. Data we plan to present includes the number of ESI and BAU sessions conducted and number of providers with active families throughout the study.
Patterns of Social Visual Engagement in the First Two Years of Life Differentially Predicts Language Abilities in Children With and Without Autism Spectrum Disorder

Koirala, Sanju; Parmaksiz, Deniz; Yuan, Stella (Yixin); Shultz, Sarah; Klin, Ami; Jones, Warren; and Edwards, Laura A.

Corresponding Author: Sanju Koirala, BA, Emory University, sanju.koirala@emory.edu
Center: Marcus Autism Center
Type: Clinical or Translational
Keyword(s): Autism Spectrum Disorder (ASD), Language Outcome
Related to Pilot Grant or Trainee Award: No
Poster Available: Yes - P38

Infants’ patterns of social visual engagement to faces change over the first two years of life. Typically developing (TD) infants tend to shift their attention from a speaker’s eyes to mouth, and this attentional shift has been shown to predict language outcomes. In contrast, infants later diagnosed with autism spectrum disorder (ASD) show atypical patterns of social visual engagement to faces, such as persistently reduced attention to a speaker’s eyes. However, the relationship between these visual fixation patterns and later language abilities is not well understood. This study examines the critical role of early social visual engagement in the acquisition and employment of functional language in a longitudinal cohort of infants at high and low familial risk for ASD. Eye-tracking measures of social visual engagement were collected and quantified as percentage of time spent visually fixated on regions-of-interest (ROIs) defined as eyes, mouth, body, and object. At 24 months, language development was measured using the Mullen scales of early learning, and clinical best estimates confirmed diagnoses of ASD. Within-group Pearson correlations revealed that TD infants’ eye-looking at the beginning of the first year of life (n=93, Mage=5.14mo) positively predicted receptive language (r=0.312, p=0.002), and mouth-looking during the second year of life (n=80, Mage=15.21mo) trended towards positive prediction of expressive language scores. Increased visual attention to eyes at the beginning of the first year of life is therefore adaptive in TD infants. In infants later diagnosed with ASD, patterns of visual fixation were unrelated to language scores. Instead, ASD infants’ early eye-looking (n=40, Mage=5.12mo) and later (n=45, Mage=15.23mo) object-looking predicted ASD severity at 24 months (r=-0.346, p=0.029, r=0.384, p=0.009 respectively). Further analyses will investigate longitudinal trajectories of social visual engagement as predictors of language outcome in both ASD and TD infants.
The Adaptive Value of Gaze to the Mouth and the First Word Milestone in Typical Development and in Autism Spectrum Disorder

Kushner, Elizabeth; Shultz, Sarah; Klin, Ami; Jones, Warren; and Edwards, Laura

Corresponding Author: Elizabeth Kushner, BS, Emory University, ekushne@emory.edu
Center: Marcus Autism Center
Type: Clinical or Translational
Keyword(s): Autism Spectrum Disorder, Language Development
Related to Pilot Grant or Trainee Award: No
Poster Available: Yes - P39

Within the second year of life typically developing (TD) infants demonstrate a shift in preferential looking to the mouth. This occurs at ages that coincide with word learning and is associated with later vocabulary (Tenenbaum et al., 2015). However, findings are variable regarding the adaptive value of mouth-looking for children with Autism Spectrum Disorder (ASD) and whether and to what extent mouth-looking is related to acquisition of first words. The present study explores the adaptive value of mouth-looking during the period of early word learning in TD and ASD groups. We predict a significant relationship between mouth-looking and language at the onset of word learning, for both TD and ASD groups. Age-matched ASD (n=48; Mage(SD)=14.85(4.348)months) and TD (n=109; Mage(SD)=13.01(3.001)months) children were assessed using the MSEL expressive and receptive language scales and eye-tracking measures of social visual engagement (percentage of time spent fixated on different facial regions of interest (ROIs)). Children were classified into four groups based on early word learning status (MSEL item-11-scale: no words (n=39), one word (n=31), two-to-seven words (n=57), and eight-or-more words (n=25). Within each category, we tested for between-group differences in visual fixation to eye and mouth regions, and within-group associations between visual fixation and concurrent expressive and receptive language.

Once children with ASD reached eight-or-more words, they displayed significantly less mouth-looking than TD children at the same stage (p=0.049); there were no other differences in amount of eye- or mouth-looking. TD children without words exhibited a positive associated between mouth-looking and concurrent receptive language (r=0.654,p<0.001), and an inverse relationship between eye-looking and expressive language (r=−0.564,p=0.002). Those at the first word milestone (one word) exhibited a positive association between mouth-looking and concurrent expressive language (r=0.448,p=0.036). Eye- and mouth-looking were unrelated to language in ASD children at all stages of word learning. Findings suggest that for TD children, the adaptive value of mouth-looking changes around the first word milestone; however, we did not find this pattern within ASD. Future analyses will explore associations between visual fixation and language in a cohort followed longitudinally across the first word transition, and potential heterogeneity in this relationship within ASD.
INTRODUCTION: Growing evidence suggests that parents of children with autism have greater risk of poor mental and physical health compared to parents of typically developing children. While child behavior problems and caregiving stress have been identified as factors associated with poor mental health, the relative contribution of other child and parent characteristics, including child sleep problems, remains unclear. The purpose of this study is to determine the relative contribution of child and caregiver characteristics on mental and physical health in parental caregivers of children with autism.

METHODS: Using data from the 2016 NSCH, this secondary data analysis examined child (sleep problems (≤ 8 hours), behavior problems, and autism severity) and parental (caregiving stress, caregiving demands, emotional support, time spending care, and time spent coordinating care) characteristics as predictors of mental and physical health in parental caregivers of children (ages between 4 to 17) with autism. Multivariate logistic regression was used to determine predictors of mental and physical health in parental caregivers of children with autism while controlling for covariates.

RESULTS: Bivariate analyses indicated that child getting enough sleep (> 8 hours) and caregiver status (mothers, fathers, non-father male, non-mother female) were significantly associated with reported good physical (respectively, $\chi^2=60.77$, $p < 0.001$; $\chi^2=27.43$, $p < 0.001$) and mental health (respectively, $\chi^2=31.04$, $p < 0.001$; $\chi^2=39.96$, $p < 0.001$). Logistic regression analyses indicated that parents of children getting adequate sleep had better mental health (OR = 1.37, 95% CI [1.15-1.63]) and physical health (OR = 1.38, 95% CI [1.18-1.62]) compared to those with inadequate. Compared to fathers, mothers had lower odds of better mental health (OR = 0.75, 95%CI [0.61-0.93]).

DISCUSSION: While having a child with autism was not uniquely associated with poor mental and physical health status, child sleep problems are significantly associated with greater odds of worse mental and physical health in both maternal and paternal caregivers of children with autism. Further studies are warranted to examine the difference in nighttime caregiving activities related to child sleep problems in parents of children with autism.
INTRODUCTION: Establishing growth curves of white matter tract development in typical infancy is critically needed for identifying deviations therefrom in infants later diagnosed with autism spectrum disorder (ASD)1. Diffusion tractography is the only available method for mapping infant white matter connectivity non-invasively and longitudinally2. However, traditional methods of delineating infant white matter tracts suffer from several limitations: (1) it is time consuming to delineate anatomical tracts in a large cohort; (2) it requires extensive expertise in neuroanatomy; (3) large experimenter variability exists. Due to these limitations, we implemented a framework to automatically delineate a large number of anatomically meaningful tracts in infants without the need for manual interventions.

METHODS: Participants: 33 typical developing infants (113(54) days) were enrolled. DTI data were collected from each infant at three pseudorandom time points between birth and 6 months, yielding a total of 63 scans. Data Acquisition: Data were collected on a 3T Siemens Trio system with 32-channel head coil. Data were preprocessed for susceptibility and eddy-current distortions using FSL. A two-tensor with Unscented Karman Filter method was used to delineate whole-brain tractography in each individual’s original diffusion space4. After whole-brain tractography was derived, these tracts from infants were mapped onto a fiber atlas created from the Human Connectome Project (HCP). 58 deep fiber tracts and 198 superficial fiber tracts were delineated5.

RESULTS: Whole-brain tractography in infants remain similar in terms of morphology as that in adults, resulting in less challenges in aligning whole-brain tracts. 58 deep white matter tracts were identified to form anatomically meaningful tracts in each infant’s original space. Figure 1 shows that using this method, subsidiary association fibers with protracted development such as SLF III can be probed and studied for their developmental changes.

DISCUSSION: The current framework overcomes these issues by performing tractography analysis on a large number of tracts, in the infant’s original diffusion space, and without requiring manual delineations. Thus, this framework may potentially generate more reproducible results and can be leveraged to study a large number of white matter tracts in infants.
Infants preferentially attend to facial areas that support their developmental goals. Attention to the eyes supports social development, while attention to mouths varies depending upon the mouth’s adaptive value for learning. For example, when first making speech sounds, attention to mouths increases; while once language skills become advanced, it decreases. Relatedly, trajectories of preferential looking to eyes and mouths vary by language exposure: as compared to monolingual infants, bilingual infants preferentially attend to talking mouths for a protracted developmental period. These processes, however, have not been extensively studied in children exposed to multiple languages who also have either autism spectrum disorder (ASD) or other developmental disabilities. Here, we investigate how language exposure affects attention to faces in toddlers with ASD or with other developmental disabilities.

Parent-reported home language and eye-tracking data were collected from N=74 toddlers (Mage(SD)=25.09(6.54) mos.); all had some degree of English and non-English exposure. For language-exposure level, those reporting <40% non-English were labeled “partially bilingual” (n=48), while those reporting 40-70% non-English were labeled “fully bilingual” (n=26). Each group was individually-matched to a comparison monolingual sample on chronological age, non-verbal cognitive function, sex, and diagnosis (ASD/Non-ASD). All children were developmentally assessed using standardized instruments. Eye-tracking data were collected while toddlers watched videos of daycare settings and were quantified by measuring percentage of fixation to eyes, mouths, bodies, and objects.

ANOVA analyses revealed no main effect of language exposure on fixation time (all p>0.05) and no interaction between diagnosis and language exposure (all p>0.05). Analyses showed a significant main effect of diagnosis on fixation to ROIs: toddlers with ASD fixated on eyes and mouths significantly less than toddlers without ASD and significantly more on bodies and objects (all p<0.05).

In a sample of toddlers with and without ASD, language exposure did not significantly affect visual fixation to eyes and mouths. Instead, although toddlers in this sample without ASD had other developmental disabilities, their levels of fixations to eyes and mouth were greater than children with ASD, regardless of language exposure. These results show promise for the use of eye-tracking-based measures to identify children with ASD with varying home languages.
Typically developing (TD) infants show shifting patterns of visual engagement to faces over the first two years of life. These patterns of visual engagement differ in infants with autism spectrum disorder (ASD). ASD diagnoses are 4 times more common in males than females, and studies of early social visual engagement (SVE) suggest that ASD females demonstrate increased attention to faces relative to ASD males during parallel play. ASD females without intellectual disability also exhibit more developmentally-appropriate language skills than ASD males. The present study examines sex-based differences in associations between SVE throughout the first two years of life, and language development/social disability at age two, in a longitudinally-followed cohort of ASD and TD infants. We collected eye-tracking data at 5 (NASD=40(10F),NTD=93(44F)) and 15 months (NASD=45(12F),NTD=80(34F)), while infants watched videos of naturalistic caregiver interactions. Eye-tracking data were quantified as percentages of fixation to regions-of-interest (eyes/mouth/body/object). Language and autism symptomatology were assessed using the Mullen Scales of Early Learning and Autism Diagnostic Observation Schedule (ADOS), respectively, at 24 months. Associations between eye-tracking measures of SVE and language/autism symptomatology were tested via Spearman correlations. Eye-looking during infancy positively predicted receptive language scores at 24 months in the TD group (rfemale=0.2981,pfemale=0.0494; rmale=0.3085,pmale=0.0305), while increased mouth-looking during the second year of life was a significant positive predictor of expressive language scores at 24 months in TD females only (rfemale=0.3771,pfemale=0.0279). Patterns of SVE to the face were unrelated to later language in the ASD group, but eye-looking negatively predicted ADOS total scores; this association was driven by ASD females (rfemale=-0.6728,pfemale=0.0330; rmale=-0.1163,pmale=0.5406). In ASD females only, mouth-looking positively predicted ADOS total scores (rfemale=0.7784,pfemale=0.0080). These findings indicate that during the first two years of life, patterns of visual fixation to the face have differential adaptive value, based on sex and diagnosis. Future research will investigate sex- and diagnosis-based trajectories of SVE to the face, and their differential relationships to later language and social ability outcome measures.
The Systematic Observation of Red Flags for Autism in the First Year of Life for Infants at Risk

Pileggi, Moira; Brane, Natalie; Bradshaw, Jessica; and Wetherby, Amy

Corresponding Author: Moira Pileggi, MS, Emory University, moira.lewis@choa.org
Center: Marcus Autism Center
Type: Clinical or Translational
Keyword(s): autism screening
Related to Pilot Grant or Trainee Award: No
Poster Available: No

BACKGROUND: Sibling studies demonstrate ASD symptoms can be detected as early as 12-months (Bryson, et. al, 2008; Watson et. al, 2013; Zwaigenbaum et. al, 2005), highlighting opportunity for earlier screening. Systematic Observation for Red Flags (SORF, Wetherby et. al, 2016) is an observational tool based on DSM-5 criteria to quantify ASD-symptoms. The SORF was validated to differentiate 16-24 month-olds with ASD versus developmental delay and typical development (Dow et al., 2017). Given the pressing need for earlier detection, this study examined utility of the SORF among 12-month-old high-risk (HR) infants.

In a prospective sibling study, we examined: 1) SORF sensitivity & specificity at 12-months for predicting ASD at 24-months, 2) associations between 12-month SORF and 24-month phenotypic/developmental outcome.

122 infants were assessed at 12/24-months of age. Participants were HR (N=45,) or low-risk (LR; N=77). CSBS-DP and Mullen Scales were administered at 12-months. The SORF was coded via video review of the CSBS, measuring ASD symptoms across 2 domains: Social Communication & Social Interaction (SC) and Restricted Repetitive Behaviors (RRB). At 24-months, participants received a diagnostic evaluation and clinician best-estimate diagnosis.

RESULTS: 12-month-olds later diagnosed with ASD scored significantly higher than TD infants on SORF SC and RRB domain, and overall composites. Infants with ASD had significantly more SC and total red flags (RFs), with differences in RRB RFs approaching significance. Highest sensitivity & specificity was observed for SORF Composite cutoffs of 18, which identified 24 of 31 ASD infants, yielding sensitivity of .77 and specificity of .76. Optimal cutoff for SORF Total RFs was 7, with 20 of 31 infants having 7+ RFs at 12 months, yielding sensitivity of.65 and specificity of.75. SORF composite and SC domain scores at 12-months were highly correlated with ADOS-2 CSS and verbal and nonverbal skills at 24-months.

CONCLUSIONS: 12-month-olds later diagnosed with ASD exhibited significantly greater SORF total and SC domain scores compared to TD-infants. SORF Composite cutoffs of 18 resulted in higher sensitivity & specificity than SORF RF cutoff of 7. The high sensitivity and specificity at 12-months highlights the need for earlier screening studies including larger samples of high-risk and community-based infants.
Engagement is critical for learning – information that does not engage cognition, even when looked at, will go unprocessed and will not be learned (Simons & Chabris, 1999, Perception). Consequently, successful social adaptive action depends upon selectively engaging with things that have the greatest behavioral relevance. Despite the individualized ways that viewers engage with the social world, no studies have quantified what viewers themselves perceive to be engaging. Therefore, the way in which engagement modulates social brain activity remains unknown. Here, we examine brain activation when typically-developing children look at faces that they themselves perceive as highly engaging compared to faces that they perceive as less engaging. Simultaneous functional MRI and eye-tracking data were collected while 8- to 12-year-old children (n=12) watched naturalistic videos of children interacting. Eye-tracking was used to identify moments when viewers looked at a face, assessed via eye-fixation data, and moments when viewers were ‘highly engaged’ or ‘less engaged’ with those faces, assessed via patterns of eye-blinking. Eye-blinking indexes engagement by capitalizing on the fact that eye-blinks interrupt the flow of visual information; as a result, viewers unconsciously adjust the timing of their eye-blinks in order to avoid missing critical information: probabilistically, the more engaging the information is to the viewer, the more likely she will be to inhibit blinking (Shultz, Klin, & Jones, 2011, PNAS). Whole-brain voxelwise regression analyses were performed using FSL’s FEAT, with each condition (‘highly engaging faces’ and ‘less engaging faces’) modeled with a boxcar function convolved with a single-gamma hemodynamic response function. Preliminary whole-brain analyses (z=2.3, cluster corrected at p<.05) reveal increased activation in bilateral occipital cortex, left middle temporal gyrus, bilateral posterior cingulate, left frontal orbital cortex, inferior frontal gyrus, right angular gyrus, and right fusiform gyrus, when viewing faces perceived as ‘highly engaging’ compared to ‘less engaging’. These findings suggest that even when viewing the same stimulus category (e.g. faces), one’s own engagement with the stimulus modulates brain activation, even in canonical face processing areas like the fusiform gyrus. This novel insight is critical for understanding how subjective engagement with the world shapes typical development of the social brain.
High levels of concordance in eye- and mouth-looking between monozygotic twins, paired with reduced concordance in dizygotic twins, highlight the influence of genetic variation on social visual engagement (Constantino et al., 2017; Kennedy et al., 2017). Here, we present data from a dizygotic twin pair discordant for MECP2, the X-linked gene encoding methyl-CpG-binding protein 2 (MeCP2): one twin is typically-developing, while the other has MECP2 duplication syndrome. Children with MECP2 duplication syndrome share many phenotypic features with ASD and are often diagnosed with ASD (Ramocki et al., 2009). By examining concordance in social visual engagement between these twins, we hope to illuminate specific genetic factors that influence behaviors relevant to the early ontogenesis of autistic social disability.

Each twin participated in longitudinal eye-tracking sessions, from which developmental trajectories of social visual engagement were produced. Twin-twin concordance analyses quantified degree of similarity between these trajectories. Additionally, Mullen and ADOS assessments measured expressive and receptive language, motor skills, and degree of social disability.

Each twin showed distinct trajectories of social visual engagement: in the affected twin, face-looking declined dramatically in the first 2 years, from greater than 90% to ~10%, offset by markedly increased body- and object-looking; in contrast, face-looking in the unaffected twin increased, from ~70% to ~90%, with diminishing body- and object-looking. For eye- and mouth-looking specifically, MECP2 duplication clearly disrupts twin-twin concordance. Notably, absolute levels of eye-looking are most disrupted; when magnitude differences are accounted for, twins’ relative levels of eye-looking are surprisingly concordant (ICC=0.64, p=0.04). This effect is specific to eye-looking: relative levels of mouth-looking are uncorrelated and remain so even when magnitude differences are corrected (ICC=0).

These results indicate that disruption in social visual engagement in these twins appears specific to active seeking of social information in the eyes. In addition, while prior results suggested similar levels of genetic influence on both eye- and mouth-looking, the current comparisons indicate separable influence, likely driven by genetic influence on eye- more than mouth-looking. Together, this reveals a means by which a specific genetic alteration may impact social visual engagement and emergence of autistic social disability.
Socioeconomic status (SES) impacts many aspects of child development, including language acquisition, but the mechanisms by which SES interacts with ASD risk to affect outcome are not well understood. It is unclear whether two main factors, access to resources (household income) and parental environment (education and occupation), show profiles that differ for ASD, and whether any such differences are significant in relating autism to language.

The goal of this study was to test whether SES indices measured at birth predict early trajectories of vocal development and later language outcome, in autism and typical development.

As part of an NIH Autism Center of Excellence, we tracked vocal development in 45 high-risk siblings and 35 low-risk controls by making monthly home audio recordings of each child from 0-24 months. Using speech recognition technology, we counted the number of vocalizations per hour for child and adult, and the rate of contingent interactions. Using Functional Data Analysis, we calculated developmental trajectories for each child. We modeled each trajectory as an exponential curve with scale and exponent parameters to separately characterize overall dosage and change over time. Mullen receptive and expressive language scores were collected at 24 months. SES indices included household income and the Hollingshead Four-Factor Index.

Comparing the two SES indices, we found significant differences between risk groups, with the high-risk group scoring lower on both. We found significant correlations between both SES indices and expressive/receptive language scores overall, but no significant difference between risk groups, indicating that SES impacts language outcome regardless of autism risk. We found significant correlations between both SES indices and the scaling parameter of adult volubility trajectories, but no other parameters, suggesting that SES affects the amount of parental input, but does not affect either child volubility or vocal contingency, or change over time. In contrast, our previous research showed that rate of change over time in vocal contingency, not adult and child volubility, is the main predictor of diagnosis and language outcome in high-risk infants. This suggests that the mechanism by which SES influences language outcome is common across risk groups and unrelated to autism.
A Randomized Controlled Trial (RCT) Comparing Parent-Implemented Versus Clinician-Implemented Naturalistic Developmental Behavioral Intervention in Infant Siblings Identified as At-Risk for Autism Spectrum Disorder (ASD): Preliminary Results

Reed, Sandra; Woodard, Camille; Bedol, Hallie; Coe, Elizabeth; and Stapel-Wax, Jennifer

Corresponding Author: Sandra Reed, BA, Emory University, sandra.reed@choa.org
Center: Marcus Autism Center
Type: Clinical or Translational
Keyword(s): Autism Spectrum Disorder
Related to Pilot Grant or Trainee Award: No
Poster Available: Yes - P42

BACKGROUND: Children with ASD demonstrate improved outcomes from early intervention including vastly improved chances of both learning functional language and placement into a typical kindergarten class. The median age of diagnosis is 4-5 years (Baio, 2018), contributing to the paucity of research for evidence-based intervention targeting very young children. Given that a reliable diagnosis can be made by 24 months of age, earlier intervention that maximizes early social and language development is key to better outcomes. Additionally, McDonald et. al. (2019) provided a familial recurrence rate of 16-36% for the disorder; thus there are many children potentially at risk who can benefit from early intervention. There is a need for more research into early intervention, specifically targeting younger siblings of children with ASD. Prior research targeting children under 24 months has focused on parent training and implementation of naturalistic approaches (Bradshaw et. al., 2014).

METHODOLOGY: One study of a P50 Autism Center of Excellence grant initiated a 2-stage sequential multiple assignment randomized trial (SMART) design to study intervention with the total sample of high and low risk infants and their families. At assignment #1 at 6 months of age, families are randomized to usual care versus an online portal of guides to their infant’s development. At assignment #2, the families are re-assigned to usual care versus an online portal of guides to their child’s development or a parent implemented versus clinician implemented intervention for infants found to be at risk for ASD at 12 months of age.

PRELIMINARY RESULTS: To date, 172 participants have been enrolled. At 6 months of age, fifty have been randomized to receive preventative parent education and forty-three to usual care. At 12 months of age, thirty toddlers have reached assignment #2 and have been randomized to either usual care or preventative parental education. Thirty-eight of the enrolled total sample are considered “high risk” due to a confirmed diagnosis of ASD in an older sibling. Twelve toddlers have been identified with early symptoms of ASD. This review will illustrate the methodology, provide preliminary descriptive results of toddlers eligible for treatment and highlight variables impacting future research.
Exploring 5-HT2 Receptors as Targets for Treating Epilepsy in Fragile X Syndrome: A Preclinical Study of Fmr1 Knock-out Mice

Saraf, Tanishka; Chen, Yiming; Armstrong, Jessica; and Canal, Clinton

Corresponding Author: Tanishka Saraf, BS, Mercer University, Tanishka.Satyajit.Saraf@live.mercer.edu
Center: Marcus Autism Center
Type: Basic
Keyword(s): Fragile X Syndrome, Seizures, Lorcaserin
Related to Pilot Grant or Trainee Award: No
Poster Available: Yes - P43

Recent clinical data report that the serotonin 2 receptor (5-HT2R) agonist, lorcaserin abates seizures in children with Dravet and Lennox-Gastaut syndromes. Lorcaserin’s neuropharmacotherapeutic effects are dependent on 5-HT2CR activation. At high doses, it also activates other 5-HTRs, including 5-HT2ARs. The present study evaluates the efficacy and possible 5-HT2AR and/or 5-HT2CR mechanism(s) of lorcaserin to treat audiogenic seizures (AGS) in juvenile (P23–P25), Fmr1 knock-out (KO) mice, a genetic model useful for studying fragile X syndrome (FXS). FXS is a monogenetic, neurodevelopmental disorder characterized by cognitive disabilities, severe anxiety, autism, and sensory hypersensitivities. AGS in Fmr1 KO mice closely models seizures seen in ~30% of individuals with FXS. The experimental design includes male and female, wild-type (WT) and Fmr1 KO mice (FVB strain) treated with vehicle (WT and Fmr1 KO mice), 1, 3, 5.6 or 10 mg/kg lorcaserin (Fmr1 KO mice only), that are exposed 30min later to a 120-dB alarm for 5min. Separate groups are pre-treated with M100907 (0.03 mg/kg) to discern contributions of 5-HT2ARs. Scored behaviors include wild-running and jumping, tonic-clonic seizures with full recovery, and tonic-clonic seizures progressing to respiratory arrest. Membranes from vehicle-treated WT and Fmr1 KO mice brains are collected for saturation binding experiments to determine binding site densities of 5-HT2ARs (frontal cortex) and 5-HT2CRs (striatum and choroid plexus) using [3H]Ketanserin and [3H]Mesulergine, respectively, with appropriate cold ligands to block off-target binding. AGS prevalence in vehicle-treated, WT and Fmr1 KO mice was ~10% (2 of 21) and 75% (15 of 20), respectively. Lorcaserin 3 mg/kg decreased AGS prevalence [~56% (10 of 18)] and lethality caused by AGS by 32% (P=0.0585, Fisher’s exact test), relative to vehicle. AGS onset latency was unaffected by lorcaserin 3 mg/kg, however, it was significantly increased with 5.6 mg/kg (P=0.0479, Kruskal Wallis test). Lorcaserin also did not affect the duration of seizure. M100907 pre-treatment 10 min before lorcaserin 5.6 mg/kg administration did not affect prevalence, seizure onset latency or seizure duration. Preliminary results from 5-HT2AR and 5-HT2CR saturation binding assays suggest decreased expression of both receptors. Results from this project could guide repurposing of 5-HT2 agonists to treat seizures in FXS.
Direct Instruction Language for Learning Program to Promote Expression in Children With Autism Spectrum Disorder (NCT02483910)

Scahill, Lawrence; Shillingsburg, Alice; and McCracken, Courtney

Corresponding Author: Lawrence Scahill, PhD, MSN, RN, Emory University, Lawrence.Scahill@emory.edu
Center: Marcus Autism Center
Type: Clinical or Translational
Keyword(s): autism
Related to Pilot Grant or Trainee Award: No
Poster Available: No

BACKGROUND: Language impairment is a common parental concern for children with ASD. Untreated language impairment is highly predictive of negative long-term outcomes for children with ASD. Direct Instruction-Language for Learning (DI-LL) is a commercially available intervention package with demonstrated effectiveness in children with language delays due to disadvantaged backgrounds, learning disabilities, or a primary language disorder – but it has not been carefully studied in ASD.

OBJECTIVE: Describe essential features of DI-LL and present preliminary results of a completed randomized clinical trial of DI-LL in 83 children with ASD (>4 and < 7 years 11 months) and moderate language delay.

METHODS: Children were randomly assigned in 1:1 ratio to continue treatment as usual (TAU) or DI-LL plus TAU for six months. The primary outcome was the Clinical Evaluation of Language Fundamentals administered at baseline and endpoint by a speech pathologist who was blind to treatment assignment. The key secondary outcome was the Improvement item of the Clinical Global Impression, rated by clinician also blind to treatment assignment. DI-LL was delivered twice per week for 90 minutes per session for 6 months by bachelor-level research staff trained to reliability. The study was approved by the Emory IRB.

RESULTS: 137 children were screened; 83 (85% male, mean age of 5.6 + 1.0 years) were randomized to TAU or DI-LL + TAU. Children with mild or severe language delay, those not in a stable TAU program or on stable medication, or those in need of alternative treatment were excluded. Based on parent report, 45% were black, 22% white, 11% Asian, 16% other. The average IQ and CELF scores were 74.7 +17.1 and 53.3 +10.5, respectively. Enrollment stopped on October 1, 2019; 79 children have completed the randomized trial. The remaining 4 participants will reach endpoint by March 20, 2020. Preliminary results will be available by May 2020.

CONCLUSION: DI-LL is a carefully scripted and sequenced program designed to teach basic and increasingly complex language skills. If this study supports the efficacy of DI-LL in children with ASD, it will provide an exportable intervention for a wide range of practitioners.
A Case Control Study of Food Selectivity and Bone Health in Boys With Autism Spectrum Disorder (ASD)

Sharp, William; Burrell, Lindsey; Wawrzonek, Addam; Berry, Rashelle; McCracken, Courtney; Gillespie, Scott; Loechner, Karen; and Scahill, Lawrence

Corresponding Author: William Sharp, PhD, Emory University, william.sharp@choa.org
Center: Marcus Autism Center
Type: Clinical or Translational
Keyword(s): Bone Density; Autism
Related to Pilot Grant or Trainee Award: 2018, MAC, A Case Control Study of Food Selectivity and Bone Health in Children with Autism Spectrum Disorder (ASD) (MPI: William Sharp, PhD and Karen Loechner, MD, PhD)
Poster Available: No

BACKGROUND: Childhood and early adolescence are critical periods for bone accrual that influence bone mineral density (BMD) and future fracture risk. Available evidence suggests children with autism spectrum disorder (ASD) may be at elevated risk of decreased BMD. Diet may contribute to compromised BMD in ASD. Food selectivity is common in children with ASD and ranges from mild to severe. The purpose of this pilot study is begin a line of research to confirm the association between severe food selectivity and decreased BMD.

OBJECTIVE: To evaluate the feasibility of using clinical methods to define levels of food selectivity and whole body DXA scan as an outcome, we conducted a case control study in school-age boys with ASD.

METHOD: In a case control study, we compared bone health measures in school-age boys with ASD and severe food selectivity to boys with ASD and mild food selectivity, as well as healthy boys without ASD or food selectivity. We set out to enroll 30 boys (6-10 years of age) divided into three groups: (1) boys with ASD and severe food selectivity (n=10); (2) boys with ASD with mild or no food selectivity (n=10); and (3) boys without ASD and mild/no food selectivity (n=10). The comprehensive assessment included nutritional status, height, weight, routine blood and urine tests and whole body DXA scan to assess BMD. The study was approved by the local IRB.

RESULTS: 120 children were screened; 28 were enrolled as of 3/10/2020 (93% of 30 recruitment goal). We anticipate recruiting two participants by April 2020. Feasibility outcomes will include successful completion of all study measures. Preliminary results on key outcome measures (BMD; nutrition status) will be available by May 2020.

CONCLUSION: As a prerequisite for future large-scale studies, this study demonstrated that parents and children can successfully complete study measures including DXA scans. The findings will support extramural grant applications to replicate and extend the study of food selectivity and bone health in children with ASD and to evaluate the efficacy of intervention models (e.g., behavioral methods to expand diet, the role of vitamin supplementation) and cost-effectiveness of these approaches.
Signal or Noise? Investigating Discrepancies Between Eye-Tracking and Clinician-Based Assessments of Social Function


Corresponding Author: Shalini Sivathasan, MA, Emory University, shalini.sivathasan@choa.org
Center: Marcus Autism Center
Type: Clinical or Translational
Keyword(s): autism, assessment
Related to Pilot Grant or Trainee Award: No
Poster Available: No

BACKGROUND: Eighty percent of parents of children with autism spectrum disorder (ASD) report suspecting developmental problems before their child’s second birthday. Yet, despite the presence of early concerns, the median age for diagnosis using best practice behavioral measures remains between 4-5 years of age. Eye-tracking technologies show promise for objectively quantifying biomarkers of atypical social visual engagement, and have been shown to reliably detect differences between toddlers with and without ASD. However, better understanding of the discrepancies between eye-tracking and clinical behavioral assessments is needed.

OBJECTIVES: To examine, in a sample of clinically referred patients, cases that are discrepant between eye-tracking-based measures of social disability and clinical gold standard assessments of ASD in children between 16-42 months.

METHODS: We focus here on 2 groups of participants in which eye-tracking measures and standardized assessments were at odds, with one set of measures indicating relatively intact social developmental functioning while the other indicated impairment. Participants were recruited through a rural community clinic and an urban academic medical center. Social visual engagement data were collected via eye-tracking. Psychologists blind to eye-tracking results completed comprehensive developmental assessments (e.g., Mullen Scales of Early Learning, ADOS-2).

RESULTS: Among participants whose eye-tracking measures indicated impaired social developmental functioning—but who did not meet criteria for an ASD diagnosis (i.e., false positives, n=30)—~77% instead received a clinical best estimate diagnosis of another diagnosis. In contrast, participants who received an ASD diagnosis and had impaired or delayed scores—but did not have eye-tracking measures indicating impaired social functioning (i.e., false negatives, n=29)—fell into two sub-groups: one with seemingly-engaged eye-tracking data, and another in which eye-tracking measures were of poor quality. Additional analyses of individual participant metrics and demographics against standardized assessments and diagnoses given will be presented.

CONCLUSIONS: The goal of this ongoing work is to investigate the clinical utility of eye-tracking-based measures of social developmental functioning for identifying ASD in early childhood. Preliminary results indicate that, in cases when eye-tracking-based measures of social disability are discrepant from other clinical assessments, there is meaningful signal that may aid in early identification generally and in identification of complex cases in particular.
Impacting Early Language Development Through Pre-Licensure and Established Practice Training for Nurses in Three Settings: An Exploration of Implementation Variables

Brasher, Susan; Ross, Kimberly; Becklenberg, Amy; and Stapel-Wax, Jennifer

<table>
<thead>
<tr>
<th>Corresponding Author:</th>
<th>Jennifer Stapel-Wax, PhD, Emory University, <a href="mailto:jennifer.stapel-wax@emory.edu">jennifer.stapel-wax@emory.edu</a></th>
</tr>
</thead>
<tbody>
<tr>
<td>Center:</td>
<td>Marcus Autism Center</td>
</tr>
<tr>
<td>Type:</td>
<td>Clinical or Translational</td>
</tr>
<tr>
<td>Keyword(s):</td>
<td>Infants and Toddlers, Language Nutrition, Nurse Training,</td>
</tr>
<tr>
<td>Related to Pilot Grant</td>
<td>No</td>
</tr>
<tr>
<td>or Trainee Award:</td>
<td>No</td>
</tr>
<tr>
<td>Poster Available:</td>
<td>No</td>
</tr>
</tbody>
</table>

BACKGROUND: Language Nutrition is the use of language that is sufficiently rich in engagement, quality, quantity, and context that nourishes the child neurologically, socially and linguistically. Language learning is highly influenced by the responsive and engaging nature of interactions between young children and their caregivers, showing an impact from the first three years of life on language and cognitive skills more than 10 years later. Maximizing the power of the nursing workforce is a key strategy to increasing caregiver awareness of the importance of language nutrition and empowering families to enhance the quality and quantity of interactions with their child. Talk With Me Baby (TWMB) is a language nutrition coaching initiative that trains professionals, who interact with families, to talk with their babies in order to create a language-rich environment that supports healthy early brain development.

AIMS: The goal of this project is to understand the most effective time and setting to introduce the concept of early language coaching to a nurse—pre-licensure or during established practice—in order for the concepts to be adopted and implemented in practice, and spread throughout the workforce. Further, we aim to examine barriers and facilitators to feasibility of TWMB training including acceptability, adoption and sustainability. Through a mixed-methods approach using focus groups and survey design, this project focuses on three groups: nursing students, public health nurses and NICU nurses, all of which have received the TWMB training. Findings from this study have the potential to enhance future implementation of TWMB trainings.

RESULTS: To date, we have developed three focus group interview guides to conduct qualitative focus groups of NICU nurses, public health nurses, and pre-licensure nursing students. We have also created an online survey to be disseminated to each of these three groups. The pre-licensure focus group consisting of seven nursing students has been completed and the data analyzed, providing extensive and insightful feedback about their experience with TWMB training and application of TWMB techniques in the clinical setting. Focus groups with NICU nurses and public health nurses are forthcoming. Results of this study will be analyzed and presented at the time of this conference.
Examination of Tube Feeding Schedules and Oral Intake: A Retrospective Chart Review

Waddle, Caitlin

| Corresponding Author: | Caitlin Waddle, MS, Children's Healthcare of Atlanta, Caitlin.Waddle@choa.org |
| Center: | Marcus Autism Center |
| Type: | Clinical or Translational |
| Keyword(s): | Tube Feeding, Feeding Disorders |
| Related to Pilot Grant or Trainee Award: | No |
| Poster Available: | Yes - P44 |

BACKGROUND: Children who have complex feeding disorders with organic and behavioral red flags require treatment with a multidisciplinary feeding team, but children with milder feeding difficulty can benefit from consultation with a pediatric dietitian 1,2. Often parents are encouraged to feed their child orally, but are unsure how to adjust the enteral feeding schedule to optimize provision of oral intake. If parents are not provided guidance on adjusting the child's enteral feeding schedule, it may lead to lack of progress with oral feeding. Stimulation of appetite is a critical component of increasing oral intake and weaning tube feeding 3. Intermittent daytime bolus feeding is often recommended for appetite stimulation as it is thought to be more physiological and preserves hunger and satiety cycles. 4,5,6,7 Overnight feeding is recommended, as well, to enhance appetite, but little research has compared the use of overnight feeding to a daytime bolus schedule in terms of improving oral intake. 6,7

METHOD: We will conduct a chart review of tube dependent children that were evaluated by the multidisciplinary feeding team between January 1, 2018 to May 31, 2019. Eligible participants will have received enteral nutrition via nasogastric or gastrostomy tube. Children will be excluded if they were receiving appetite stimulant medications at the time of evaluation. Children will be divided into two groups: continuous feeds and daytime bolus feeds.

RESULTS: The percentage of calories from enteral nutrition versus oral nutrition will be compared between groups of children with continuous and daytime bolus enteral feeds to evaluate any differences in oral food consumption between groups.

CONCLUSION: We anticipate that children on a daytime bolus tube feeding schedule will have a higher percentage of calories from oral food intake versus children on a continuous feeding schedule. These results could have significant impact on clinical recommendations for tube feeding regimen to promote oral intake.
Social Visual Engagement Patterns Have Differential Adaptive Value in Predicting Early Language Outcomes

Yuan, Yixin; Koirala, Sanju; Parmaksiz, Deniz; Shultz, Sarah; Klin, Ami; Jones, Warren; and Edwards, Laura

Corresponding Author: Yixin Yuan, BS, Emory University, yixin.yuan@emory.edu

Center: Marcus Autism Center
Type: Clinical or Translational
Keyword(s): Autism Heterogeneity
Related to Pilot Grant or Trainee Award: 2016, MAC, Neural Correlates of Perceived Salience in Children with Autism Spectrum Disorders (PI: Sarah Shultz, PhD)
Poster Available: Yes - P45

Heterogeneity in autism spectrum disorder (ASD) presents obstacles in identifying causes of the disorder and designing targeted interventions to increase social functioning. Measures capturing the core underlying features of ASD, such as reduced interest in socially adaptive stimuli, may provide a means for parsing phenotypic heterogeneity in ASD. Given the developmental nature of ASD, our study aims to examine whether ASD subjects characterized by different levels of social disability diverge in their social visual engagement strategies even in the earliest years of life, and whether such differences reflect or predict distinct adaptive strategies for language development and outcomes. 59 ASD subjects were divided into subgroups based on their 24-month ADOS social affect (SA) scores. The high-ADOS group encompassed 33 ASD subjects with SA≥10 (24 males; MSA=13.58, SDSA=2.62); the low-ADOS group comprised 26 ASD subjects with SA<10 (18 males; MSA=5.69, SDSA=2.54). Between-group differences in percent fixation to regions of interest (ROIs) were assessed using repeated-measures ANOVA. Within-subgroup correlations were tested for associations between fixation to ROIs and 24-month Mullen Receptive Language (RL) and Expressive Language (EL) age equivalent scores. We found that high-ADOS group subjects looked less at the eyes and more at the mouth, body, and objects than those in the low-ADOS group, with differences reaching statistical significance in the second year of life (Mage=18.56months, group*ROI F=3.62, p=0.0145). While social visual engagement was unrelated to 24-month language outcomes in the high-ADOS group, in low-ADOS subjects higher levels of eye-fixation in the first and second years of life predicted higher EL outcomes at 24 months (Mage=4.07, r=0.625, p=0.0042; Mage=24.73, r=0.504, p=0.0073, respectively). In conclusion, less socially impaired infants with ASD look more at the eyes throughout early development, and show increased social adaptive value of eye-looking, compared to more socially impaired ASD infants. This suggests that fundamentally different social learning processes may be at play within subgroups of individuals with ASD, from the first years of life. Future work will use longitudinal modelling procedures to examine trajectories of social visual engagement as predictors of language and social outcomes within these subgroups of children with ASD.
Automatic and Early Detection of IV Infiltration Using Non-Invasive Sensing

De, Subhendu; Mabrouk, Samer; Inan, Omer; Maher, Kevin; Basu, Rajit; Antes, Traci; and Rodriguez, Zahidee

Corresponding Author: Subhendu De, MD, Emory University, sde3@emory.edu
Center: Pediatric Technology Center (PTC)
Type: Technology
Keyword(s): IV Infiltration
Related to Pilot Grant or Trainee Award: No
Poster Available: No

BACKGROUND: Intravenous (IV) therapy is received by more than 85% of hospitalized patients in the US. A risk of IV use is dislodgement of the IV catheter or compromise to the vein leading to extravasation of an irritant into the surrounding perivascular or subcutaneous tissue (infiltration), which can lead to local edema, discomfort, pain, ischemia, and necrosis (death of surrounding tissue). Adult patients receiving IV therapy can communicate pain and swelling associated with IV infiltration leading to repositioning or removal. However, infants and young children receiving IV therapy cannot or do not communicate in time, which can lead to skin, muscle, and tendon compromise leading to debilitation and disfigurement. Current methods of IV infiltration detection include caregiver protocols assessing IVs at regular intervals for swelling, color change, and decreased perfusion and determining extravasation risk (medication, concentration, volume). Automatic detection of an IV infiltration within 30 minutes would represent an improvement in patient safety over the standard of care.

METHODS: In a pilot study involving patients receiving IV therapy in the Cardiac and Pediatric Intensive Care Units, an investigational non-invasive sensor will be used to continuously monitor IV sites alongside institutional IV monitoring protocols. Data collection will include changes in electrical bioimpedance, strain (swelling), and skin temperature. We hypothesize that a change in electrical bioimpedance of more than 10% will be detected by sensors with infiltrations greater than 5ml before observation of symptoms by caregivers. All patients will be enrolled after informed consent, and age, extremity, and treatment will be collected.

RESULTS AND FUTURE DIRECTION: 200 patients in the CHOA between the ages of 0-21 receiving continuous IV infusions will be included. Data collected from the devices will be analyzed using custom algorithms. Cases of IV infiltration will be compared to cases without infiltration (controls). Data collection is ongoing and results are pending and will be available at the time of the conference. Results will be used to guide improvement of the device and detection algorithms with the eventual goal of decreasing the incidence of IV infiltration.
Using Virtual Agents and Activity Monitors to Track and Assess Autonomous, Self-Determined Physical Activity Among Young Children: A 6-Week Feasibility Field Study

Hahn, Lindsay; Rathbun, Stephen; Schmidt, Michael; Johnsen, Kyle; Annesi, James; and Ahn, Sun Joo (Grace)

Corresponding Author: Lindsay Hahn, PhD, University of Georgia, lindsay.hahn@uga.edu
Center: Pediatric Technology Center (PTC)
Type: Technology
Keyword(s): virtual reality, obesity
Related to Pilot Grant or Trainee Award: No
Poster Available: No

The majority of youth fail to get the recommended amount of physical activity (PA), and there is a precipitous decline in PA among children as they get older. Guided by self-determination theory and social cognitive theory, we designed an interactive, mixed reality PA intervention for 6-10-year-old children. Capitalizing on the features of consumer-grade interactive communication technologies, the intervention features a kiosk-based system that houses a virtual agent programmed to encourage children to set autonomous PA goals. This intervention aims to resolve many practical challenges in designing and administering a personalized, intrinsically motivated PA intervention for this age group. We tested the feasibility of this kiosk across six weeks with N = 42 child-parent dyads. The kiosk tracked and logged children’s daily PA and engagement with the intervention without having to rely on human reporting, provided tailored evaluation and feedback whenever children requested it, informed parents about their child’s PA progress, and employed a virtual agent (a dog) to offer social support to children. The virtual agent prompted users to set PA goals, and as children met these goals over time, their personalized dog became happier, more fit, and better at tricks. Each time a child engaged with the kiosk the system automatically sent a text message to his/her parent with details about the child’s PA progress. The current study demonstrated the kiosk’s feasibility in the field over 6 weeks, illustrating the potential of using interactive technologies as tools for disseminating self-sufficient, and truly self-determined health interventions for children at scale.
Artificial Intelligence With Convolutional Neural Networks Predicts Clinical Status in Neonates Following the Norwood Procedure

Aljiffry, Alaa; Xu, Yanbo; Sun, Jimeng; Biswal, Siddharth; Hong, Shenda; and Maher, Kevin

Corresponding Author: Kevin Maher, MD, Emory University, maherk@kidsheart.com
Center: Pediatric Technology Center (PTC)
Type: Clinical or Translational
Keyword(s): machine learning, intensive care
Related to Pilot Grant or Trainee Award: 2017, CDD, Screening for Agents that Stimulate HIV-1 Replication and Expression in Macrophages for HIV Cure (PI: Christina Gavegnano, PhD)
Poster Available: No

BACKGROUND: Artificial Intelligence is expected to revolutionize medicine, allowing clinicians to obtain knowledge from large data sets. We utilized our BedMaster(TM) waveform data set along with the electronic medical record to develop an algorithm that can detect differences in clinical status in neonates following the Norwood procedure. The Norwood procedure is performed on patients with Hypoplastic Left Heart Syndrome and remains one of the highest risk surgical operations performed in children, with a persistent morbidity and mortality associated with the procedure. Having additional information to determine the clinical status would enhance the care of these patients.

METHODS: A convolutional neural network (CNN) algorithm was designed and trained on 250,000 thirty-second ECG waveforms collected from 51 neonates who were postoperative from the Norwood procedure, utilizing several billion data points. The algorithm was a 34-layer convolutional neural network based on ResNeXt 1 architecture was developed in our lab and applied to the waveform and EMR data set. We formulated our problem as a binary classification task: labeling all the patients’ data observed in the first two ICU days after surgery as ‘critical’ (class 0), and labelled the 44 patients’ data on the last day prior to transfer from the ICU as ‘healthy’ (class 1). The model was tested on a cohort of 10 consecutive infants undergoing the Norwood procedure.

RESULTS: The algorithm reached a 92.4 (±12.1)% Receiver Operating Characteristic curve (ROC-AUC) when using a single lead ECG, increasing to 95.2 (±7.8)% for three ECG Waveforms leads, distinguishing the critically ill vs the stable patient. The final model that combined all input signals obtained produced a ROC-AUC of 98.0 (±3.4).

CONCLUSIONS: Application of a Convolutional Neural Network algorithm to ECG waveforms and EMR data can accurately detect changes in clinical status as patients progress from critically ill to stable following the Norwood procedure. This represents a novel application of artificial intelligence in the management of the critically ill pediatric patient and may enhance patient assessment and clinical decision making.
Effect of Powered Exoskeleton Assistance on the Gait Pattern of Pediatric Patients With Walking Disabilities

McLain, Bailey; Lee, Dawit; Kang, Inseung; and Young, Aaron

Corresponding Author: Bailey McLain, BS, Georgia Tech, bmclain3@gatech.edu
Center: Pediatric Technology Center (PTC)
Type: Technology
Keyword(s): robotic exoskeleton
Related to Pilot Grant or Trainee Award: 2020, PTC Imlay Innovation Fund, Enhancing Rehabilitation in Children with Genu Recurvatum Walking Gait Using Robotic Exoskeletons (PI: Aaron Young, PhD)
Poster Available: No

In the US, approximately 500,000 children have Cerebral Palsy (CP) (cerebralpalsy.org). Of those, 66% walk with crouch gait (excessive knee flexion) and 15% walk with Genu Recurvatum (knee hyperextension) (S.A. Rethlefsen et al. 2017). Current treatment options, including invasive surgical operations and botulinum toxin injections, lack supporting evidence for long-term improvement (C. Bleyenheuft et al. 2009, S.A. Galey et al. 2017). Therefore, there is a need for a successful, noninvasive treatment for children with these walking patterns that demonstrates long-term effectiveness and does not lead to adverse effects.

Utilizing a low-profile, robotic bilateral knee exoskeleton controlled with an impedance controller, we investigated the effect exoskeleton assistance has on the patient’s gait. The impedance controller utilizes an encoder at the knee joint and a foot switch placed on the shoe. The controller assists the user by providing appropriate flexion or extension assistance throughout the gait cycle to help attain a more optimal kinematic pattern. Data were collected with one subject exhibiting genu recurvatum, with the exoskeleton on the subject’s paretic leg. The subject walked over ground and on the treadmill without the exoskeleton, with the exoskeleton unpowered, and with the exoskeleton assistance. Motion capture, electromyography, force plates, gait parameters from a Gait Mat, and subjective subject preference data were collected.

Kinematic results display increased peak knee flexion on the affected leg in the powered exoskeleton condition (34.6 ± 0.4°) compared to no exoskeleton (28.1 ± 1.0°) when walking over ground. Additionally, walking with the assistance resulted in a decrease in step length asymmetry index (ASI). The average step length ASI for no exoskeleton (16.9 ± 11.0) was further from perfectly symmetric (0) than the powered exoskeleton condition (13.5 ± 13.7). Furthermore, the unpowered condition had the highest average ASI value (29.6 ± 4.5).

The results indicate the smart controlled robotic exoskeleton can improve the gait pattern of children with atypical walking patterns. Future works include investigating the effect of the assistance on other biomechanical outcomes, including muscle activity and knee joint load, collecting additional subject data, and studying the rehabilitation effect from multiple training sessions with the exoskeleton.
Remote Monitoring of Lung Sounds Using a Novel Wearable Device: A Diagnostic Aid to Improve Management of Patients With Chronic Respiratory Disease

Powers, Richard; Au, Yu Kan; Muqeem, Tanziyah; Delmonico, Nicholas; Fauveau, Valentin; Capp, Noah; Kroh, Jason; and Glass, Mitchell

Corresponding Author: Richard Powers, MBA, Georgia Tech, richard@stradoslabs.com
Center: Pediatric Technology Center (PTC)
Type: Technology
Keyword(s): pulmonology, wearable device, digital health
Related to Pilot Grant or Trainee Award: No
Poster Available: No

Pediatric patients with chronic respiratory disease such as asthma or cystic fibrosis may experience difficulty in coordination of care due to challenges articulating symptoms as well as provider assessment variability. There is currently no objective way to continuously track signs and symptoms and measure treatment response. The current standard of care consists of ad-hoc episodic checks of a patient’s respiratory status in which the true character and quality of the patient’s worsening respiratory condition may be missed because of insufficient longitudinal data. The Strados Remote Electronic Stethoscope Platform (RESP) is a novel wearable device being developed as a diagnostic aid for clinicians to extend the temporal and geographic reach of traditional stethoscopy. RESP is comprised of a wearable device, a mobile application, and a clinician portal through which clinicians can review lung sounds. Here, we present the feasibility and utility of the platform as well as the data that has been collected from the device. The platform has been studied in ER, outpatient, and inpatient settings for lung sound collection, classification, and patient assessment. We demonstrate that the device is able to detect adventitious breath sounds similar to a traditional or electronic stethoscope, but that its wearable form allows for greater longitudinal collection of sounds. With the advent of machine-learning technology, frequent lung sound monitoring at home is a practical and cost-effective tool in the management of chronic respiratory diseases. We also present our preliminary results using machine learning algorithms for the classification of lung sounds collected using RESP, demonstrating greater than 90% accuracy. The platform is currently being tested and expanded for use in pediatric patients. The Strados RESP can be used in acute care and home settings to provide data that has largely been difficult to collect, allowing clinicians to glean new insights and potentially intervene early before disease decompensation causes significant morbidity and mortality. Additionally, this platform can provide standardization of objective signs and symptoms no matter where the patient presents (ER, outpatient office, inpatient, home). This technology may be especially useful in pediatric patients who suffer from chronic respiratory disease and lack continuity in their care.
A Machine Learning Model to Detect Anatomical Regions of Interest on Pediatric Foreign Body Series Radiographs

Rostad, Bradley; Richer, Edward; Riedesel, Erica; and Alazraki, Adina

Corresponding Author: Bradley Rostad, MD, Emory University, brostad@emory.edu
Center: Pediatric Technology Center (PTC)
Type: Technology
Keyword(s): Artificial intelligence, Foreign body
Related to Pilot Grant or Trainee Award: No
Poster Available: No

PURPOSE OR CASE REPORT: Foreign body ingestion is common in children. Timely diagnosis of the nature and location of the foreign body is important. A button battery which lodges in the esophagus can quickly cause severe esophageal and mediastinal injury. Machine learning that can detect anatomical regions of interest is an important step in computerized foreign body localization and may result in prioritization of radiographs with mediastinal foreign bodies. The purpose of this study is to develop a machine learning model to identify anatomical regions of interest on pediatric foreign body series radiographs.

METHODS & MATERIALS: The institutional review board approved this retrospective study. The training dataset was created from 357 images selected from foreign body series radiographs acquired between 2007-2017. The images consisted of a variety of frontal views of the neck, chest, abdomen, and pelvis. These images were labeled with bounding box annotations for the following anatomical regions: neck (262), mediastinum (317), abdomen (352), and pelvis (302). This training dataset was used to create the model using the object detector toolkit of the Turi Create framework and YOLOv2 model with a Darknet base network on an iMacPro. The testing dataset consisted of all foreign body series frontal radiographs acquired between 2017-2018, a total of 1679 images. The model was tested on the testing dataset and model sensitivity and specificity for each anatomic region was calculated. Only completely imaged anatomic regions were considered in the analysis; partially imaged anatomic regions were not considered (e.g., the abdomen on a chest radiograph or the mediastinum on an abdominal radiograph).

RESULTS: Sensitivities for identifying anatomical regions of the neck, mediastinum, abdomen, and pelvis were 93.9%, 97.8%, 88.1%, and 90.3%, respectively. Specificities for identifying anatomical regions of the neck, mediastinum, abdomen, and pelvis were 99.8%, 99.8%, 100.0%, and 99.7%, respectively. Overall model accuracy was 94.1%.

CONCLUSIONS: Machine learning can be used to identify anatomical regions of interest on pediatric foreign body series radiographs. This model could be combined with a foreign body detection model to prioritize those exams with mediastinal foreign bodies for radiologist interpretation.
A Machine Learning Model to Detect Ingested Button Batteries and Coins on Pediatric Foreign Body Series Radiographs

Rostad, Bradley; Richer, Edward; Riedesel, Erica; and Alazraki, Adina

Corresponding Author: Bradley Rostad, MD, Emory University, brostad@emory.edu
Center: Pediatric Technology Center (PTC)
Type: Technology
Keyword(s): Artificial intelligence, Button battery
Related to Pilot Grant or Trainee Award: No
Poster Available: No

PURPOSE OR CASE REPORT: Timely diagnosis of foreign body ingestion in children is important, particularly in the case of an ingested button battery. A button battery which lodges in the esophagus can quickly cause severe esophageal and mediastinal injury. It is also important to distinguish an ingested button battery from a coin; a button battery may be misdiagnosed a coin because of its similar radiographic appearance. The purpose of this study is to develop a machine learning model to identify button batteries and coins on pediatric foreign body series radiographs.

METHODS & MATERIALS: The institutional review board approved this retrospective study. The training dataset was created from 228 images selected from foreign body series radiographs acquired between 2007-2017. It included 114 images with ingested button batteries, 57 images with ingested coins, and 57 normal images. For simplicity, only frontal radiographs were used. The type of foreign body was either endoscopically proven or confirmed by the consensus of three pediatric radiologists. The button batteries and coins were labeled with bounding box annotations. This training dataset was used to create the model using the object detector toolkit of the Turi Create framework and YOLOv2 model with a Darknet base network on an iMacPro. The testing dataset consisted of all foreign body series radiographs acquired between 2017-2018, a total of 1678 images (37 button batteries, 347 coins, 211 other foreign bodies, 1083 normal). The model was tested on the testing dataset and model sensitivity and specificity calculated.

RESULTS: The sensitivity and specificity of the machine learning model for button battery detection was 81% and 92%, and for coin detection was 83% and 96%. Only 1% of normal images were false positive for a coin or button battery, but 58% of images with other foreign bodies (not a coin or button battery) were false positive for a coin or button battery. The overall accuracy of the model was 88%.

CONCLUSIONS: Machine learning can be used to identify and differentiate button batteries and coins on pediatric foreign body series radiographs. Further development with a larger training dataset is needed to improve model accuracy.
An Object Detection Machine Learning Model to Identify Rickets on Pediatric Wrist Radiographs

Meda, Karthik; Milla, Sarah; and Rostad, Bradley

Corresponding Author: Bradley Rostad, MD, Emory University, brostad@emory.edu
Center: Pediatric Technology Center (PTC)
Type: Technology
Keyword(s): Artificial intelligence, Rickets
Related to Pilot Grant or Trainee Award: No
Poster Available: No

PURPOSE OR CASE REPORT: Machine learning that can identify and localize objects in an image using a labeled bounding box is called object detection. The purpose of this study is to demonstrate object detection in identifying rickets on pediatric wrist radiographs.

METHODS & MATERIALS: The institutional review board approved this retrospective study. The radiology information system was searched for radiographic examinations of the wrist performed for the evaluation of rickets from 2007-2018 in children less than 7 years old. Inclusion criteria were an exam type of “Rickets Survey” or “Joint Survey 1 View” with reports containing the words “rickets” or “rachitic.” Exclusion criteria were reports containing the words “renal,” “kidney,” or “transplant.” Two pediatric radiologists reviewed the images and classified them as either rickets or normal. The images were annotated according to their classification by drawing a labeled bounding box around the distal radial and ulnar metaphases. The training dataset was created from those images acquired between 2007-2017 inclusive. This included 264 normal wrists on 142 images and 104 wrists with rickets on 61 images (most images had bilateral wrists). This training dataset was used to create the object detection model using the Turi Create framework and YOLOv2 model with a Darknet base network on an iMacPro. The testing dataset consisted of those images acquired during 2018. This included 37 normal wrists on 20 images and 20 wrists with rickets on 10 images. The model was tested on the testing dataset and model sensitivity and specificity calculated.

RESULTS: Of the 20 wrists with rickets in the testing set, 16 were correctly identified as rickets, 2 incorrectly identified as normal, and 2 were not labeled. Of the 37 normal wrists in the testing set, 33 were correctly identified as normal, 2 incorrectly identified as rickets, and 2 were not labeled. This yielded a sensitivity and specificity of 80% and 95% for wrists with rickets and 89% and 90% for normal wrists. Overall model accuracy was 86%.

CONCLUSIONS: Object detection can be used to identify rickets on wrist radiographs. Further development is needed to improve model accuracy and validate model generalizability.
Promoting Oral Wound Healing Using Immunomodulatory FTY720-loaded Polymer Scaffolds

Toma, Afra; Kamalakar, Archana; Amanso, Angelica; McDermott, Anna; Ballestas-Naissir, Samir; Kaiser, Jarred; Willett, Nick; and Goudy, Steven

Corresponding Author: Afra Toma, MS, Georgia Tech, atoma6@gatech.edu
Center: Pediatric Technology Center (PTC)
Type: Clinical or Translational
Keyword(s): wound healing, drug delivery
Related to Pilot Grant or Trainee Award: 2017, PTC Child Impact Grant, Immune Modulatory Nanofibers for Cleft Palate Repair (PI: Edward Botchwey, PhD)
Poster Available: No

BACKGROUND: Orofacial clefts are the most prevalent congenital defect and require palate surgery to allow proper feeding and maxillary growth. Due to adverse healing, 60% of these surgeries fail, leading to oronasal fistula (ONF). The ONF affects the child’s ability to eat, talk, and thus, the overall quality of life. Current clinical care to repair ONF uses human donor tissue but carries risk of infection and allograft rejection. As the oral microbiome is bacteria laden, proper wound healing is difficult without immunomodulatory intervention. We recently showed that locally delivering immunomodulatory drugs using scaffolds can promote a pro-regenerative oral environment and reduce off-target side effects. We hypothesize that delivering FTY720-loaded polymer scaffolds will enhance oral wound healing and reduce the occurrence of ONF.

METHOD: ONF was modeled as a critically sized defect in the oral cavity of C57/B6 mice. On day 0, a midline 1.5 mm injury in the hard palate mucosa was created. Following ONF creation, polymer scaffolds were implanted: blank PLGA thin films and FTY720-loaded PLGA thin films. Mass spectroscopy was used to evaluate polymer scaffolds for drug loading and release kinetics. At days 1, 3, 5, and 7 post ONF formation, mice were euthanized, and hard palate mucosa was harvested. To examine the effects of FTY720 on ONF closure, defect size was quantified using microscopy images and histological staining.

RESULTS AND CONCLUSION: ONF persisted in all treatment groups 1d post injury. All mice, regardless of treatment, had a significant reduction of ONF area over time (p<0.05). In this pilot study, we observed a greater effect of FTY720-loaded PLGA thin films in reducing the ONF defect size compared to control at 7 days (p=0.1). We are now in the process of repeating a fully powered study to observe effects FTY720-loaded scaffolds in complete ONF closure. Future studies will help characterize the mechanism by which localized delivery of FTY720 promotes regenerative environment through recruitment of pro-regenerative immune cells. These findings are of extreme importance as harnessing the effects of immunomodulation for oral wound healing provides greater implication for more personalized and efficacious treatment options.
A method of quick and simple detection of wound infections has yet to become readily available as patients are left to their own discretion of whether to seek medical attention for a possibly infected wound. Biochemically, the bacteria in an infected wound destroy the extracellular matrix of one’s skin, producing basic byproducts. Studies show that the pH of wounds infected by *S. Epidermidis*, *S. Aureus*, or *Enterobacteria* rise by a factor of at least one within a 24 hour period. Moreover, a pH increase consistently precedes the onset of clinical symptoms by at least 24 hours. The goal of this engineering project was to develop a wound dressing that uses pH to allow for the rapid detection of a possible wound infection. This project was executed by obtaining an autoclaved sample of *S. Epidermidis* in LB broth; this process was followed to mimic the exudates of an infected wound, which are a product of an inflammatory response and maintain the same pH as the wound. Meanwhile, I constructed five band-aids using a different band-aid and pH indicator for each. After, I micropipetted 100 µL to 1 mL of LB broth onto the band-aids and revised the construction based on the clarity of the visual color change. I determined that a Water-Block-Clear Band-Aid with a Hydrion 4.5 to 8.5 pH indicator strip is the clearest binary visual guide to determine when the pH of a wound signals infection.
Information About Pilot Grants and Trainee Awards

Pediatric Research Alliance Pilot Grants
Pilot grants referenced with a pediatric research center acronym or “JFF” (Junior Faculty Focused) were awarded through the Pediatric Research Alliance pilot grant program. This program is designed to stimulate new research projects, build new collaborations, and increase extramural funding for pediatric research. The current application cycle ends July 1, 2020. Please click here for more information.

Pediatric Technology Center (PTC) Pilot Grants
The Children’s Healthcare of Atlanta Pediatric Technology Center at Georgia Institute of Technology offers grants through the Imlay Innovation Fund that are intended to support pediatric innovation and discovery efforts between Georgia Tech and Children’s, focusing on practical steps that will lead to clinical impact as well as potential commercial opportunities. These include Quick Wins and Innovation Investment, as well as historical Child Impact Grants. Click here for more information.

Dudley Moore Nursing and Allied Health Research Fund
This fund was established to foster new and grow existing pediatric translational research among Children’s nursing staff and allied health professionals. More information is available here.

Trainee Awards
The Warshaw Fellow Research Award and Buchter Resident Research Award were created to encourage pediatric trainees to engage in high quality research, provide a mechanism to fund trainee projects, and enhance the research environment at Children’s Healthcare of Atlanta. Funds may be used for any type of child health-related research, including basic, clinical, translational, and outcomes research. The next application cycle is expected to occur in spring 2021. Please click here for more information.
All posters provided by abstract authors are available in the following 45 pages, labeled P-1 to P-45.
Impact of cryopreservation on clinical sample transcriptomes assessed by single cell profiling.
Swati S Bhasin¹, Harimander Khalsa², Beena Thomas¹, Georgios Theocharidis², Aristidis Veves², Manoj K Bhasin¹

1 Dept. of Pediatrics, Emory University School of Medicine, 2 Joslin-Beth Israel Deaconess Foot Center, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts, USA

Abstract: Single cell transcriptome profiling has emerged as a powerful technique for dissecting cellular heterogeneity in many different diseases. Traditional bulk analysis approaches average out the gene expression signals which complicates analysis of diverse cell types present in a given tissue sample. In diseases like cancer, it is highly desirable to identify the extent of immune infiltrate as well as the presence of immunosuppressive cell populations for a timely evaluation of response necessary for determining the course of treatment. A major limitation of single cell transcriptomics is that along with freshly prepared high viability single cell suspensions, the technique requires considerable expertise and specialized equipment which is not readily available. One alternative to using fresh single cell suspensions is to viably freeze single cells harvested from a patient. Frozen samples offer many advantages including flexibility to process at a later time and/or ship to another lab/collaborator who have the capability to process single cell samples. Our group is standardizing the protocol for single cell transcriptomics assay of viable frozen bone marrow single cell suspensions of AML patients at Emory University. The aim of our study is to generate a much more granular map of the cellular landscape in AML critical for understanding the disease progression and response to therapy. We have evaluated the efficacy of using frozen samples by comparing the transcriptomes of fresh and viably frozen cells from skin biopsy samples collected in a clinical setting from diabetic and healthy control subjects. Gene expression profiles, cellular compositions and pathways of matched 8 fresh and 6 frozen samples cryopreserved for over 3 months have been compared. In both fresh and frozen samples, we are able to generate transcriptome landscape of major cell types including fibroblasts, adipocytes, immune cells. Our analysis indicates that overall, the single cell transcriptome landscapes of fresh and viably frozen samples are quite similar. Furthermore, the comparative analysis of transcriptome profiles at genes as well as at pathways level shows similar significant. In conclusion, cryopreservation has subtle impact on the transcriptome landscape that does not impact biological conclusions of the studies.

Results1

Comparison of cell numbers and distribution in skin samples from diabetic patients: Fresh vs Frozen

UMAP Plots depict sticking similarity between Fresh and Frozen transcriptome landscape.

Pathways and Regulators based comparison of Fresh and Frozen Clusters

UMAP based comparison of healthy skin tissue

UMAP based comparison of Diabetic foot ulcers

Subtle differences Fresh and Frozen

Cluster 0 Frozen vs Cluster 1 Fresh

Cluster 3 Frozen vs Cluster 3 Fresh

Ribosomal genes among the upregulated genes

Comparable signatures across clusters.

Conclusions

• Overall fresh and frozen transcriptomes are comparable irrespective of diseased or healthy samples.
• Pathways and regulators maps are very similar between fresh and frozen samples.
• Freezing creates subtle changes in expression profiles of mitochondrial and ribosomal genes.
• Cluster differentiating markers do not depict significant differences between fresh and frozen.

Future Plan:

• Dissect the transcriptional landscape in Acute Myeloid Leukemia and T-acute Lymphoblastic leukemia by single cell profiling of frozen bone marrow samples.
• Integrate Single cell proteomics and transcriptomics in same assay to better characterize immune cell populations.
• Identify expression pathways correlating with disease progress/response to therapy in specific cell types.

Funding: We are thankful to Diaomp initiative from NIDDK for funding this study.
Videoconference Support of Siblings of Pediatric Stem Cell Transplant Recipients: A Pilot Study
Rachel S. Hianik\textsuperscript{a} and Mary C. Thomson\textsuperscript{a}, Rebecca J. Schuetz\textsuperscript{b}, Elyse W. Bryson\textsuperscript{b}, Margie Dixon\textsuperscript{a}, Ann E. Haight\textsuperscript{b,c}, Rebecca D. Pentz\textsuperscript{a,c}
\textsuperscript{a}Winship Cancer Institute of Emory University, Atlanta, Georgia; \textsuperscript{b}Children’s Healthcare of Atlanta, Aflac Cancer and Blood Disorders Center, Atlanta, Georgia; \textsuperscript{c}Emory School of Medicine

**Background**
- The American Academy of Pediatrics has recognized child life services as an important tool for helping families adjust to a child’s illness.\textsuperscript{1}
- Previous research has identified that the concerns and needs of donor and non-donor siblings of children undergoing hematopoietic stem cell transplant (HSCT) are often unmet.\textsuperscript{2}

**Objective**
- A pilot study to test an intervention providing siblings of HSCT recipients with certified child life specialist services through videoconferencing.

**Methods**
- We identified families of children undergoing HSCT who had siblings living at home between the ages of 7 and 18.
- Weekly videoconference sessions between the sibling and the certified child life specialist (CCLS) were attempted for 100 days post-transplant.
- The CCLS completed evaluations after each session. After 100 days, parents and siblings were interviewed. Interviews were qualitatively coded and analyzed.

**Results**
- Eight families (12 parents and 8 siblings) were recruited. In total, 43 videoconference sessions were completed.
- On a 10-point scale of usefulness, with 10 being most useful, siblings rated the intervention 8.5 and parents 9.6.
- The most commonly cited positive outcomes were the sibling receiving medical education and 1-on-1 attention from the CCLS.
- Difficulties were encountered with scheduling and internet connection. Suggestions for improvement include reminder messages and a pre-intervention meeting between parents and CCLS.

**Conclusions**
- Providing child life services via videoconferencing to siblings between the ages of 7 and 18 of children undergoing HSCT proved possible, effective at addressing sibling concerns, and was well received by families.

**References**
**Background**
- The majority of children undergoing therapy for B-cell acute lymphoblastic leukemia (B-ALL) develop an infection during their treatment.
- Severe infections can cause increased hospitalizations, delays in chemotherapy, and significant morbidity.
- Intravenous immunoglobulin (IVIG) is used for infection prevention in pediatric B-ALL, but evidence for its efficacy for infection prevention is lacking.

**Objective**
- To describe patterns of hypogammaglobulinemia in pediatric B-ALL and compare characteristics of IVIG recipients and non-recipients.

**Methods**
- IRB-approved retrospective cohort study, participants identified through institutional cancer registry, LEARN cohort, and through querying the electronic medical records for a diagnosis of B-ALL.
- Included: de novo B-ALL, age 1 to 21 years at diagnosis between January 1, 2010 and December 31, 2017.
- Excluded: IVIG receipt prior to diagnosis, participants who moved to another hospital prior to the maintenance phase of chemotherapy.
- Variables for manual and automated abstraction from the electronic medical record.
- Patient demographics, disease characteristics, medication records for IVIG receipt.
- IVIG recipients: Serum IgG value just prior to IVIG initiation.
- IVIG non-recipients: lowest documented serum IgG value.
- Mann-Whitney test and Chi-Square tests for comparisons of characteristics of IVIG recipients and non-recipients.

**Results**

**Overall cohort characteristics**
- 387 participants.
- Median age was 5.2 years (IQR 3.2–9.8).
- 31.7% (n=123) were IVIG recipients.
- For IVIG non-recipients, 54% had an IgG level checked.

**Group comparisons**
- IVIG recipients were younger than non-recipients (5.5 vs 7.6 years, p<0.01).
- Median IgG level differed between non-recipients (669 mg/dL, IQR 490–516) and recipients (410 mg/dL, IQR 311–490) (p<0.01).
- MRD status differed between recipient groups (p=0.05).
- IgG levels of ≤400 mg/dL were present in 45.8% of IVIG recipients vs 6.3% of IVIG non-recipients (p<0.01).

**Conclusion**
- Age, race/ethnicity, MRD, and serum IgG levels differed between IVIG recipients and non-recipients.
- Race/ethnicity differences will be explored, with attention to other disease and treatment variables that may explain this difference.
- Multivariable analyses will examine the predictors of IVIG receipt, and the efficacy of IVIG in recipients.
- Further studies are needed to understand hypogammaglobulinemia in pediatric B-ALL and inform practices of IVIG administration.

**Funding**
- TL1 Postdoctoral Clinical & Translational Research Training Grant/Award Number: TL1TR002382; National Center for Advancing Translational Sciences, Grant/Award Number: UL1TR002378; National Institutes of Health
- Leukemia: Electronic Abstraction of Records Network (P1T Miller)
- Presence of ≥0.01% of leukemia cells on bone marrow flow cytometry evaluation at end of induction chemotherapy
- Died during induction.
Developing and Testing a Portable Psychosocial Intervention for Adolescents with Cancer and Sickle Cell Disease

Sarah E. Moran1, Laura G. McKee1, Lindsey L. Cohen1,2, Soumitri Sii1, Brady Jones3, Meghan Goyer1, Caitlin Shneider1, Jena Michel1, & Matthew Donati1

1Georgia State University, 2Children’s Healthcare of Atlanta, 3University of St. Francis

Introduction

Background:
- Adolescents with sickle cell disease (SCD) and cancer face unique challenges such as higher rates of anxiety and depression (Ketten et al., 2013; Steere et al., 2015) and need for access to effective psychosocial interventions (Edwards & Edwards, 2010; Zierler & Jacobson, 2012).
- Narrative psychology is concerned with the way individuals think about themselves and their experiences and the stories they tell that reflect their perspectives; these narratives are predictive of mental and physical health (Perera & Sagar, 1999).
- Utilizing the Hero’s Journey, a common literary template used across cultures characterized by stages an individual must experience in order to become a hero (Campbell, 1949), may be an effective strategy in constructing a photo-narrative intervention to address psychosocial concerns for adolescents with SCD and cancer.

Phase 1: Methods

- Participants:
  - 9 patients with cancer
  - Mean age = 15.11, SD=2.03
  - Sex: 5 female, 4 male
  - 12 patients with sickle cell disease
  - Mean age = 15.09, SD=2.17 (1 age unreported)
  - Sex: 4 female, 8 male

- Procedures:
  1. Patients invited to be “members of the research team” and to provide feedback to develop a time-limited, online photo-narrative intervention using the Hero’s Journey entitled FOCUS (Framing Opportunities and Challenges Using Stories).
  2. Patients presented with the Hero’s Journey video and recorded presentation reviewing condensed stages of Hero’s Journey: Participants watched two videos reviewing the Hero’s Journey—an animated video explaining the 12 steps of the Hero’s Journey and a recorded presentation that condensed the Hero’s Journey stages—and responded to questions about their experience.
  - (1) Were you familiar with the Hero’s Journey? (2) What did you like and not-like about the videos? (3) How would you describe the Hero’s Journey? (4) What is confusing about the Hero’s Journey?
  3. Patients presented with intervention: Participants were presented with the following intervention and responded to questions about the feasibility of the intervention.

  Intervention: Every day for 10 days, starting after hospital discharge, complete the following activities:
  - 1. Consider where you are in your own Hero’s Journey and tell us what stage you are in.
  - 2. Take a photo of something that was challenging about your day (i.e., challenge photo) and take a photo of something that brought you joy (i.e., opportunity photo) during your day and write a caption each as well as advice for another patient dealing with a similar situation.

- Questions:
  - On a scale of 1 to 10, how motivated would you be to do what we are asking?
  - On a scale of 1 to 10, how confident are you that you will be able to do what we are asking?
  - What might get in the way of doing these activities? What would help you to get past these barriers?

- Patients asked to apply their own experiences to the FOCUS intervention to determine comprehension of the Hero’s Journey and Intervention procedures.

- Indicate which stage of the Hero’s Journey you are currently in.

- Give an example photo of something that was challenging about your day and a photo of something that brought you joy and share what you would write for a caption and what advice you would provide.

- Based on transcripts and coded responses to the questions above, changes were made to the materials subsequently presented in remaining qualitative interviews of Phase 1.

Phase 1: Adjustments Integrated During Phase 1

- 12 stages of the Hero’s journey was rated as confusing
- The recorded presentation reviewing the stages was rated as not interesting

Phase 1: Results

- Adolescents with SCD were both motivated (M=7.54, SD=2.31) and confident in their ability (M=8.41, SD=1.69) to engage in the intervention (see Table 1).
- Adolescents with cancer were also motivated (M=7.81, SD=2.00) and confident (M=8.08, SD=1.59) in their ability to engage with and carry out the intervention (see Table 1).
- Independent samples t-test analyses were utilized to determine if confidence and motivation differed by diagnoses, and results revealed differences were non-significant. Thus, all additional feedback and recommendations will be presented together regardless of diagnosis.

Phase 2 Current Status

- Phase 2, a pilot randomized control trial, is underway to determine if the FOCUS intervention refined in Phase 1 is effective in improving psychosocial functioning in adolescents with SCD and cancer.
- Participants complete the following:
  - 1) Baseline assessment including measures assessing psychosocial functioning
  - 2) Random assignment to intervention (photo taking + daily survey group) or control (daily survey only) condition and are instructed accordingly
  - 3) Upon discharge, participants receive text messages with the web link to indicate their current stage in Hero’s Journey, upload photos, captions and advice, and to complete the daily survey
  - 4) After 10 days, participants complete the follow-up battery assessing current psychosocial functioning.

- Data collection is ongoing (N=48, 6 intervention, control); however, COVID-19 has halted recruitment efforts. Currently, efforts are underway to adjust recruitment efforts and adapt all materials to an online format to safely enroll participants who are predominately immunocompromised.

Table 1: Motivation and Confidence Levels to Carry Out Intervention

<table>
<thead>
<tr>
<th>Intervention</th>
<th>Motivation</th>
<th>Confidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCD</td>
<td>7.54</td>
<td>8.41</td>
</tr>
<tr>
<td>Cancer</td>
<td>7.81</td>
<td>8.08</td>
</tr>
</tbody>
</table>

Table 2: Barriers

<table>
<thead>
<tr>
<th>Barriers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pain</td>
</tr>
<tr>
<td>Fatigue</td>
</tr>
<tr>
<td>Anxiety</td>
</tr>
<tr>
<td>Depression</td>
</tr>
</tbody>
</table>

Table 3: Motivators to Engage in Intervention

<table>
<thead>
<tr>
<th>Motivators</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hero’s Journey</td>
</tr>
<tr>
<td>Opportunity for growth</td>
</tr>
<tr>
<td>Satisfaction with progress</td>
</tr>
</tbody>
</table>

Final Intervention Presentation for Phase 2:

- Present Hero’s Journey video paired with the simplified model of the 3-stages and brief description each stage.
- Participants asked to apply the Hero’s Journey stages to own experience with SCD or cancer.
- Present daily activities to be completed for 10-days after discharge from inpatient: Challenge and opportunity photos along with captions and advice for other teens with SCD and cancer with examples and information on how to submit these to the daily survey.
- Participants asked to think of novel examples of challenges and opportunity photos, captions, and advice.
- Present the final letter of advice to complete after the 10-day intervention: Letter of advice to other teen with SCD or cancer with example letter.

References


MERTK Tyrosine Kinase is a Potential Therapeutic Target in Pediatric Bone Sarcomas

Jessica Yeung¹, Olivia Santos¹, Sherr K. Smari², Xiaodong Wang³, Stephen V. Frye²,³, Shelton H. Earp¹,³, Douglas K. Graham¹,³, Deborah De Ryckere²

¹Emory University College of Arts and Sciences, Atlanta, GA; ²Akh Cancer and Blood Disorders Center; ³Children’s Healthcare of Atlanta and Department of Pediatrics, Emory University, Atlanta, GA; ⁴Center for Integrative Chemical Biology and Drug Discovery and Division of Chemical Biology and Medicinal Chemistry; ⁵Emory School of Medicine; ⁶Children’s Healthcare of Atlanta; ⁷Children’s Healthcare of Atlanta at Chapel Hill; Chapel Hill, NC; ⁸Department of Medicine, UNC Lineberger Comprehensive Cancer Center; Chapel Hill, NC; ⁹Department of Pharmacology, School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC

ABSTRACT

Osteosarcoma (OS) and Ewing’s Sarcoma (EWS) are the most common pediatric bone tumors, yet treatment options are limited. MERTK receptor tyrosine kinase is aberrantly expressed and promotes tumorigenesis in numerous cancer types. We investigated MERTK as a potential therapeutic target in OS and EWS. Publicly-available data were used to demonstrate MERTK RNA expression in OS and EWS cell lines. MERTK mutations in OS patient samples, and functional dependence on MERTK in CRISPR-based screens. MERTK protein was also expressed in three of four OS and both EWS cell lines tested. Treatment with MRX-2843, a MERTK tyrosine kinase inhibitor that is currently in clinical development, decreased cell density in cultures of MERTK-expressing OS and EWS cell lines, with EC50 values ranging from 180 to >1000 nM. The SAOS-2 OS, RD-ES EWS, and SK-ES-1 EWS cell lines were most sensitive, with EC50 values less than 250 nM. Although the remaining MERTK-expressing OS cell lines were less sensitive to MRX-2843 alone, MERTK inhibition enhanced chemosensitivity in numerous cancer types and may enhance the response to chemotherapy in OS and EWS as well. The SAOS-1 OS cell line did not express MERTK and was not sensitive to MRX-2843. These data implicate MERTK as a promising therapeutic target in OS and EWS and support continued investigation of MRX-2843 alone and with cytotoxic chemotherapy for treatment of pediatric bone tumors.

BACKGROUND

- Ewing’s Sarcoma (EWS) and Osteosarcoma (OS) are the two most common forms of pediatric bone tumors.
- Standard treatments for EWS and OS have significant short and long term side effects.
- New treatments are urgently needed. The receptor tyrosine kinase (RTK) MERTK promotes tumor cell survival and proliferation, metastasis, and chemoresistance in a variety of cancer types.
- MERTK is a member of the TAM family of receptors consisting of TYRO3, AXL, and MERTK.

HYPOTHESIS

- MERTK plays a critical role in the proliferation and survival of EWS and OS cells and is a potential therapeutic target in this context.

RESULTS

EWS and OS are functionally dependent on MERTK and associated with MERTK mutations.

REFERENCES


FUNDING & CONFLICT OF INTEREST

DD, DKG, HSE, SVF, and XW hold equity in Merex, Inc. The company is developing MRX-2843, a product evaluated in this research.

ACKNOWLEDGEMENTS

This work was supported by funding from Swim Across America.
Comparison of the Gut Microbiome between Children with Solid Tumors Post-chemotherapy and Age-, Gender-, and Race-matched Healthy Controls

Shuqi Zhou, BSNc\textsuperscript{1}; Melissa Martin, CPNP\textsuperscript{2}; Christie Powell, CPNP\textsuperscript{2}; Thomas Olson, MD\textsuperscript{1,2,3}; Deborah Watkins Bruner, PhD, RN, FAAN\textsuperscript{1,4}; Jinbing Bai, PhD, MSN, RN, FAAN\textsuperscript{1,4}

\textsuperscript{1} Nell Hodgson Woodruff School of Nursing; \textsuperscript{2} Aflac Cancer and Blood Disorders Center, Children’s Healthcare of Atlanta; \textsuperscript{3} School of Medicine; \textsuperscript{4} Winship Cancer Institute

Emory University, Atlanta, GA

**Background**

- Cancer therapies can alter the composition and function of the gut microbiome (GM).
- Characteristics of the GM in children with solid tumors across chemotherapy is unknown.
- Studying the GM with solid tumors post-chemotherapy could help identify potential gut microbes associated with treatment-related gastrointestinal symptoms and toxicity.

**Purpose**

- Profile the GM in children (aged 7-21 years) with solid tumors post-chemotherapy
- Compare GM profiles of these children with age-, gender-, and race-matched healthy controls

**Methods**

- A case-control study was conducted.
- Children with solid tumors post-chemotherapy within 1 year, and healthy controls recruited from CHOIA.
- GM was assessed using stool specimens collected at home following a revised Human Microbiome Project protocol.

**Methods cont’d**

- The bacterial 16S rRNA V4 gene region was extracted and sequenced based on standardized 16S sequencing protocol.
- All bioinformatic analyses (diversity, taxonomy, and abundance) were conducted using QIIME 2.

**Results**

- 27 cancer cases and 22 healthy controls were enrolled in the study with no significant differences in age, gender, race, and BMI (all p>0.05) between two groups.
- There were no significant differences in alpha-diversity metrics (all p>0.05)
- Case and control groups differed significantly in GM beta-diversity based on Jaccard distance (p=0.009) (Figure 1)

**Results cont’d**

- There is a trend of difference (p=0.074) in Unweighted UniFrac distance between two study groups (Figure 2).
- Figures 3 and 4 show the dominant bacterial taxa phyla and genera.

**Conclusions**

- Children with solid tumors showed different GM profiles in beta-diversity and taxa abundance compared to healthy controls.
- Associations between taxa profiles and cancer treatment-related symptoms should be studied.

**Acknowledgements**

- Study participants and their families.
- American Nurses Foundation/Southern Nursing Research Society (PI: Bai); Oncology Nursing Foundation (PI: Bai).
Preliminary Data on Eosinophilic Esophagitis in African American Children from Georgia

Sofia Edwards-Salmon, MD¹, Elizabeth Sinclair, MD¹, Seth Marcus, MD¹,² and Patrice Kruszewski, DO¹,²

1-Emory University School of Medicine, Atlanta, Georgia; 2-Children’s Healthcare of Atlanta, Atlanta, Georgia

BACKGROUND

• Eosinophilic esophagitis (EoE) is an increasingly common inflammatory condition that affects adults and children.

• The demographics of pediatric eosinophilic esophagitis are thought to mainly affect Caucasian males, mirroring adult data¹.

• However, demographics of pediatric EoE have not been thoroughly studied and the state of Georgia is comprised of a significant proportion of African-Americans (30.5% of the population according to 2010 census).

OBJECTIVE & METHODS

• To characterize EoE in African American children in Georgia seen at one of two urban pediatric centers in Atlanta, GA.


RESULTS

• 57.5% of patients had abnormal mucosa at diagnosis and over 40% had a high eosinophilic load (Table 1).

• The most common presenting symptoms were vomiting/regurgitation, dysphagia/feeding difficulties, and reflux, whereas food impaction was rare (Figure 1).

• 50% of African American females presented with reflux compared to 38.5% of African American males

• Fewer females presented with dysphagia (28.5%) compared to males (34.6%).

• Remission was achieved in 21/40 patients, utilizing a variety of single and combination therapies (Table 2).

• Remission results suggest that African American children with EoE present with a higher variety of symptoms including vomiting/regurgitation, abdominal pain, and reflux compared to Caucasians who predominantly present with dysphagia¹,³.

• African American females were more likely to present with reflux than males.

• Remission was most often achieved via PPI alone or combination of PPI and food elimination/elemental diet.

• Further study will detail a larger cohort of pediatric African Americans with EoE and compare them to our overall pediatric EoE population.

CONCLUSION

• Preliminary results suggest that African American children with EoE present with a higher variety of symptoms including vomiting/regurgitation, abdominal pain, and reflux compared to Caucasians who predominantly present with dysphagia¹,³.

REFERENCES

1. Leigh, Lyvia Y. et al. An in-depth characterization of a large cohort of adult patients with eosinophilic esophagitis. Annals of Allergy, Asthma & Immunology, Volume 122, Issue 1, 65 - 72.e1


Table 1: Peak eosinophils (eos/hpf) on diagnostic esophageal biopsy

<table>
<thead>
<tr>
<th>Eosinophils/hpf</th>
<th>Percentage (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15-30</td>
<td>20% (8)</td>
</tr>
<tr>
<td>30-50</td>
<td>17.5% (7)</td>
</tr>
<tr>
<td>50-70</td>
<td>20% (8)</td>
</tr>
<tr>
<td>&gt;70</td>
<td>42.5% (17)</td>
</tr>
</tbody>
</table>

Table 2: Number of patients achieving remission categorized by therapy regimen.

<table>
<thead>
<tr>
<th>Therapy</th>
<th>Number of Patients (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proton Pump inhibitor (PPI)</td>
<td>6</td>
</tr>
<tr>
<td>Food Elimination</td>
<td>1</td>
</tr>
<tr>
<td>PPI + Food Elimination</td>
<td>9</td>
</tr>
<tr>
<td>Topical corticosteroids + Food Elimination</td>
<td>4</td>
</tr>
</tbody>
</table>
Identification of Abnormal Ca²⁺ Transient Data from Human Induced Pluripotent Stem Cell-Derived Cardiomyocytes by Machine Learning Method

Hyun Hwang¹, Rui Liu¹,², Joshua T. Maxwell¹, Jingjing Yang³,⁴, Chunhui Xu¹,⁴,*
¹ Division of Pediatric Cardiology, Department of Pediatrics, Emory University School of Medicine and Children’s Healthcare of Atlanta, Atlanta, GA, 30322, USA
² Department of Pediatrics, The Third Xiangya Hospital of Central South University, Changsha, Hunan 410013, China
³ Center for Computational and Quantitative Genetics, Department of Human Genetics, Emory University School of Medicine, Atlanta, GA, 30322, USA
⁴ Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University, Atlanta, GA, 30033, USA

BACKGROUND

- Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) can serve as cellular models for studying basic biology as well as drug discovery.
- Ca²⁺ transient recording is a popular tool for investigating cardiac functionalities but is labor-intensive and time-consuming.
- Juhola et al. (2015) proposed an analytical algorithm capable of detecting cycling Ca²⁺ transient peaks, extracting peak-level variables, and assessing peak and signal abnormalities

OBJECTIVE

Develop a machine learning pipeline capable of using human expert assessment of Ca²⁺ transient data, as well as analytical algorithm peak/cell signal assessment.

METHODS

Figure 1. Overall workflow of our machine learning method. A. Calcium transient data of 200 cells and 1893 peaks were collected and analyzed to train our peak- and cell-level SVM models, which were validated via LOOCV. B. Testing data of 54 cells and 454 peaks were used to implement our machine learning tool to yield final cell status prediction.

Figure 2. Cells under fluorescence and their peak signals. A. An example of iPSC-CMs stained with Fluo-4 fluorescing under 488nm light. B. An example of calcium transient signal visualized with detected peaks marked. Calcium transient signal of cell number 1 from well B3 is shown. Number of frames on the x-axis and fluorescence intensity on the y-axis. C. Examples of Ca²⁺ transient signal visualized by human expert. Red arrows denote abnormal peaks and green arrows denote inconsistent periods.

RESULTS

- Figure 3. An example peak, its first derivative, second derivative, and peak-level variables. Out of sixteen peak-level variables, eight are indicated: peak left amplitude (A_l), peak right amplitude (A_r), left peak duration (D_l), right peak duration (D_r), maximum value of left side first derivative (Dy_max), absolute minimum of right side first derivative (Dy_min), maximum of right side second derivative (D2y_max), and absolute minimum of right side second derivative (D2y_min). The peak variables extracted were used for peak status prediction via SVM modeling.

- Figure 4. ROC curve plot. A. Training data ROC curve plot. B. Testing data ROC curve plot.

CONCLUSIONS

- The trained machine learning pipeline was tested on testing dataset, which yielded 87% accuracy, 89% sensitivity, and 83% specificity.
- Analytical algorithm from Juhola et al (2015), on the other hand, yielded 83% accuracy, 83% sensitivity, and 83% specificity when applied to the testing dataset.

ACKNOWLEDGEMENTS

We thank Anita Saraf and Antonio Rampildi at the Division of Pediatric Cardiology, Department of Pediatrics, Emory University School of Medicine and Children’s Healthcare of Atlanta for their support on cell culture. We also thank Myra S. Chao at Emory University College of Arts and Sciences for her help with data collection.
Missed Opportunities for Adolescent HIV Diagnosis: Targeted Testing vs. Universal Screening in a Pediatric Emergency Department (PED)

Naomi P. Newton; Claudia R. Morris, MD; Andres Camacho-Gonzales, MD; Lauren Middlebrooks, MD; Elizabeth Duda; Ryan Alevy; Katherine Palmer; Mark A. Griffiths; Colleen K. Gutman, MD; Department of Pediatrics, Emory University School of Medicine, Atlanta, GA and Children’s Healthcare of Atlanta, Atlanta, GA

BACKGROUND

Universal HIV Screening

- CDC recommends starting at age 13 in all healthcare settings, including Emergency Departments (EDs).
- Successfully implemented in adult EDs.
- Targeted testing is the standard of care in pediatric EDs (PEDs); this relies on HIV risk assessment.

Adolescent HIV Screening: Georgia & Nationwide

- 56% of U.S. adolescents with HIV are aware of their diagnoses.
- 2017: Georgia #1 for HIV diagnosis rate
- Targeted testing in high-prevalence PEDs misses HIV until later stages.

OBJECTIVES

- Compare adolescents with PED HIV screening via targeted testing & universal screening.
- Compare providers’ documentation to adolescents’ perception of HIV risk assessment in the PED.

METHODS

Prospective study concurrent with 4-month universal screening pilot
Survey on HIV risk & knowledge if > 16 yrs.

Inclusion Criteria
Ages 13-18, Primarily English- or Spanish-speaking

Exclusion Criteria
- Level 1 on Emergency Severity Index
- Unable to consent (altered, developmental delay)
- Ineligible, per attending physician

Analysis
Descriptive/Agreement statistics, Chi-squared analysis

RESULTS

Study Design Flow Chart

- 3204 Total Adolescent PED Visits (Jun 20-Oct 24, 2019)
- 1480 Adolescent PED Visits when Research Staff Present
- 1345 Adolescents Eligible for Participation
- 432 Not Approached
- 107 Targeted Testing
- 344 Universal Screening
- 462 Declined Screen
- 17 completed survey
- 95 completed survey
- Further Assessment of all 451 Tested Adolescents
- 326 Asked about risk assessment
- 436 with chart review

HIV Risk Assessment by PED Providers

<table>
<thead>
<tr>
<th>Provider Documented HIV Risk Assessment (n = 154)</th>
<th>No Provider Documented HIV Risk Assessment (n = 292)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age, years ± SD</td>
<td>15.8 ± 1.4</td>
<td>15.1 ± 1.5</td>
</tr>
<tr>
<td>Age, N (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>1/152 (0.7%)</td>
<td>53/282 (18.1%)</td>
</tr>
<tr>
<td>14</td>
<td>25/152 (16.4%)</td>
<td>58/281 (20.3%)</td>
</tr>
<tr>
<td>15</td>
<td>14/152 (9.2%)</td>
<td>60/281 (21.4%)</td>
</tr>
<tr>
<td>16</td>
<td>41/152 (27.0%)</td>
<td>60/281 (17.4%)</td>
</tr>
<tr>
<td>17</td>
<td>5/152 (3.3%)</td>
<td>67/281 (23.5%)</td>
</tr>
<tr>
<td>18</td>
<td>10/152 (6.6%)</td>
<td>7/281 (2.5%)</td>
</tr>
</tbody>
</table>

Low-moderate agreement between documentation & perception of HIV risk assessment (k = 0.61, 95% CI 0.41-0.74).

CONCLUSIONS

- Clear disparities in characteristics of adolescents receiving HIV risk assessments & targeted, provider-led HIV testing during PED visits.
- At-risk & HIV+ adolescents missed by targeted testing.
- Adolescent risk factor disclosure may not be reliable.
- Universal screening tests more patients & reduces disparities in PED HIV testing.

LIMITATIONS

- Convenience sample at single center, not randomized.
- Parents present for question about perception of HIV risk assessment, but no difference in kappa on sensitivity analysis of this subpopulation.
**INTRODUCTION**

- Every hour, an American teen dies by prescription stimulant overdose, adding up to over 10,000 deaths in 2019.
- Prescription stimulants, drugs commonly prescribed to teens to treat attention-deficit-hyperactivity disorder (ADHD) or increase alertness, were misused by over 15% (additions of Adderall and Amphetamine use) of high school and college students in 2018.

**PERCENTAGE OF HIGH SCHOOL SENIORS WHO ABUSED PRESCRIPTION DRUGS IN 2018**

<table>
<thead>
<tr>
<th></th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sedatives</td>
<td>25.0%</td>
</tr>
<tr>
<td>Opioids</td>
<td>25.0%</td>
</tr>
<tr>
<td>Antidepressants</td>
<td>3.7%</td>
</tr>
<tr>
<td>Mood Stabilizers</td>
<td>4.0%</td>
</tr>
<tr>
<td>Antianxiety</td>
<td>4.5%</td>
</tr>
<tr>
<td>Transmitters</td>
<td>4.4%</td>
</tr>
<tr>
<td>Allosterics</td>
<td>2.5%</td>
</tr>
<tr>
<td>Amphetamines</td>
<td>1.0%</td>
</tr>
</tbody>
</table>

- As shown in the graph above, generic amphetamines and Adderall (the most common brand name for prescription stimulants) were misused by 12th graders in the US more commonly than opioids and other sedatives.

**METHODS**

- **Step 1:** Materials: Arduino Uno board, breadboard, photometric sensor, TFT display, Wi-Fi modul, jumper wires, Arduino Nano board, EDA sensor electrodes
- **Step 2:** Materials (Gold Nanoparticle Test): Diagnostic Measure, pH Test – (Control Measure). Hydrogen Tetroxidetricloro (III)Trihydroxide (BaA/C4 +HEO), Trisodium Citrate Dihydrate (CBS1Na2O7 +2H2O), Benzoic Acid (CBSOOOH), Hydrochloric Acid (HCl), Sodium Hydrosulf (NaSH), Sodium Chloride (NaCl), Antoinch and litmus indicator
- **Design and development:** Arduino Uno prototyped a mobile system that incorporates eight factors to detect an overdose in real-time.

**PROPOSED SOLUTION**

- **Trial Number**
- **Score (SMS analysis)**
- **Score when composite score**
- **Score when child asks**
- **Score when composite score**
- **Score when SMS was sent**
- **Score when child asks**

**RESULTS – MOOD SWINGS**

- **Determining Optimal Threshold (GBM)**
- The figures below show accuracy, sensitivity, and specificity as the threshold between case and control changes for GBM.
- For euphoria (left), in testing, 0.51 was the score threshold with maximum accuracy, and 0.49 was the score threshold where all three measures are optimal.
- For melancholy (right), in testing, 0.49 was the score threshold with maximum accuracy, and 0.48 was the score threshold where all three measures are optimal.

- **Accuracy**
- The Receiver Operating Characteristic curve plots the False Positive Rate (1 – specificity) against the True Positive Rate (sensitivity).
- For euphoria, GLM testing had an AUC of 0.7852, GBM testing had an AUC of 0.6924, and MLP testing had an AUC of 0.8409 (a 5.57% increase).
- For melancholy, GLM testing had an AUC of 0.7762, GBM testing had an AUC of 0.8384, and MLP testing had an AUC of 0.8618 (a 4.56% increase).
- For euphoria, the average precision increased by 6.59% between GLM and MLP, and for melancholy, the percent increase was 8.10%.

**RESULTS – SWEAT COMPOSITION**

- **Control Measure – pH Test**
- The absorbance spectra for the final anthocyanin-limbo indicator solutions at pH 1, 5, and 8 are shown to the right.
- Two lambda max values were selected based on the spectra, one in which acidic solutions absorbed optimally (520 nm) and one in which basic solutions absorbed optimally (680 nm).
- Absorbance across the pH spectrum at these wavelengths was then measured (shown below) to find the pH range and pKa value.
- pKa value was found to be ~4.15, and based on the fact that the anthocyanin indicator will change color at a pH of approximately 4.75, when 25 microliters of sweat are added, the pH of the strip must surpass pH 4.5 to exhibit a significant color change.
- The volume of the original pH 3 solution to be placed on the strip was calculated as 0.815 microliters. This value was approximated down to 0.750 microliters during strip testing to account for slightly acidic sweat.

**RESULTS – SPASM DETECTION**

- **Network Architecture**
- The architecture of the feed-forward network is shown above. The MLP contains an input layer, two hidden layers, and an output layer.
- The Recutced Linear Unit (ReLU) activation function is used for the hidden layers, and the Sigmoid activation function is used for the output layer.

**RESULTS – PROTOTYPE TESTING**

- **Prototype 1**
- **Overall Strip Evaluation**
- The figures to the right display the final color changes on the strip.
- The bottom half of the strip is the control measure and changes color for all sweat (no matter the composition) and the top half is the diagnostic test.
- The control test indicates that the strip has come in contact with adequate sweat. This would prevent false positives.

**CONCLUSIONS**

- The trial sign algorithm performs with an accuracy of 97.86%, which is much higher than previous PPG-based algorithms.
- The spasms detection algorithm performs under 5 seconds.
- The final mood swing detection model had an accuracy of over 85%. 0.50 was selected as the threshold for emotion determination.
- The system would exhibit a color change which can be detected by a sensor prototype with 97.65% accuracy.
- All eight factors are implemented into the Hero mobile application, and are based on devices that a teen would likely already use in their life.
INTRODUCTION

- Every 100 minutes, another teen takes their life, often with no perceivable reason.
- Suicide is the third-leading cause of death in young people age 15 to 19, and the fear of the prevalence has been in detecting its top risk factor: mental health disorders, particularly depression and other mood disorders.

MOOD DISORDERS

- Mental health disorders affect over 400 million people worldwide, more than cancer, diabetes, or heart disease.
- According to WHO, 1 in 5 adults worldwide is struggling with a mental health disorder.
- 45% of mental health patients begin manifesting symptoms by age 18, but the earliest, most 15 to 19 percent of individuals in high-income countries are not diagnosed.
- The prolonged delay between the initial appearance of symptoms and diagnosis allows for the condition to become significantly worse, often impacting social behavior.

This study focuses on the presence of depression and its top risk factors as described by the DSM-5 Criteria. Whether it be as a pure depressive disorder or as the depressed period of a bipolar disorder.

CURRENT SOLUTIONS

- In 2016, clinical guidance was released by the American Medical Association advising practitioners to screen all patients for mental health disorders.
- According to the National Institute of Mental Health, 43.2% of adults in the United States met criteria for a mental health disorder in 2017.

This app can easily identify detection of mood disorders, and the sooner that the disorder is detected, the less therapy the individual will ultimately need.

School Counselor’s Perspective

- Currently, the number of students who may have a mood disorder includes teachers observing deviations from normal patterns, calling in, and student calling in counselors themselves.
- The school’s platform with 160 mental health organizations, and a counselor visits every week to kids who may be able to afford private mental health counselors.

GENERAL PROJECT INFORMATION

Research Questions: Can a mathematical model identify and quantify the risk factors measured to individually identify language use in comparison to that of an individual with a mood disorder? Hypothesis: [H1] Qualitative linguistic use is compared to that of an individual with a mood disorder. There exist differences in terms of linguistic biomarkers which can be identified by a mathematical model.

Independent Variable: Linguistic Biomarkers

Specific inputs include a group of words:
- Structure (inclusive pronoun, self-referent word usage, etc.)
- Certain topics (reference to drugs, display of outward insecurity, etc.)

Dependent Variable: Mental state of the individual.

MOOD DISORDERS

- A category of mental illness in which the underlying condition primarily affects a person’s persistent emotional state.
- Can be divided into two broad groups: unipolar depressive disorder and bipolar disorder.

This study focuses on the presence of depression and its top risk factors as described by the DSM-5 Criteria. Whether it be as a pure depressive disorder or as the depressed period of a bipolar disorder.

PRODUCT FEATUER

- Gregariousness
- Distractibility
- Meal Dysregulation Disorder
- Depressive Episode Disorder
- Other Mood Disorders
- Major Mood Disorders

RESULTS – GENERALIZED LINEAR MODEL

- Figure 5 shows the number of tweets in each class with the highest percentage of observations per class.

Model Accuracy

- The model was trained on the training data and the accuracy was measured in terms of different factors. The accuracy of the model was 0.7665, which indicates that the model did not overfit to the training data.

RESULTS – GRADIENT BOOSTING MACHINE

- Figure 7 shows a scatter plot of the AUC curve and the standard deviation curve. The AUC curve shows the performance of the model, and the standard deviation curve shows the variation of the performance.

Model Accuracy

- The model accuracy was calculated using the average precision at different thresholds. The average precision was found to be 0.7662, which indicates that the model is performing well.

RESULTS – MULTILAYER PERCEPTRON

- Figure 8 shows the model architecture of the feed-forward deep neural network. The network consists of multiple layers, where each layer processes the data and passes it to the next layer.

Model Accuracy

- The model accuracy was calculated using the average precision at different thresholds. The average precision was found to be 0.7662, which indicates that the model is performing well.

LINGUISTIC ANALYSIS

- As shown in Figures 12, 23, and 35, all four of the linguistic patterns had significant validation scores.
- Linguistic Patterns: indications of outward insecurity, enlarged use of self-referent words, reference to drugs, and phobias.

CONCLUSIONS

- The hypothesis was supported because there exists a meaningful difference in the language use of an individual with a mood disorder when compared to a healthy individual’s language.

APP DESIGN/FUNCTIONS

- Figure 20 shows how the number of coefficients increases as the objective function’s landscape changes in a noisy case.
- Figure 21 shows the number of coefficients selected, AUC increases by 0.017, and the number of coefficients selected is similar to 0.17. The number of coefficients selected is similar to 0.17.

PARAMETER OPTIMIZATION

- The table shows how Mean AUC and LogLikelihood Mean change at different combinations of parameters and training conditions (3 trains x 3 conditions).
- The probabilities depicted in the first two rows are used as the final parameters.

DEVELOPMENT

- iSense is a fully-functional mobile app backed by an Artificial Intelligence model designed for a target audience.

- The next iterations of the existing models could be trained to distinguish between healthy and at-risk teens.

- The model was trained on the training data and the accuracy was measured in terms of different factors. The accuracy of the model was 0.7665, which indicates that the model did not overfit to the training data.

- The model accuracy was calculated using the average precision at different thresholds. The average precision was found to be 0.7662, which indicates that the model is performing well.

- The hypothesis was supported because there exists a meaningful difference in the language use of an individual with a mood disorder when compared to a healthy individual’s language.

- The model accuracy was calculated using the average precision at different thresholds. The average precision was found to be 0.7662, which indicates that the model is performing well.

- The next iterations of the existing models could be trained to distinguish between healthy and at-risk teens.

- The model was trained on the training data and the accuracy was measured in terms of different factors. The accuracy of the model was 0.7665, which indicates that the model did not overfit to the training data.

- The hypothesis was supported because there exists a meaningful difference in the language use of an individual with a mood disorder when compared to a healthy individual’s language.

- The model accuracy was calculated using the average precision at different thresholds. The average precision was found to be 0.7662, which indicates that the model is performing well.

- The next iterations of the existing models could be trained to distinguish between healthy and at-risk teens.

- The model was trained on the training data and the accuracy was measured in terms of different factors. The accuracy of the model was 0.7665, which indicates that the model did not overfit to the training data.

- The hypothesis was supported because there exists a meaningful difference in the language use of an individual with a mood disorder when compared to a healthy individual’s language.

- The model accuracy was calculated using the average precision at different thresholds. The average precision was found to be 0.7662, which indicates that the model is performing well.

- The next iterations of the existing models could be trained to distinguish between healthy and at-risk teens.

- The model was trained on the training data and the accuracy was measured in terms of different factors. The accuracy of the model was 0.7665, which indicates that the model did not overfit to the training data.

- The hypothesis was supported because there exists a meaningful difference in the language use of an individual with a mood disorder when compared to a healthy individual’s language.

- The model accuracy was calculated using the average precision at different thresholds. The average precision was found to be 0.7662, which indicates that the model is performing well.

- The next iterations of the existing models could be trained to distinguish between healthy and at-risk teens.

- The model was trained on the training data and the accuracy was measured in terms of different factors. The accuracy of the model was 0.7665, which indicates that the model did not overfit to the training data.

- The hypothesis was supported because there exists a meaningful difference in the language use of an individual with a mood disorder when compared to a healthy individual’s language.

- The model accuracy was calculated using the average precision at different thresholds. The average precision was found to be 0.7662, which indicates that the model is performing well.

- The next iterations of the existing models could be trained to distinguish between healthy and at-risk teens.
Cell-specific DNA Methylation Patterns of Peripheral Blood Reveals ITGB7 Regulation in CD4+ T Cells Associates with Crohn’s Disease

Kalifa Shabazz, MS1, Sureesh Venkateswaran, PhD2,3, Jason Matthews, PhD2, David Cutter, PhD4, Karen Connelly, PhD1,4, Alicia Smith, PhD1,5, Subra Kugathasan, M.D., M.B.1,2,3
1Genetics and Molecular Biology PhD Program, GDBBS, Atlanta, GA | Emory University School of Medicine, Atlanta, GA | Children’s Healthcare of Atlanta, GA | Emory University Department of Human Genetics, Atlanta, GA | Emory University Department of Gynecology and Obstetrics, Atlanta, GA

BACKGROUND

Crohn’s Disease (CD) is a chronic, remitting and relapsing disorder of the gastrointestinal tract. Recently we showed that peripheral blood cells of CD patients having distinct DNA methylation (DNAm) patterns. However, this study controlled for differences in cell composition between CD cases and controls, which prevented it from identifying differences in specific blood cell types. Mapping DNAm signatures to specific cell types during CD is fundamental in understanding the role of epigenetics in the onset and progression of disease. Therefore, we sought to distinguish cell-specific methylation signatures to identify immune cell type-specific DNAm differences during CD.

METHODS

Blood samples were obtained from 164 CD pediatric patients and 74 non-IBD controls (RISK cohort). Genome-wide DNAm was profiled at ~850,000 sites using MethylationEPIC array. TOOLS for the Analysis of heterogeneous Tissues (TOAST) was used to test for cell-type specific DNAm differences in granulocytes, monocytes, B-Cells, T-Cells (CD4 AND CD8), and natural killer cells that associated with CD. The statistically significant sites were identified after multiple test correction with a false discovery rate of <0.05.

RESULTS

Table 1. Demographics at Baseline and 36 months Follow-Up

<table>
<thead>
<tr>
<th>Risk</th>
<th>Crohn's Disease</th>
<th>Non-IBD</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=164</td>
<td>n=74</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean Age</td>
<td>12.5 +/- 2.56</td>
<td>11.9 +/- 3.13</td>
<td>2.37E-01</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>68 (41.5%)</td>
<td>34 (40%)</td>
<td>5.23E-01</td>
</tr>
<tr>
<td>Male</td>
<td>96 (58.5%)</td>
<td>40 (54%)</td>
<td></td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>126 (76.8%)</td>
<td>67 (72.8%)</td>
<td>9.60E-01</td>
</tr>
<tr>
<td>African American</td>
<td>27 (16.5%)</td>
<td>18 (19.6%)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>11 (6.7%)</td>
<td>7 (7.6%)</td>
<td></td>
</tr>
</tbody>
</table>

CONCLUSIONS

Epigenetic regulation of CD4+ T-cells during CD leads to lower methylation of ITGB7, likely decreasing ITGB7 expression and down regulating lymphocyte trafficking to/from gut-associated lymphoid tissues. Therefore, our results are providing the further evidence that the dysregulation of epigenetic factors affecting CD4+ T-cell function may be a contributor to CD-associated gut inflammation.

ACKNOWLEDGEMENTS

REFERENCES

Expanding the View of Research Impact: Altmetrics of the Georgia CTSA Pediatrics Program

Amber Weber, MPH¹, Anne Fitzpatrick, PhD, RN, CPNP, MSCR¹,², & Eric Nehl, PhD¹, & Nicole Llewellyn, PhD¹

¹Emory University. ²Children’s Healthcare of Atlanta

INTRODUCTION

• Past research was limited in its ability to contextualize different types of research impact over time. Altmetrics offers new ways to showcase attention and influence of research, as a complement to traditional, citation-based metrics.
• Since 2007 the Georgia CTSA Pediatrics Program has supported 93 investigators in authoring 250 pediatric publications. These articles have accrued almost 10,000 academic citations, which is more than 3 times the expected rate.
• These 250 articles have also accrued more than 15,000 Altmetric citations in media, community, and public documents.

RESULTS: PEDIATRIC PUBLICATION ALTMETRICS

OUTCOMES

• Altmetric Attention Scores are a new, innovative way to provide a preliminary picture of how much interest is shown to a publication, an early indicator of the eventual utility and impact that research is expected to have.
• Cumulative Citations, a more traditional-based metric, in academic and public forums provide a measure of the extent of downstream influence an article has had over time.
• Both reflect opportunities for a line of research to move forward along the translational spectrum.
• This research allows us to show how two complimentary metrics can provide a more holistic understanding of aggregate impact of support provided, and identifies the types of research that will have the most eventual impact.

DATA COLLECTION

• Internal records of Georgia CTSA support (e.g. clinical/expert support, pilot/training grants) and acknowledgement of grant support identified a portfolio of pediatric articles published over the past 11 years.
• To assess short-term impact, or ‘splash’ made by articles, we collected Altmetric Attention Scores from Dimensions:
  • 343 news stories
  • 3,441 tweets
• To assess long-term impact, or ‘ripple effects’ of articles, we collected citation data from iCite and Dimensions:
  • 8 patent applications
  • 25 policy documents
• Altmetric citations served as an indicator of research influence and gave examples of how each publication was referenced across various platforms.
• We used overall Altmetric Attention scores and diversity of topics to select 4 example publications.

CONTACT

Contact: amber.adia.weber@emory.edu
Biochemical Characterization of Leukemia Specific SAMHD1 Mutants

Nicole E. Bowen1, Caitlin Shepard1, Jessica Holler1, and Baek Kim1,2

1 Department of Pediatrics, School of Medicine, Emory University, Atlanta, GA. 2 Center for Drug Discovery, Children’s Healthcare of Atlanta.

Background

- In order to preserve genomic integrity, cells must regulate deoxynucleotide triphosphate (dNTP) concentrations such that they are low when the cell is not synthesizing new DNA1.
- SAMHD1 is a triphosphohydrolase that cleaves dNTPs into deoxynucleosides (dNAs) and triphosphates when the cell is not in S phase2.
- Mutations in SAMHD1 were initially identified in Aicardi-Goutières Syndrome (AGS), a rare neurogenic immune disorder3.
- Recently, a series of novel SAMHD1 mutations were identified in several types of leukemia. The impact of these mutations on cancer development remains unknown4,5.

Goal: Characterize the enzymatic activity and structural integrity/stability of four Leukemia-associated SAMHD1 mutants (R145Q, Y155C, P158S, and R366C) in order to elucidate the mechanistic link between SAMHD1 and cancer.

Methods

Protein Purification: The HD domain of the wild type and mutant proteins were expressed in E. Coli using an inducible T7 system. These proteins were purified using a Ni2+ column followed by thrombin cleavage of the polyhistidine tag and subsequent size exclusion chromatography.

dNTPase Assay: Purified proteins were incubated with 1mM of the specific dNTP and 50μM of dgTP, an activator nucleotide, for 40 min at 37°C. Reactions were heat inactivated for 10 min at 65°C. HPLC was used to measure dNTP product formation.

Thermal Shift Assay: Purified proteins were incubated with dgTP and SYPRO Orange dye. A thermocycler was used to monitor the binding and fluorescence emission of SYPRO Orange to the denatured proteins during temperature increase. Tm was determined by plotting the first derivative of the fluorescence emission as a function of temperature, where Tm is represented by the lowest point of the curve.

In vivo Protein Stability: HEK 293T cells were transfected with plasmids expressing SAMHD1 (pLVX-IRES-mCherry). Transfection efficiency was confirmed using flow cytometry and protein levels were analyzed using Western Blots.

Tetramerization Assay: Proteins were incubated with indicated dgTP on ice for 30 min. An equal volume of 2% formaldehyde was added followed by a 15 min incubation at 37°C. 0.25M glycine was used to quench the reaction. Cross-linked proteins were analyzed using SDS-PAGE.

SAMHD1 Leukemia mutants have reduced dNTP triphosphohydrolase activity

Conclusions

- All Leukemia-associated SAMHD1 mutants tested have significantly impaired dNTP triphosphohydrolase capabilities.
- Mutants R145Q, Y155C, and P158S are less stable both in vitro and in vivo than wild type SAMHD1. This instability is likely responsible for their observed reduction in enzymatic activity.
- This work has identified Leukemia mutation R366C as useful for the continued study of the role of SAMHD1 in cancer because the mutant has lost dNTPase activity without global structural changes.

Future Directions

- Experiments are underway to test whether R366C has defects in other SAMHD1 functions that could mediate a cancer phenotype. These include:
  - involvement in homologous recombination after dsDNA break
  - stimulating the repair of stalled replication forks
  - interaction with cell cycle proteins
- Additional experiments are being conducted to test the effect of R366C on cellular proliferation, cell cycle, and apoptosis.

References

1. [References]

Acknowledgements

This work was supported by NIH K120861 and AI054651.

Figure 1: Structural Model of SAMHD1. SAMHD1 forms an active tetramer upon binding dgTP in allosteric site 1 and any dNTP in allosteric site 2. Residues R145, Y155, and P158 are located in these allosteric sites. The active tetramer contains four catalytic sites that can bind and hydrolyze any dNTP. Residue R366 is positioned in the catalytic site and is known to stabilize the y-phosphate of the substrate dNTP. PDB 4dio.

Figure 2: Purified SAMHD1 Leukemia-associated mutant proteins show reduced enzymatic activity. A. SDS-PAGE gel of overexpressed wild type and Leukemia mutant SAMHD1 HD domains after purification protocol. The HD domain of SAMHD1 is predicted to be 58 KDa. M: Molecular weight marker. B. HPLC based dNTPase assay using purified proteins shows that all tested Leukemia SAMHD1 mutants have a reduced ability to hydrolyze substrate dNTPs compared to the wild type (WT) protein. Graphs display mean product formation normalized to wild type protein formation + SD, n=3, ***p<0.01, **p<0.001.

Figure 3: Protein stability and structural integrity profiles of SAMHD1 Leukemia-associated mutants. A. Thermal shift assay displays protein denaturation, indicated by increased relative fluorescence, as a function of temperature. Melting temperature, Tm, is represented by the inflection point of the curve. R145Q, Y155C, and P158S have altered thermal shift curves and lower Tm, suggesting protein instability and global structural changes. R366C, however, retains a thermal shift profile and Tm, comparable to wild type. B. Western Blot showing cellular expression levels of the Leukemia-associated mutants after transfection. R145Q, Y155C, and P158S have reduced protein levels, suggesting these proteins are also unstable in vivo, whereas R366C maintains wild type expression levels. M: Molecular weight marker C. SDS-PAGE gel of chemically crosslinked purified proteins. R366C is able to undergo dgTP activated tetramerization comparable to the wild type protein. Y155C, which was predicted to be unstable, is unable to form this tetramer structure. The SAMHD1 HD domain monomer is predicted to be 69 KDa, the dimer is predicted to be 112 KDa and the tetramer is predicted to be 224KDa. M: Molecular weight marker.

SAMHD1 R366C is the only Leukemia mutant to retain protein stability and structural integrity
Healthcare Access and End-Stage Renal Disease Detection in Children

Nikhila Gandrakota MBBS MPH1; Chia-shi Wang MD MSc1,2; Margret W Kamel PhD1; Laurence A Greenbaum MD PhD1,2
1Emory University, Atlanta, GA; 2Children’s Healthcare of Atlanta, Atlanta, GA

METHODS
• We performed a retrospective chart review of all children who underwent renal transplantation at Children’s Healthcare of Atlanta between 1/1/2010 and 12/31/2018 (N=239)
• Study criteria:
  1. Age 2-17 during ESRD diagnosis
  2. Residing in the Atlanta Metropolitan Statistical Area (MSA).
  3. ESRD diagnosed between 1/1/2010 and 12/31/2018
• We collected patient demographics, insurance status, ESRD cause, insurance type of the patients who met our study criteria (n=98). We examined the proportion of patients with access to primary or nephrology care prior to ESRD diagnosis
• We performed logistic regression on SAS 9.4 to assess the difference in the healthcare access for primary care and nephrology care depending on insurance status & insurance type and calculated the unadjusted odds ratios

RESULTS
• Among all the patients, 7% did not have identified primary care physician and 36% didn’t have an established care with a Nephrologist prior to ESRD diagnosis
• Among uninsured patients at ESRD diagnosis, 13% didn’t have an identified primary care physician and 50% didn’t have established care with a nephrologist prior to ESRD diagnosis
• Among insured patients at ESRD diagnosis, 6% didn’t have an identified primary care physician and 33% didn’t have established care with a nephrologist prior to ESRD diagnosis
• Logistic Regression models showed neither insurance status nor the insurance type is associated with access to primary care physician or nephrologist prior to ESRD diagnosis

CONCLUSION
• A high proportion of children with ESRD did not have prior access to primary or nephrology care. Those without insurance had less access to primary or nephrology care, though the findings are not statistically significant in our small study.

Table 1: Sociodemographic Characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Median(IQR)/N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at ESRD diagnosis</td>
<td>12.5 (7.0)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>60 (61.2)</td>
</tr>
<tr>
<td>Female</td>
<td>38 (38.8)</td>
</tr>
<tr>
<td>Race</td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>46 (46.9)</td>
</tr>
<tr>
<td>African American</td>
<td>38 (38.8)</td>
</tr>
<tr>
<td>Asian</td>
<td>6 (6.1)</td>
</tr>
<tr>
<td>Native Hawaiian or Pacific Islander</td>
<td>1 (1.0)</td>
</tr>
<tr>
<td>American Indian/Alaska Native</td>
<td>2 (2.0)</td>
</tr>
<tr>
<td>Unknown/Not reported</td>
<td>5 (5.0)</td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
</tr>
<tr>
<td>Hispanic</td>
<td>18 (18.4)</td>
</tr>
<tr>
<td>Non Hispanic</td>
<td>80 (81.6)</td>
</tr>
<tr>
<td>Insurance status at ESRD presentation</td>
<td></td>
</tr>
<tr>
<td>Uninsured</td>
<td>16 (16.3)</td>
</tr>
<tr>
<td>Insured</td>
<td>82 (83.7)</td>
</tr>
<tr>
<td>Insurance Type</td>
<td></td>
</tr>
<tr>
<td>Private</td>
<td>36 (39.1)</td>
</tr>
<tr>
<td>Medicaid</td>
<td>56 (60.9)</td>
</tr>
<tr>
<td>Military/VA</td>
<td>2 (2.2)</td>
</tr>
<tr>
<td>Other</td>
<td>1 (1.1)</td>
</tr>
</tbody>
</table>

Table 2: Access to care by insurance status and type of insurance

<table>
<thead>
<tr>
<th>Primary Care Odds Ratio P-value</th>
<th>Nephrology Care β1 (95% CI) P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insurance status*</td>
<td>0.46 (0.8)</td>
</tr>
<tr>
<td>Insurance Type**</td>
<td>2.27 (0.4)</td>
</tr>
<tr>
<td>Insurance Status*</td>
<td>0.49 (0.2)</td>
</tr>
<tr>
<td>Insurance Type**</td>
<td>1.61 (0.3)</td>
</tr>
</tbody>
</table>

*Insured vs Uninsured(reference)
**Private vs Medicaid(reference)
African American Females with Pediatric Autoimmune Hepatitis Present with More Severe Disease and Have Worse Clinical Outcomes

James P. Stevens, MD\(^1,2\), Courtney E. McCracken, PhD\(^1\), Sirish K. Palle, MD\(^1,2\), Kushal B. Naik, MBBS, MPH\(^2,3\), Vasantha Kolachala, PhD\(^1\), Nilika A. Gupta, MD\(^1,2\)  
\(^1\)Emory University School of Medicine, Department of Pediatrics, Atlanta, GA; \(^2\)Children’s Healthcare of Atlanta, Transplant Services, Atlanta, GA; \(^3\)Emory University, Rollins School of Public Health, Dept of Epidemiology, Atlanta, GA

**OBJECTIVE**  
- To analyze a cohort of patients diagnosed with pediatric autoimmune hepatitis (AIH) and assess the relationships between race, biological sex, the severity of presentation, treatment course, and long-term clinical outcomes.

**INTRODUCTION**  
- Autoimmune hepatitis (AIH) is a chronic, often progressive inflammatory condition of the liver. It is characterized by elevated liver enzymes, autoantibody seropositivity, and classic histologic changes on liver biopsy such as interface hepatitis and lymphoplasmacytic inflammation. The underlying etiology is not fully understood.\(^1,2\)
- Clinical presentation is wide-ranging, from asymptomatic laboratory abnormalities to severe acute disease with liver failure (ALF). Given the variability in symptoms, some patients have progressed to end-stage liver disease (ESLD) by the time of diagnosis.\(^2\)
- While it can present at any age, AIH has a bimodal age distribution with a first large peak occurring during teenage years.\(^3,4\)
- Like many immune-mediated processes it more commonly presents in females, who make up between 60-76% of all cases of pediatric AIH.\(^1,4\)
- With the exception of a recent paper by our group, there are few studies analyzing the complex relationships between biological race or gender and AIH specifically in the pediatric population.\(^5\)

**METHODS**  
- Single-site, retrospective chart review within Children’s Healthcare of Atlanta (Atlanta, GA).
- Data search of EMR for all cases of pediatric AIH from January 2000-April 2016, as identified by both ICD-9 and ICD-10 codes.
- Manual chart review of every case for analysis of the following: patient demographics, presentation by labs, imaging and diagnostic codes to determine type of AIH (1,2, or undifferentiated); severity and chronicity at presentation (acute hepatitis, ALF, or ESLD); markers of immune activation at diagnosis; and long-term patient outcomes including remission/relapse, progression to liver transplantation, recurrence after transplant, and death.

**RESULTS**  
- **Table 1: Patient Demographics, AIH Type, and Disease Severity/Chronicity at Diagnosis**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Male</th>
<th>Female</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (Years), Median (IQR)</td>
<td>12 (4-15)</td>
<td>13 (2-15)</td>
<td>0.26</td>
</tr>
<tr>
<td>Gender &amp; Race (n)</td>
<td>Male/AA</td>
<td>Female/AA</td>
<td>Odds</td>
</tr>
<tr>
<td>Non-AA vs AA Females vs AA Males</td>
<td>12/28/7</td>
<td>13/30/10</td>
<td>0.58</td>
</tr>
<tr>
<td>Type 1 (+LMK Ab) AIH</td>
<td>8/8/0</td>
<td>13/13/0</td>
<td>0.68</td>
</tr>
<tr>
<td>Secondary Autoimmune Hepatitis</td>
<td>2/2/0</td>
<td>8/8/0</td>
<td>0.39</td>
</tr>
<tr>
<td>IgG at Presentation, Median</td>
<td>3.6 (2.1-5)</td>
<td>4.2 (2.4-7)</td>
<td>0.096</td>
</tr>
<tr>
<td>High-Dose Steroid Use, N (%)</td>
<td>2/2/0</td>
<td>7/7/10</td>
<td>0.89</td>
</tr>
<tr>
<td>Follow-up (Months), Median</td>
<td>33.1 (10-8)</td>
<td>16 (10-8)</td>
<td>0.05</td>
</tr>
</tbody>
</table>

**Table 2: Patient Treatment Course and Outcomes**

- **Figure 1: Patient Presentation Based on Race and Biological Sex**

**DISCUSSION**  
- We identified 109 individuals in our healthcare system that were diagnosed with pediatric AIH.
- Females comprised 72% of our cohort, consistent with prior reports.\(^6,7\) 34% of our patients were AA Females.
- Males in our cohort presented more often with Type 2 AIH than females, contrary to the same prior studies.\(^7,10\)
- Compared to other patients, AAF had higher rates of ESLD (33% vs. 18%) and ALF (8% vs. 1%) at time of diagnosis (p=0.018).
- Non-AA females had the second highest rates of ESLD (26%, p=0.013 all females vs. males) and ALF (3%).
- ALF was only seen in female patients (5% vs 0%).
- AA patients of both sexes had higher immunoglobulin G levels at diagnosis versus non-AA’s (p=0.05), which may suggest a more significant auto-inflammatory response and may correlate with more severe disease presentation.
- AAF had higher rates of liver transplantation for AIH than other patients (38% vs. 19%, p=0.037), with lower overall transplant-free survival (59% vs. 79%, p=0.029).
- Recurrence after transplantation was higher in AA than non-AA patients (47% vs. 8%, p<0.023).
- Death was only seen in female patients, 10% of females in our cohort died. (14% AAF, 7% non-AA females, 0% all males).

**CONCLUSIONS/FUTURE DIRECTIONS**  
- Female sex and AA race are separately correlated with more severe presentation and worse outcomes in AIH, with AA females having the worst prognosis of all groups both at diagnosis and long-term.
- Future studies must be done to further delineate the biological, psychosocial, and healthcare systemic factors contributing to disparities in AAF with AIH disease with an overall goal to eliminate such disparities.

**REFERENCES**
Assessment of pre-transplant memory T cell phenotypes in children associated with costimulation blockade resistant rejection in adults

DUNETON, C1, GEORGE, R2, FORD, ML1, WINTERBERG, PD2
1 Emory Transplant Center, Emory University Department of Surgery, 2 Pediatric Nephrology, Emory Department of Pediatrics and Children’s Healthcare of Atlanta

BACKGROUND
• Belatacept (CTLA4-Ig targeting the CD28-CD80/86 T cell costimulation pathway) improves patient and graft survival in renal transplant recipients compared to standard-of-care calcineurin inhibitors.
• However, clinical adoption of Belatacept has been limited due to increased early acute rejection rates.
• Our group recently reported that pre-transplant frequencies of CD4+ CD28+ Effector Memory T cells (CCR7-CD45RA-) in adults experiencing early-onset rejection on Belatacept were comparable to healthy controls.
• Conversely, adults that remained rejection-free post-transplant had lower frequencies of CD4+ CD28+ TEM, decreased cytokine production, and increased expression of T cell co-inhibitory pathways.1 2

OBJECTIVES
We aimed to determine if similar T cells populations associated with rejection episodes under costimulation blockade therapy in adults are detectable in children awaiting kidney transplant.

METHODS
We analyzed existing flow cytometry data studying memory T-cell populations and examined expression of CD28, CD57, and PD-1 on CD4+ memory T-cells. Population frequencies were compared via Mann-Whitney test.

RESULTS
Belatacept (CTLA4-Ig targeting the CD28-CD80/86 T cell costimulation pathway) improves patient and graft survival in renal transplant recipients compared to standard-of-care calcineurin inhibitors.

Figure 1. Patient flow diagram. We included 30 children on dialysis and 18 healthy controls from our previously published study of T cell phenotypes.4

Table 1. Patient’s characteristics. Dialysis patients are compared to healthy patients using Mann-Whitney test or Fisher’s exact test.

Table 2. Characteristics of “stable like” patients.

Figure 2. Flow cytometry gating strategy. We examined expression of CCR7, CD45 RA (memory markers), CD28, CD57 and PD-1 on CD4+ T cells.

Figure 3. Children on dialysis had lower frequency of CD28+CD4+TEM (A), but none of them had frequencies as low as those observed in adults that were rejection-free on Belatacept (around 60%).2 However, 8 out of 30 (27%) children on dialysis had CD28+CD4+TEM frequencies below the minimum value we observed in healthy children (B). We next further evaluated other T cell characteristics in these patients with a “stable like” CD28+TEM profile.

Figure 4. These patients with the lowest CD28+CD4+TEM frequencies (“stable like” phenotype), also had higher frequencies of senescence-associated markers on their CD4+ TEM which is reminiscent of the pre-transplant phenotype in adults who were ultimately free of rejection on Belatacept.3 The functional characteristics of this T cell population in dialysis patients needs further study.

REFERENCES

CONCLUSION
A subset of children on dialysis awaiting kidney transplant have lower frequency of CD28+CD4+ effector memory T cells compared to healthy children. These same patients also have increased expression of senescence-associated markers on their CD4+ TEM which is reminiscent of the pre-transplant phenotype in adults who were ultimately free of rejection on Belatacept. The functional characteristics of this T cell population in dialysis patients needs further study.

6/10/2020
Maternal and Adolescent Depression: The Role of Genetic Sensitivity and Telomere Length
Thompson, A.J. & Henrich, C.

Participants:
- Mother-child dyads (N=2,884, n=2,859)
- Children (Male=1,480)
- Nearly half identified as Black or African American
- Most mothers had at most high school degree and were in their mid-twenties at childbirth
- Children in good to excellent health

Results:
- There was a significant effect of MD on maternal report of the CBCL at ages 9 and 15. Higher levels of MD had a small positive effect on child CBCL.
- There was no effect of MD on child-report of the Center for Epidemiological Studies Scale Short-Form (CESD-SF) at age 15.
- There was no support for mediation by child TL.
- There was no support for modulation by child genotype or sex.
- Maternal TL and age had a positive effect on child TL.
- Age 3 CBCL, child race, and child health had an effect on child CBCL.
- Children with higher age 3 CBCL, poorer health, and those who identified as Black or African American had higher CBCL at age 15.

Discussion: This research is the first to test the mediation by child TL hypothesis for the effect of MD on adolescent depressive outcomes. This investigation contributes to our understanding of the biological pathways through which MD leads to child depressive outcomes. We aimed to provide some clarity on how these mechanisms operate in hopes that future research might find ways to act on these mechanisms decreasing the risk for transmission of maternal depression to children.

6/10/2020
Crying Behavior in Infants with Simple Skull Fracture

Varrone, E1; Rindler, R MD, 1; Newman, S MD, 1; Gangavelli, A1; Duhaime, A MD, 2; and Chern, J MD, PhD. 3

1Emory University School of Medicine 2Massachusetts General Hospital for Children, Dept of Neurosurgery 3Children’s Healthcare of Atlanta, Dept of Neurosurgery

Introduction
Isolated, simple skull fractures in young infants commonly occur after a fall from height onto a hard surface and also may be associated with non-accidental trauma (NAT). The frequency of crying behavior in infants presenting with skull fracture has not been quantitated and could potentially affect timing of presentation to medical attention as well as assist with determination of NAT-related injuries.

Aim
The purpose of this study was to describe the frequency with which young infants presenting to the emergency department (ED) with skull fractures were observed to cry at the time of the injury, and to determine how it relates to timing of presentation following injury. A secondary aim was to identify social factors that may increase frequency of social work consults.

Method
Children < 6 months old evaluated in the ED by the neurosurgery service with isolated, simple skull fractures on computed tomography imaging without additional intracranial findings were included in this study. Variables were abstracted retrospectively from a chart review as follows: demographics, presence of crying after initial injury; timing of presentation (immediate < 6 hours, acute 6-24 hours, delayed > 24 hours); attainment of ophthalmology consult; single or two-parent household; whether the child was accompanied by one or both parents in the ED; and whether social work and/or Division of Family and Children Services (DFCS) were involved.

Each child was screened for potential NAT by social workers, per hospital protocol. Any subsequent DFCS referral was made at the discretion of social work or primary managing team. The study was approved by the institutional review board.

Results
Forty-five infants were included in the study (female= 26, 57.7%). The infants presented at a mean age of 94 days, ranging from 18 to 236 days. Most infants cried at initial injury (n=38, 84%). Three injuries were unwitnessed. Almost all children (95%) presented immediately (n=37) or acutely (n=6). Only two children presented in a delayed fashion: one unwitnessed; the other cried on injury. These two were almost 6 months old, compared to mean age of 4 months for the remaining children. Both were seen in the ED due to delayed scalp swelling.

DFCS was notified for a total of 16 patients (35.5%): 14 immediate, 1 acute, 1 delayed). Two of three (66%) unwitnessed injuries were further investigated, and only one deemed non-accidental. All 4 patients that did not cry presented immediately to the ED; 3 (75%) underwent DFCS evaluation, and 2 (50%) were found to be non-accidental.

Thirty-eight children lived in two-parent households (84%). Four (25%) DFCS investigations occurred in single-parent households, which is higher than the percentage of patients living in single-parent homes (n=7, 15.6%).

Eighteen (40%) children were accompanied by both parents to the ED, twenty-five (55%) children were accompanied solely by the mother, and one child was accompanied by the father. Ten (63%) of DFCS investigations occurred in cases where the infants were accompanied by one parent.

Conclusion
Skull fractures in infants are relatively minor injuries that could be missed if patients do not cry following such an event. In this descriptive study, most, but not all, young infants cried immediately following a head injury causing skull fracture and were promptly evaluated in the ED, regardless of their crying behavior. Patients that did not cry did not present in a delayed fashion. Notably, absence of crying did not preclude the determination that the event was caused by an accidental etiology, but only in half of instances. Delayed presentation in our cohort was rare and occurred in response to worsened symptoms. Certainly, crying behavior of children with skull fracture that never present to medical attention is unknown.

DFCS referral was common, regardless of timing of patient presentation or crying behavior. Although the rate for involvement was highest after delayed presentation and for patients that did not cry, these may be spurious results given our small sample size. The true reasons for social work consultation in this cohort is likely provider-dependent and multifactorial, and beyond the scope of this study, though our results suggest that specific social factors could be at play, which should be considered in future work.

The results suggest that although it is possible for children not to cry following injury with skull fracture, absence of crying is rare and likely not a cause of delayed presentation.

References
The Accuracy of Non-Invasive Blood Pressure Measurement in Obese Children

Christopher M. Berry 1, Asaad Beshish M.D. 2, Nikita Figueroa M.P.H. 3, Courtney McCracken Ph.D. 3, Kevin O. Maher M.D. 2, Michael P. Fundora M.D. 2

1 College of Sciences and Mathematics, Auburn University, Auburn, AL
2 Children’s Healthcare of Atlanta, Department of Pediatrics, Division of Cardiology, Emory University School of Medicine, Atlanta, GA
3 Department of Pediatrics, Emory University School of Medicine, Atlanta, GA

Introduction
Obesity and hypertension are important public health priorities in the United States, often occurring simultaneously. Accurate blood pressure (BP) measurement is critical to diagnosis and manage hypertension in children usually using automated devices. The accuracy of these devices is unknown, and may be affected by habitus, misdiagnosing hypertension. We compared intra-arterial BP to non-invasive (NI) cuff BP in obese versus non-obese children.

Methods
- Retrospective 1:1 matched case-controlled
- 100 obese (97-99th %tile body-mass index) and 100 non-obese children after cardiac surgery
- Matched age, sex, race and RACHS-1
- Simultaneous intra-arterial (IA) and cuff BP measurements.
- Intraclass correlation coefficients and Bland-Altman plots used to determine arterial vs cuff agreement
- ICC <0.75 as threshold for agreement
- Approximately 1,877 individual BP measurements were utilized.

Results

- Systolic Blood Pressure
- Diastolic Blood Pressure
- Mean Arterial Pressure

Non-invasive BP measurements underestimate hyper- and hypotension in all children when and may misdiagnose hypertension.

Conclusions
- We found no difference in the accuracy of NI-BP measurement between obese and non-obese children.
- NI-BP demonstrated consistent bias when BP was outside the limits of normal, with lower measurements during systolic hypertension and higher measurements during systolic hypotension.
- NI-BP showed poor sensitivity and high specificity for blood pressure measurements over 130 mmHg in obese and control patients suggesting many patients may have unrecognized hypertension.
Improving Ambulatory Pediatric Blood Pressure Measurement and Management

Brandy M. Compton, MD¹, Diane M. Straub, MD, MPH², Lana Soylu, MD, FAAP³

¹ Pediatric Residency Program, University of South Florida, Tampa FL
² Department of Pediatrics, University of South Florida, Tampa FL

Hypertension (HTN) is underdiagnosed in the pediatric outpatient setting. Children can be especially challenging to obtain an accurate blood pressure (BP) reading, which is affected by environmental stimulation, user training, and cuff size variation. In many instances, when retaken manually, with the correct equipment, BP falls in the normal range. Current literature reports the prevalence of HTN decreases with repeat measurements. For those patients who have true abnormal BP, repeat readings increase the odds of the patient receiving a correct diagnosis.

We aimed to enhance and assess nursing staff competency in:
- Proper technique to obtain accurate BP readings
- Special ambulatory patient populations that need their BP taken

We aimed to enhance and assess resident physician knowledge of:
- Process of verification of abnormal BP
- Correct classification of BP using the 2017 AAP guideline
- Special ambulatory patient populations that need their BP taken

Surveys were completed by 32 and 29 residents pre- and post- intervention, respectively, out of 56 residents total in the USF program. Measures evaluated resident knowledge of process of verification of abnormal BP, categories of BP classification, recognition of special populations, and variable affecting BP which included multiple choice and true/false questions.

Nurse competency was assessed pre- and post- intervention using a comprehensive multiple choice quiz.

Survey results were as follows:

- Resident Knowledge Assessment:
  - Process of verification of abnormal BP: Pre-Intervention 32%, Post-Intervention 65%
  - Categories of BP classification: Pre-Intervention 44%, Post-Intervention 79%
  - Recognition of special populations: Pre-Intervention 32%, Post-Intervention 86%
  - Variables affecting BP: Pre-Intervention 78%, Post-Intervention 79%

- Nursing Competency:
  - Technique protocol comprehension: Pre-Intervention 92%, Post-Intervention 100%

Conclusion
Nurse and resident education along with a specific protocol for BP measurement in the outpatient clinic led to an improved understanding of the AAP best practice guidelines and increased competency in performing BP measurements by nurses. Our next step is to utilize chart review to evaluate if these knowledge and performance increases correlate to clinical change in HTN diagnosis on repeat BP measurements. Additionally, we plan to coordinate a skills assessment for residents to demonstrate proper technique in obtaining BP readings.
Association between Safety Perceptions and Medical Error Reporting among Neonatal Intensive Care Unit Staff

Rachel Culbreth, PhD, MPH, RRT;1 Regena Spratling, PhD, RN, APRN, CPNP;2 Lauranne Scales, BS-RRT;3 Laryssa Frederick, MS, RRT, RPFT, RRT-NPS;1

1. Department of Respiratory Therapy, Byrdine F. Lewis College of Nursing and Health Professions, Georgia State University, Atlanta, GA
2. School of Nursing, Byrdine F. Lewis College of Nursing and Health Professions, Georgia State University, Atlanta, GA
3. Department of Respiratory Care, Piedmont Atlanta Hospital, Atlanta, GA

INTRODUCTION

• Critically ill neonates are particularly susceptible to medical errors.1-4

• Previous research has found strong associations between cultures of patient safety and reduction in medical errors.5

• However, few studies have examined these associations in the context of medical error reporting, specifically in the Neonatal Intensive Care Unit (NICU)

METHODS

• NICU staff members (n=79)

• Safety Attitudes Questionnaire (job satisfaction, teamwork climate, safety climate, perceptions of management, working conditions, and stress recognition)

• Multiple regression used to determine safety attitudes and associated predictors for each hypothetical medical error reporting scenario

RESULTS

Table 1. Safety attitudes domains among NICU nurses and respiratory therapists, (n=79)

<table>
<thead>
<tr>
<th>Safety Attitudes Domains</th>
<th>Median (QIR)</th>
<th>Respiratory Therapists (n=41)</th>
<th>NICU RN's (n=38)</th>
<th>Total (n=79)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Management</td>
<td>28 (20, 35)</td>
<td>25 (20, 27)</td>
<td>30 (20, 37)</td>
<td>28 (20, 35)</td>
</tr>
<tr>
<td>Job satisfaction</td>
<td>23 (21, 24)</td>
<td>24 (20, 27)</td>
<td>24 (20, 27)</td>
<td>23 (21, 24)</td>
</tr>
<tr>
<td>Teamwork</td>
<td>32 (21, 45)</td>
<td>33 (26, 40)</td>
<td>33 (26, 40)</td>
<td>32 (21, 45)</td>
</tr>
<tr>
<td>Work conditions (range 4-29)</td>
<td>16 (13, 19)</td>
<td>17 (14, 18)</td>
<td>18 (14, 18)</td>
<td>16 (13, 19)</td>
</tr>
<tr>
<td>Safety (range 6-30)</td>
<td>27 (24, 29)</td>
<td>27 (21, 35)</td>
<td>27 (21, 35)</td>
<td>27 (24, 29)</td>
</tr>
<tr>
<td>Stress (range 3-15)</td>
<td>12 (10, 13)</td>
<td>9 (7, 12)</td>
<td>11 (8, 13)</td>
<td>12 (10, 13)</td>
</tr>
</tbody>
</table>

Note. Higher scores represent more positive attitudes/perceptions.

DISCUSSION

• Significant differences in job satisfaction and stress were found between NICU RNs’ and RTs’.

• Positive working conditions were associated with increased reporting of major harm pertaining to blood draw errors.

REFERENCES

1. Makary MA, Daniel M. Medical error-the third leading cause of death in the US. BMJ. 2016;353:i2139. doi:10.1136/bmj.i2139
The Effect of Functional Electrical Stimulation Cycling Followed by Over Ground Dynamic Body Weight Support on Gross Motor Skills and Quality of Life In Children with Cerebral Palsy

Erie Eggebrecht, PT, DPT, NCS, Kelly Moore, PT, DPT, Els Van Den Eynde, OTR/L, MBA, Joshua Vova, MD
Children’s Healthcare of Atlanta, Atlanta, Georgia

Background and Purpose
- This study evaluated feasibility and preliminary efficacy of intensive therapy using functional electrical stimulation (FES) and over-ground body weight support on gross motor function in children with mild to moderate cerebral palsy (CP).
- The study looked at therapy completion rate and satisfaction of therapy looking at quality of life in mobility.
- Data was gathered to determine if FES during cycling combined with dynamic body weight support over ground training was superior to over ground gait re-training for children with mild to moderate CP.

Participants and Methods
- CP participated in therapy two times per week, over twelve weeks followed by a month and three month follow up.
  - Comparison group participated in 20 minutes of cycling on the Restorative Therapies 3000 FES bike followed by 15 minutes of over ground ambulation training. The comparison group used their assistive device and braces as needed.
  - Experimental group participated in 20 minutes of FES cycling followed by 15 minutes of over ground ambulation training while receiving dynamic body weight support from the Bioness® Vector gait and safety system.

Outcome Measures
- Gross Motor Functional Measure Form 88 dimensions D/E (GMFM-88, D/E)
- Pediatric Berg Balance Scale (PBBS)
- 10 meter walk test (10MWT)
- Patient Reported Outcomes Measurement Information System (PROMIS)

Results
- Both groups primarily demonstrated gains with functional skills (GMFM-88, D/E) and balance (PBBS).
- Both groups demonstrated a statistically significant difference in their average GMFM scores. Both groups also show a statistically significant change in their quality of life measure in the comparison group.
- The experimental group demonstrated positive changes in mean scores for the GMFM, PBBS, and the PROMIS quality of life measure.

Discussion
- All participants demonstrated improved outcome measures at their three month follow up compared to their pre intervention visit.
- 89% in experimental group, and 61% in the comparison group completed the study. Thus demonstrating feasibility of a 12 week intensive therapy program with follow up visits.

Conclusion
- This study supports previous FES studies and demonstrates improvements in functional skills for participants that use both over-ground or over-ground body weight support training.
- The results are limited due the small number of participants limiting power to detect small effect sizes.
- Our sample consisted of a heterogeneous group of CP children, spread over a wide range of age, which may influence the results.

References
Anxiety and Depressive Symptoms in Adolescents and Young Adults with Epilepsy: The Role of Illness Beliefs and Social Factors

Melissa L. Engel, MA1, Alicia Kunin-Batson, PhD2, Ryan Shanley, MS3, & Peter B. Scal, MD, MPH2

1. Department of Psychology, Emory University; 2. Department of Pediatrics, University of Minnesota; 3. Masonic Cancer Center Biostatistics Core, University of Minnesota

Introduction

Anxiety and depression affect 8-36% of youth with epilepsy.1 However, this may be an underestimate for adolescents and young adults (AYA) with epilepsy, who must jointly navigate the adverse neurobiological effects of seizures and antiepileptic drugs (AEDs) with the complex psychosocial demands of this period.2

Previous studies have examined the relationship between demographic and medical factors (e.g., type of epilepsy, seizure frequency, duration of illness, number of AEDs) and psychological health.3

Less is known about the relationship between factors more malleable to intervention, such as illness beliefs and social factors, with anxiety and depressive symptoms among AYA with epilepsy.

Research Aims

1) Describe the prevalence of clinically significant elevations in anxiety and depressive symptoms in a geographically diverse cohort of AYA with epilepsy

2) Examine the relative contribution of demographic and medical characteristics, illness beliefs, and social factors to emotional distress

Methods

Participants & Procedures

179 AYA with epilepsy (61% female) between 13 and 24 years of age (M = 19.2, SD = 3.6) completed a battery of questionnaires online, by phone, or through a mailed survey

Medical Characteristics

Several epilepsy-related variables were assessed via self-report and classified by an epileptologist

- Epilepsy type (generalized, partial, unknown)
- Time since last seizure (marker of seizure frequency)
- Years since diagnosis
- Number of antiepileptic medications

Illness Beliefs

Illness Cognition Questionnaire assessed beliefs about epilepsy:

- Helplessness (e.g., “My epilepsy controls my life”)
- Acceptance (e.g., “I can cope effectively with my epilepsy”)
- Perceived Benefits (e.g., “Dealing with my epilepsy has made me a stronger person”)

Social Factors

Family Functioning. APGAR Family Functioning Questionnaire assessed 5 key components of family functioning (adaptability, partnership, growth, affection, resolve)

Social Stigma. Enacted Social Stigma Scale assessed perceptions of social rejection or devaluation (e.g., “Do you feel that others have low expectations of you because of your epilepsy?”)

Connectedness. Connectedness subscale of the EPOCH Measure of Adolescent Well-Being examined satisfying and mutually supportive relationships characterized by belongingness, love, and care

Social Factors

Family Functioning. APGAR Family Functioning Questionnaire assessed 5 key components of family functioning (adaptability, partnership, growth, affection, resolve)

Social Stigma. Enacted Social Stigma Scale assessed perceptions of social rejection or devaluation (e.g., “Do you feel that others have low expectations of you because of your epilepsy?”)

Connectedness. Connectedness subscale of the EPOCH Measure of Adolescent Well-Being examined satisfying and mutually supportive relationships characterized by belongingness, love, and care

Results

36% and 35% of AYA reached the cutoff score of 10+ on the GAD-7 and PHQ-9

Linear regression predicting GAD-7 scores from demographic, epilepsy, illness belief, and social factors

<table>
<thead>
<tr>
<th>Measure</th>
<th>Coefficient</th>
<th>Lower 95% CI</th>
<th>Upper 95% CI</th>
<th>p-value</th>
<th>Partial R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>15.38</td>
<td>13.95</td>
<td>16.81</td>
<td>&lt;0.001</td>
<td>0.028</td>
</tr>
<tr>
<td>Age In Years</td>
<td>0.33</td>
<td>0.14</td>
<td>0.52</td>
<td>0.004</td>
<td>0.026</td>
</tr>
<tr>
<td>Female</td>
<td>1.67</td>
<td>0.09</td>
<td>3.25</td>
<td>0.038</td>
<td>0.013</td>
</tr>
<tr>
<td>Seizure Type PE (Y/G)</td>
<td>-0.18</td>
<td>-0.25</td>
<td>0.86</td>
<td>0.777</td>
<td>0.001</td>
</tr>
<tr>
<td>Seizure Frequency (GAD-7)</td>
<td>0.19</td>
<td>0.15</td>
<td>0.23</td>
<td>0.001</td>
<td>0.038</td>
</tr>
<tr>
<td>Years Since Last Seizure</td>
<td>0.39</td>
<td>0.04</td>
<td>0.74</td>
<td>0.001</td>
<td>0.040</td>
</tr>
<tr>
<td>Number of Medications</td>
<td>-0.49</td>
<td>-1.04</td>
<td>0.06</td>
<td>0.360</td>
<td>0.003</td>
</tr>
<tr>
<td>Test Score Depression</td>
<td>-0.56</td>
<td>-0.90</td>
<td>-0.22</td>
<td>&lt;0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>Helplessness</td>
<td>0.94</td>
<td>0.38</td>
<td>1.50</td>
<td>0.001</td>
<td>0.040</td>
</tr>
<tr>
<td>Social Stigma</td>
<td>0.36</td>
<td>0.05</td>
<td>0.67</td>
<td>0.013</td>
<td>0.019</td>
</tr>
<tr>
<td>Connectedness</td>
<td>0.12</td>
<td>-0.08</td>
<td>0.32</td>
<td>0.175</td>
<td>0.008</td>
</tr>
<tr>
<td>Acceptance</td>
<td>-0.34</td>
<td>-0.93</td>
<td>0.25</td>
<td>&lt;0.001</td>
<td>0.051</td>
</tr>
<tr>
<td>Perceived Benefits</td>
<td>-0.06</td>
<td>-1.07</td>
<td>0.95</td>
<td>0.917</td>
<td>0.001</td>
</tr>
</tbody>
</table>

GAD-7 and PHQ-9 scores were highly correlated (r = 0.8); model diagnostics and percent of variance accounted for by linear regressions were similar. Only GAD-7 results are presented due to space constraints.

Conclusions

AYA with epilepsy may experience clinically significant anxiety and depressive symptoms even in the context of more mild neurological symptomatology, supporting routine mental health screenings for this population.

The majority of variance in internalizing symptoms was accounted for not by demographic or medical characteristics, but by potentially modifiable illness beliefs and social factors.

Interventions that promote illness acceptance, enhance family functioning, and reduce social stigma may ameliorate psychological distress among AYA with epilepsy.

This work was supported by the Centers for Disease Control and Prevention as part of the Managing Epilepsy Well Network (special interest project 14-007, cooperative agreement number 1U48DP005022). The findings and conclusions in this study are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

References


@itsMelissaEngel
An Environmental Scan of Human Papillomavirus Vaccination in the State of Georgia

Adrian R. King, MPH CHES1 ; Mary M Gullatte PhD4; Robert A. Bednarczyk, PhD2,3
1. Hubert Department of Global Health, Rollins School of Public Health, 2. Department of Epidemiology, Rollins School of Public Health, 3. Cancer Prevention Center, 4. Woodruff School of Nursing, Emory University

Background

- Low Human Papillomavirus (HPV) vaccine uptake was identified as a priority in the 2014-2019 Georgia Cancer Plan. 1
- In 2017, Georgia ranked 44th of the 50 states and DC for female HPV vaccination initiation and 29th for male adolescent HPV vaccination series initiation. 2,3
- The 2018 National Immunization Survey-Teen (NIS-Teen), estimated that only 68.1% of Georgia adolescents initiated the HPV vaccine series with only 49.6% of adolescents up-to-date for the vaccination series. 4
- Georgia’s diverse population (e.g. racial, ethnic, immigrant status) illuminates a great need for state-specific research to identify effective strategies to improve HPV vaccination uptake in Georgia. 5
- This project was funded as a one-year administrative supplement to the Winship Cancer Institute Cancer Center Support Grant to conduct an environmental scan related to the uptake of HPV vaccine

Specific Aims:

1. Document and evaluate the current state of HPV vaccine promotion, coordination, and uptake, and associated barriers and facilitators in Georgia.
2. Strengthen existing and build new coalitions and collaborations involved in cancer prevention, vaccine promotion, and adolescent and young adult health.
3. Utilize information technology resources to facilitate information sharing.

Methods

- We conducted a comprehensive mix-methods environmental scan of the factors influencing HPV vaccination uptake and completion across the state of Georgia using a variety of qualitative and quantitative methods.

Activities Included:

1. A systematic review of uptake, promotion, and coordination of HPV vaccination activities, research, and efforts specific to the state of Georgia
2. Assessed adolescent vaccine coverage through analysis of Georgia Registry of Immunization Transactions and Services (GRITS) immunization records
3. HPV vaccination coverage visualization developed from this data
4. Conducted 23 focus group discussions with stakeholders involved in vaccination promotion and decision making (e.g., caregivers of adolescents, healthcare providers, community/religious leaders, Georgia HPV Working Group)
5. Has resulted in numerous qualitative and thematic analyses to understand the factors, motivators, barriers, and decision-making processes involved in HPV vaccination promotion and uptake.
6. Inclusion of previously developed EVCI now framing the basis of the EVCI-Ad
7. Developed of an online website ‘HPVCancerFreeGa.org’ to provide curated and specific messaging and education to Georgians on the importance of HPV vaccination and prevention of HPV-associated cancers.

Results

- Analysis and dissemination of results from this Environmental Scan are ongoing.
- To date, we have published findings from of our systematic review of HPV-related research in Georgia and our assessment of the Georgia Cancer Control Consortium (GC3) HPV Working Group. (See table below)
- Qualitative analyses from the collected data focuses on identification and examination of barriers and facilitators to HPV vaccination across the state.
- We have also completed analyses of the perceived motivators and barriers to HPV vaccination amongst healthcare providers in Georgia and on the strategies and techniques most commonly used by providers to recommend and promote HPV vaccination.
- We have completed a case study of a metro-Atlanta church to understand the impact that religious communities can have on HPV vaccination.
- Georgia-specific identification of motivators and barriers to HPV vaccination has been conducted. While many of these have been found to be similar to other states, the methods for addressing and NILTE each must be considered and curated to be effective in the Georgia context.
- Analyses of the communication between caregivers and healthcare providers is ongoing and aimed at understanding the communication styles and techniques which occur and identifying strategies to increase the effectiveness of HPV vaccine recommendations and HPv vaccination promotion, and adolescent and young adult health.
- Our findings on the need for more Georgia-specific information led to the development of the hpvcancerfreega.org website.

Discussion and Conclusions

- We completed a comprehensive mixed-methods examination of HPV vaccination uptake and beliefs across diverse portions of the state of Georgia.
- Addressing subnational-level vaccine uptake data, alongside analysis of facilitator and barriers to uptake, is essential to improving HPV vaccination rates especially in Georgia.
- Data collected from this project has been used to identify that while many nationally recognized and promoted strategies are effective in Georgia, there are strategies which could be considered and implemented to increase the effectiveness of these strategies.
- These include the adaptation of information and educational tools to a region specific context which could increase receptivity to the information and acknowledgment of the importance of HPV vaccination.
- Strengthened communication between healthcare providers and caregivers is needed throughout much of the state.
- Healthcare providers need to consider the benefit of flexibility and adaptability in the methods they use to promote HPV vaccination.
- Development of a trusting relationship with healthcare providers (including physicians, pediatricians, nurses, medical assistants, etc.) is critical to cultivating and improving relationships between caregivers, patients, and healthcare providers.
- Improved trust may result in increased acceptance of healthcare provider recommendations

This project was completed with limited funding. We continue to leverage our available resources, including master’s level thesis students to continue ongoing work from this initial batch of data collected by the project. These analyses result in the continued development of further understanding of HPV vaccination uptake and the factors and processes involved in improving uptake across the state.

Our findings, and ongoing analyses, will continue to support research on, and implementation of, best practices to improve HPV vaccine coverage in Georgia.

Acknowledgements

This project was supported by the National Cancer Institute through an administrative supplement to the Winship Cancer Institute Cancer Center Support Grant (5 P30 CA138292-09S2). Winship Cancer Institute Cancer Center Support Grant (5 P30 CA138292-09S2). The research project was supported by the Intervention Development, Dissemination and Implementation Developing Shared Resource at Winship Cancer Institute of Emory University. This project was also supported by Winship Research Investigators who collaboratively worked with the research team to develop and publish our website. hpvcancerfreega.org

References


STRRSX
Background

- Strong healthcare provider recommendation for HPV vaccination has been found to be a strong motivator encouraging parental acceptance of the HPV vaccine for their child. 1,2
- Uptake and completion of the HPV vaccine series remain suboptimal in the state of Georgia with parental motivators and barriers for vaccination uncovering unmet research. 1,4
- The objective of the research was to understand the current healthcare provider strategies for HPV vaccine promotion across the state of Georgia.

Methods

- Six focus group discussions (FGDs) with fifty-five (n=55) healthcare providers throughout the state of Georgia, conducted using a semi-structured FGD guide that was reviewed by the Wisconsin Cancer Institute’s Intervention Development Dissemination Shared Resource.
- Participant recruitment was facilitated by close collaboration with community based organizations (CBOs).
- Eligibility Criteria: Identify as a healthcare provider (physician/pediatrician, nurse, pharmacist, nurse practitioner, etc.), >18 years or older, English proficiency, practice in Georgia.
- Participants provided informed consent.
- All FGDs were audio recorded and verbatim transcribed.
- Questions focused on multilevel facilitators and barriers to HPV vaccine promotion and uptake alongside recommendation styles and promotion efforts at the practice-level.
- Thematic analysis using MaxQDA, guided by a deductive coding approach.
- We used coding to highlight segments of transcripts which emphasized common patterns and themes.
- Two members of the research team collaborated to complete coding of the FGD transcripts and participated in regular meetings to discuss coding strategies and to identify and resolve any coding discrepancies.
- A conceptualization was developed to assist with visualization of the common themes and patterns identified through our analysis. (Figure 1)

Results

- Participant demographics are summarized in Table 1
- Six FGDs with 55 total participants.
- ~30% of participants were nurses.
- 40.0% from southeast Georgia.
- Key themes, and representative quotes, are presented in Table 2.

Practice-Level Influence

- Inclusion of the entire clinical practice in HPV vaccination promotion and recommendation is critical.
- Changing dynamic between healthcare providers and patients/caregivers

Informed Consent

- Healthcare is now viewed as a “consumer good” that can be “rated” and “reviewed”.
- It is critical for providers to adjust to this understanding to provide relevant information and resources, and to accommodate new methods of vaccination recommendation.
- Consideration of the patient or caregiver’s needs/wants must be considered in order to provide an effective HPV vaccine recommendation.

Strategies and Best Practices

- Discussion of an array of strategies:
- GRITs to identify vaccination needs
- Provision of vaccination options
- Repetition and persistence
- Discussing the protective value and benefits of vaccination (e.g., cancer prevention)
- Answering questions and calming concerns.
- Unique strategies to overcome potential difficulties in recommending vaccination in religious and/or conservative communities (e.g. focusing on protective value of the vaccination, identifying effective communication strategies).

Sharing Knowledge

- Ability to answer questions or concerns is critical.
- Burden put on providers to “own all the research”.

Adolescent Engagement

- Somewhat common that parents discuss health-related matters with their children and provide them with some “ownership” of that situation.
- Inclusion of adolescents provides the opportunity to let their opinion be known, however providers noted that adolescents often don’t understand the risk of HPV virus alongside benefits of the vaccination.
- Provider’s belief that it is common that parents perceive their adolescent’s risk as low, however may not fully understand the risk behaviors their adolescent may be partaking.

Discussion and Conclusions

- While regional and national assessments on HPV vaccination have been conducted, only recently has Georgia-specific research been conducted on the barriers and motivators towards HPV vaccination among caregivers of adolescents and healthcare providers. 3,4
- As the population of Georgia continues to grow increasingly diverse, it is critical that providers effectively promote HPV vaccination to increase coverage across the state and among all recommended populations. 3,4
- Our provider participants identified shifts in communication strategies and techniques used across the state to better recommend HPV vaccination with patients and caregivers to increase understanding of the importance of the vaccination and to dislodge the perception that adolescent HPV vaccination encourages early sexual debut, a common concern among caregivers which has been disproven. 4
- Practices which align their vaccination promotion strategies in culturally relevant methods, and encourage all practice employees to provide the same information to all caregivers of adolescents needing vaccination may witness considerable increases in vaccination coverage.

- Providers noted that persistence and repetition, alongside a curated message to the caregiver was the most effective strategy they’ve used in order to increase HPV vaccination acceptance and uptake in their practices.
- Providers should consider utilizing strengthened communication strategies, which are both specific to the caregiver/adolescent’s needs and are culturally relevant (e.g. “meeting them where they are”), with caregivers and adolescents to increase understanding of the importance of HPV vaccination.

- Tools like pictures, videos, and media sources designed specifically for adolescents may be an effective strategy to improve vaccination acceptance by engaging adolescents in decision making among adolescents, ultimately increasing Georgia coverage levels. 6

Table 1. Key Themes and Healthcare Provider Illustrative Quotes

<table>
<thead>
<tr>
<th>Practice-Level Influence</th>
<th>Discussion Level of Health Care Providers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthcare is now viewed as a “consumer good” that can be “rated” and “reviewed”.</td>
<td>Healthcare Provider, Southeast Georgia</td>
</tr>
<tr>
<td>It is critical for providers to adjust to this understanding to provide relevant information and resources, and to accommodate new methods of vaccination recommendation.</td>
<td>Health Department, Southeast Georgia</td>
</tr>
</tbody>
</table>

4. Reference

4. References

Implementation of a How-to Guide for Updating Pediatric Practice Websites with Vaccine Content: A Georgia Based Pilot Project

Cristina Meza, MPH1; Adrian R. King, MPH CHEST2; Paula M. Frew, PhD MA MPH3; Laura A. Randall, MPH3; Walter Orenstein, MD2; Robert A. Bednarczyk, PhD2,4,5; and Allison T. Chamberlain, PhD5

1 Gaganvasa Department of Environmental Health, Rollins School of Public Health, Emory University
2 Hubert Department of Global Health, Rollins School of Public Health, Emory University
3 Emory Vaccine Center, Emory University
4 Department of Epidemiology, Rollins School of Public Health, Emory University

Background

• In response to a National Vaccine Advisory Committee (NVAC) call to action we completed a cooperative agreement project from July 2016 - March 2019 with the National Vaccine Program Office (NVPO) to bolster parent and provider vaccine confidence.
• Vaccine hesitancy has been increasing in the U.S in recent years. 1,2
• In recent years, various evidence-based communication strategies have emerged as being helpful for communicating vaccine-related topics. 3,4

Discussion and Conclusions

• Preliminary findings support the acceptability and usability of the vaccine-related How-to Guide for pediatric practices interested in more effectively using their websites to communicate vaccine-related information to their specific patient populations.
• Understanding that participating staff still grappled with exactly what new content to add (e.g. verbiage of answers to frequently asked questions), practices were receptive to the idea of additional support from national, and state-level, professional organizations like the American Academy of Pediatrics to provide additional website-related resources. In addition to promoting the how-to-guide itself, this support could include:
  • Provision of templates which providers might utilize from AAP or GAAAP websites to include on their website.
  • A repository of structured responses to frequently asked questions which pediatric practices could pull onto their own practice websites. This could enable them to further curate revised website content but ensure their content is accurate, up-to-date and appropriate for the specific concerns heard most frequently from their patient populations.
• Participants felt the guide was easy to use and that the section devoted to appealing to patients’ core values when crafting answers to commonly asked questions would be most useful.
• Highlights the importance of targeted and curated messaging to effectively educate about and recommend vaccinations.

Methods

• We conducted six qualitative focus group discussions (FGDs) and six in-depth interviews (IDIs) with 35 total healthcare providers (e.g. nurses, pediatricians, practice managers).
• Prior to enactment of any website changes, we completed unstructured observations of participating practice websites to identify vaccine information currently on practice websites.
• Thematic analyses of FGDs, IDIs, and practice website updates were conducted. We aimed to:
  • Identify themes related to the usability and usefulness of the How-to-Guide.
  • Examine frequently encountered vaccine-related questions and concerns.
  • Understand providers’ perceptions of vaccine-related messages they most wanted to convey to their patient populations.
  • Describe providers’ perceptions of the usefulness of presenting vaccine-specific information on practice websites.

Timeline of Emory Website Pilot Project

• Participating practices were located in metro-Atlanta and varied by practice size (e.g. number of physicians, patient population).
• Participants of both FGDs and IDIs included mostly nurses, medical assistants, and nurse practitioners while most of the IDI participants were physicians. Women constituted 97% of the FGD & IDI participation (N = 541) ranging from 23 – 51 years of age.
• Healthcare providers believed that presenting vaccine-specific information on their practice website would be well-received by parents.
  • This was a common belief even among those practices who considered themselves to be “vaccine friendly,” defined as a practice which accepts patients who are non-vaccinating or on alternate vaccine schedules.
• Parental concerns most frequently encountered by providers related to:
  • The number of vaccinations given at once (and related questions about alternate schedules).
• Vaccine ingredients, specifically worried about ingredients like mercury and aborted fetal cells.
• Specific concerns about human papillomavirus and influenza vaccines.
• Potential and perceived vaccine side effects.

Summary of Key Questions/Concerns Providers Received from Patients

• Multiple practices expressed a desire to make or have videos available on their website to describe vaccine information
• Despite the “self-help” intention of the guide, some participating staff still expressed confusion about what new verbiage to develop and post to their websites.
• Bi-weekly check-ins with participating practices are ongoing.

Practice Website before (left) and after (right) implementation of the How-to-Guide

Summary of Key Messages Providers are Interested in Relaying to Patients

• It is better to experience side effects from the vaccine than from the disease itself (Side effects show the immune system is responding appropriately)
• Delays vaccinations and increasing vaccine only visit will increase a child’s anxiety
• It is safe to give several vaccines on one visit
• Vaccines are safe and effective

References


Acknowledgements

This project was supported by the Emory Vaccine Center at Emory University. In addition, we would like to thank the Georgia Chapter of the American Academy of Pediatrics (GA AAP) and Mrs. Noreen Dahill for their work and support of this project. We would also like to acknowledge the previous research team (HHS NVPO award number VSRNV00000—01—00) which worked to develop the guide that was pilot tested for this project.
Does Race Moderate the Association Between Parent Strategies for Managing Children’s Violent Media Exposure and Child Anxiety?

Hillary Ortiz, Emily Ronkin, Katie Murphy, Susanna McQuarrie, Ph.D., & Erin B. Tone, Ph.D.
Department of Psychology, Georgia State University

Introduction

Violent media exposure has been strongly associated with child anxiety (Comer & Kendall, 2007; Madan, Mrug, & Wright, 2014).
- Children spend as much as 7 hours watching TV and other media (Kaiser Family Foundation, 2010).
- In a year, a child may be exposed to as many as 12,000 acts of violence in the media (Kalin, 1997).

Parents’ behavior can mitigate or amplify child’s anxiety associated with media violence exposure (Padilla-Walker & Coyne, 2011; Valkenburg et al., 1999).
- One strategy that parents report using to handle their children’s responses to violent media is Scaring for Safety (SS) - scaring children on purpose in order to keep them safe (McQuarrie & Caporino, 2017).

This strategy may impact child anxiety differently, depending on whether children come from groups at high or low risk for real life exposure to violence.
- Family race may moderate the association between use of the SS strategy and child anxiety.
- SS use may be related to increased anxiety, but only in children from groups at a relatively low risk for real-life violence exposure.

Current Study

Purpose
- To examine whether race moderates the association between use of the Scaring for Safety strategy to manage child violent media exposure and child anxiety.

Hypotheses
- Black caregivers will report greater use of Scaring for Safety than White caregivers.
- Anxiety will be positively related to parent use of Scaring for Safety among White children and unrelated in Black children.

Methods

Study 1
Caregivers and Children
- 516 White
- 56 Black/African American

Measures
- The Caregiver Responses to Youth Media Exposure (CRYME; McQuarrie & Caporino, 2017)
- The Screen for Child Anxiety Related Disorders (SCARED; Birmaher et al., 1997; Birmaher et al., 1999)

Data Analyses
- T-test
- Pearson’s Product Moment Correlation
- Multiple Linear Regression
- Compared Effect Sizes

Study 2
Caregivers and Children
- 66 Black/African American

Measures
- The Caregiver Responses to Youth Media Exposure (CRYME; McQuarrie & Caporino, 2017)
- Behavior Assessment System for Children (BASC; Reynolds & Kamphaus, 1992)

Results

Study 1
Regression Predicting Child Anxiety

<table>
<thead>
<tr>
<th>B</th>
<th>β</th>
<th>SE</th>
<th>t</th>
<th>Semi-Partial Correlations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Step 1 Constant</td>
<td>63.88</td>
<td>1.65</td>
<td>38.62</td>
<td></td>
</tr>
<tr>
<td>SES</td>
<td>-1.84</td>
<td>.17***</td>
<td>.45</td>
<td>-4.14</td>
</tr>
<tr>
<td>Step 2 SES</td>
<td>-1.89</td>
<td>.18***</td>
<td>.45</td>
<td>-4.22</td>
</tr>
<tr>
<td>Race</td>
<td>.99</td>
<td>.04</td>
<td>.97</td>
<td>1.02</td>
</tr>
<tr>
<td>Step 3 SES</td>
<td>-1.65</td>
<td>.15***</td>
<td>.44</td>
<td>-3.76</td>
</tr>
<tr>
<td>Race</td>
<td>1.60</td>
<td>.07</td>
<td>.96</td>
<td>1.68</td>
</tr>
<tr>
<td>Quadratic Scaring for Safety</td>
<td>.42</td>
<td>.23***</td>
<td>.07</td>
<td>5.60</td>
</tr>
</tbody>
</table>

Note: N = 572; R² = .**p < .001

Step 1: T-test: Black caregivers reported greater use of Scaring for Safety than White caregivers (t(568) = 2.69, p < .05).
Correlations: Greater use of Scaring for Safety related to greater child anxiety in White caregivers (r = .248, p < .05), but not for Black caregivers.
Regression: With SES covariates, Scaring for Safety was significantly and positively associated with child anxiety in study 1 but not study 2. Race was not a significant predictor in Study 1.
Closer examination of the association between Scaring for Safety and child anxiety among White children in Study 1 yielded evidence of a quadratic relationship; both low and high levels of Scaring for Safety were associated with higher levels of anxiety.
Effects sizes compared: SR₁ = 0.232 (N = 572) and SR₂ = 0.039 (N = 66), z = 1.49, p > 0.05

Study 2
Regression Predicting Child Anxiety

<table>
<thead>
<tr>
<th>B</th>
<th>β</th>
<th>SE</th>
<th>T</th>
<th>Semi-Partial Correlations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Step 1 Constant</td>
<td>51.20</td>
<td>4.72</td>
<td>10.85</td>
<td></td>
</tr>
<tr>
<td>SES</td>
<td>-.16</td>
<td>-.01</td>
<td>1.36</td>
<td>-.12</td>
</tr>
<tr>
<td>Step 2 SES</td>
<td>-.18</td>
<td>-.02</td>
<td>1.37</td>
<td>-.13</td>
</tr>
<tr>
<td>Scaring for Safety</td>
<td>.06</td>
<td>.04</td>
<td>.19</td>
<td>.31</td>
</tr>
</tbody>
</table>

Note: N = 66; R² = .**p < .001

Discussion

- Consistent with our hypothesis, Black caregivers reported significantly more use of Scaring for Safety.
- This supports previous research reporting Black families use of harsh parenting significantly more than White families (e.g., Flink et al., 2012; Deater-Deckard et. al, 1996).
- For White caregivers, Scaring for Safety showed a quadratic association with child anxiety, which may suggest that there is a moderate amount of use of Scaring for Safety that is healthy.
- Contrary to our hypothesis, use of the Scaring for Safety strategy was linearly associated with low levels of child anxiety for Black caregivers.
- Findings suggest that Scaring for Safety works well in Black families in warning children of dangers to keep themselves safe.
- Effect sizes did not show significant difference between studies.

Limitations

- There was not a fair representation of Black/African Americans in study 1.
- We don’t know how much the children in the study viewed violent media.
- Both studies used different measures of child anxiety.
- Parents may (either intentionally or not) inaccurately report the strategies they use to manage child media violence responses.

Future Directions & Implications

- Future studies should investigate a variety of races and examine how culture can influence parenting strategies.
- A longitudinal study could help give a better understanding of parental practices on children after a violent event.

Correspondence regarding this presentation should be directed to: Hillary Ortiz
Email: hortiz@student.gsu.edu
Impact of Acute Pancreatitis order Set Implementation on Pediatric Clinical Outcomes

Meera Shah, Traci Leong, A. Jay Freeman

1Emory University, Department of Pediatrics, Pediatric Residency Training Program, 2Rollins School of Public Health, Emory University, 3Children’s Healthcare of Atlanta and Emory University Department of Pediatrics, Division of Gastroenterology, Hepatology, Nutrition, Children’s Healthcare of Atlanta Advanced Pancreatic Care Program

Background

- Pediatric acute pancreatitis (AP) management previously derived from adult literature
- New guidelines were developed by NASPghan in 2017
- Evaluation of these guidelines impact on clinical outcomes still needed
- CHOA implemented guideline based AP order set January 1st, 2018
- Average length of stay 5 days
- Cost per admission for acute pancreatitis is estimated to be $4000-6700 based on age

~770,000 children aged 12-17 misuse opioids and between 1999 to 2016, pediatric mortality rate from opioid poisoning increased more than twofold.

Objectives

- To evaluate the impact of AP guidelines through CHOA’s implementation of pancreatitis order set in 2018

Methods

- Patients admitted for AP in 2017 and 2018 before and after AP order set implementation were included
- Analysis of patients admitted in 2018 comparing order set use vs. no use
- Patient demographic and clinical information such as fluid type and rate, early feeding, narcotic use, order set use collected
- Outcome variables: Length of stay, PICU admission, 30-day re-admission, and narcotic use

Data

Table 1. Patient characteristics by year

<table>
<thead>
<tr>
<th>Variable</th>
<th>All (n = 175)</th>
<th>2017 (n = 69)</th>
<th>2018 (n = 106)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender, n (%)</td>
<td></td>
<td>0.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>107 (61.1)</td>
<td>50 (72.5)</td>
<td>57 (53.8)</td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>68 (38.9)</td>
<td>19 (27.5)</td>
<td>49 (46.2)</td>
<td></td>
</tr>
<tr>
<td>Race, n (%)</td>
<td></td>
<td></td>
<td>0.759*</td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>56 (32.0)</td>
<td>23 (33.3)</td>
<td>33 (31.1)</td>
<td></td>
</tr>
<tr>
<td>Asian/Indian</td>
<td>7 (4.0)</td>
<td>2 (2.9)</td>
<td>5 (4.7)</td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>72 (41.1)</td>
<td>26 (37.7)</td>
<td>46 (43.4)</td>
<td></td>
</tr>
<tr>
<td>Hispanic</td>
<td>40 (22.9)</td>
<td>18 (26.1)</td>
<td>22 (20.8)</td>
<td></td>
</tr>
<tr>
<td>Age, M (SD)</td>
<td>12.9 (4.2)</td>
<td>12.7 (4.5)</td>
<td>13.0 (4.0)</td>
<td>0.59</td>
</tr>
<tr>
<td>Hospital, n (%)</td>
<td></td>
<td></td>
<td>0.5382**</td>
<td></td>
</tr>
<tr>
<td>EG</td>
<td>82 (46.9)</td>
<td>35 (50.7)</td>
<td>47 (44.3)</td>
<td></td>
</tr>
<tr>
<td>HS</td>
<td>1 (0.6)</td>
<td>0 (0.0)</td>
<td>1 (0.9)</td>
<td></td>
</tr>
<tr>
<td>SR</td>
<td>92 (52.6)</td>
<td>34 (49.3)</td>
<td>58 (54.7)</td>
<td></td>
</tr>
<tr>
<td>GI service, n (%)</td>
<td>145 (82.9)</td>
<td>59 (85.5)</td>
<td>86 (81.1)</td>
<td>0.59</td>
</tr>
</tbody>
</table>

*Comparison combines Asian and Indian races into 1 category  **Comparison between EG and SR

Table 2. Outcomes by year

<table>
<thead>
<tr>
<th>Variable</th>
<th>All (n = 175)</th>
<th>2017 (n = 69)</th>
<th>2018 (n = 106)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 day readmission, n (%)</td>
<td></td>
<td></td>
<td>0.1287</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>163 (93.1)</td>
<td>67 (97.1)</td>
<td>96 (90.6)</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>12 (6.9)</td>
<td>2 (2.9)</td>
<td>10 (9.4)</td>
<td></td>
</tr>
</tbody>
</table>

Discussion

- The implementation of the pancreatitis order set based on NASPghan guidelines demonstrates decreased length of stay and total narcotic use without increasing readmission rates

References

Beta-blockers Reduce Exercise Induced Left Ventricular Outflow Tract Obstruction in Pediatric Hypertrophic Cardiomyopathy

Mansi Gaitonde1, Megan Simpson1, Martha Wetzel1, Patricia Simpson2, Megan Stark3, William Border1,2, Ritu Sachdeva1,2, Matthew E. Ferguson1,2, Robert Whitehill1,2

1Emory University, Atlanta, GA; 2Children's Healthcare of Atlanta, Atlanta, GA

Introduction

- Inducible left ventricular outflow tract (LVOT) obstruction in patients with Hypertrophic Cardiomyopathy (HCM) can lead to functional and hemodynamic impairment during activity
- In pediatric patients, beta-blockers have been used to improve heart failure symptoms but has not been studied to reduce LVOT obstruction during exercise
- The aim of this study was to assess the whether beta-blockers can lessen inducible LVOT obstruction during exercise in pediatric patients with HCM

Pediatric HCM patients who develop elevated LVOT gradients during exercise have a reduction in inducible LVOT gradients with beta blocker therapy.

Methods

- Reviewed charts of patients < 22 years with a diagnosis of HCM who underwent an exercise stress echocardiogram from Jan 2009 - Dec 2019 at our center.
- Patients included if treated with beta blocker therapy and underwent a staged exercise stress echocardiogram both before and after initiation of beta-blocker therapy
- Demographics, clinical status, and echocardiographic indices were assessed
- LVOT gradients were evaluated at rest, stage 1 of exercise, and peak exercise both before and after beta-blocker therapy
- Statistical significance was assessed using Wilcoxon signed rank tests with p-value set at <0.05

Results

![LVOT Gradients During Exercise Before and After Beta Blocker Therapy](image)

Conclusions

- Pediatric HCM patients who develop elevated LVOT gradients during exercise have a reduction in inducible LVOT gradients with beta blocker therapy.
- Improvements are seen in the submaximal and peak stages of exercise.
Introduction
• Cardiovascular disease (CVD) remains the leading cause of death among women in the U.S.
• Preventing CVD risk factors early in life can reduce lifetime CVD burden.
• Adolescent and young adult females are rarely the target population of CVD prevention campaigns.
• This study aims to understand how adolescent and young adult females receive cardiovascular health information and how well-informed about CVD they believe they are.

Methods
• Surveyed 331 females ages 15-24 years between September 2017 and January 2018 at two practices in Boston, MA.
• Determined the prevalence of the following:
  1. Exposure to sources of CVD information over past 12 months.
  2. Whether participants felt informed regarding heart disease or stroke.
  3. Preferences and motivations to seek information on CVD.
• Logistic regression models were used to examine associations between exposure to CVD information sources and whether participants felt informed about CVD and stroke.

Results

<table>
<thead>
<tr>
<th>Information Source</th>
<th>n (%)</th>
<th>OR (95% CI) for being informed about HD</th>
<th>OR (95% CI) for being informed about stroke</th>
</tr>
</thead>
<tbody>
<tr>
<td>TV</td>
<td>116 (36.9)</td>
<td>1.59 (0.88, 2.87)</td>
<td>1.37 (0.75, 2.52)</td>
</tr>
<tr>
<td>Social Media</td>
<td>92 (28.8)</td>
<td>2.04 (1.09, 3.82)</td>
<td>1.56 (0.84, 2.89)</td>
</tr>
<tr>
<td>Web</td>
<td>19 (28.5)</td>
<td>2.73 (1.41, 5.26)</td>
<td>1.91 (1.02, 3.57)</td>
</tr>
<tr>
<td>Friend/Family</td>
<td>63 (18.9)</td>
<td>2.65 (1.43, 4.9)</td>
<td>1.43 (0.76, 2.99)</td>
</tr>
<tr>
<td>Healthcare Professionals</td>
<td>97 (29.4)</td>
<td>2.65 (1.36, 5.17)</td>
<td>1.43 (0.71, 2.99)</td>
</tr>
<tr>
<td>None</td>
<td>85 (25.7)</td>
<td>0.10 (0.05, 0.22)</td>
<td>0.37 (0.19, 0.72)</td>
</tr>
</tbody>
</table>

All models adjusted for age, race, ethnicity, and caregiver education. Significant associations are in **bold**

Table 1: Demographic characteristics of 331 adolescent and young adult females completing the modified American Heart Association Women's Health Survey.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
</tr>
<tr>
<td>15-17</td>
<td>91 (27.5)</td>
</tr>
<tr>
<td>18-21</td>
<td>160 (42.3)</td>
</tr>
<tr>
<td>22-24</td>
<td>79 (23.8)</td>
</tr>
<tr>
<td>Missing</td>
<td>31 (9.4)</td>
</tr>
<tr>
<td>Race</td>
<td></td>
</tr>
<tr>
<td>Black</td>
<td>91 (27.5)</td>
</tr>
<tr>
<td>White</td>
<td>74 (22.4)</td>
</tr>
<tr>
<td>Other (includes Asian, Native American, and participants reporting more than one race)</td>
<td>112 (33.8)</td>
</tr>
<tr>
<td>Missing</td>
<td>54 (16.3)</td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
</tr>
<tr>
<td>Hispanic</td>
<td>122 (37.2)</td>
</tr>
<tr>
<td>Non-Hispanic</td>
<td>162 (48.9)</td>
</tr>
<tr>
<td>Missing</td>
<td>46 (14.1)</td>
</tr>
<tr>
<td>Caregiver Education</td>
<td></td>
</tr>
<tr>
<td>Less than college</td>
<td>168 (47.7)</td>
</tr>
<tr>
<td>College or above</td>
<td>163 (49.1)</td>
</tr>
<tr>
<td>Missing</td>
<td>42 (12.7)</td>
</tr>
</tbody>
</table>

Figure 1: Self-reported awareness of CVD and stroke among 331 adolescent and young adult females.

Table 2: Association between various information sources and feeling informed about CVD and stroke among 331 adolescent and young adult females.

Figure 2: Prevalence of preferred methods of being informed about CVD among 331 adolescent and young adult females.

Figure 3: Prevalence of motivating factors to become more informed about CVD among 331 adolescent and young adult females.

Future Directions
1. Develop public health campaigns for youth that focus on the importance of cardiovascular health and wellness.
2. Use media channels frequently accessed by this demographic, including social media, to promote knowledge regarding cardiovascular health and wellness.
3. Encourage health care providers to discuss CVD risk factor modification among younger patients to promote cardiovascular health across the life course.

LESS THAN HALF OF ADOLESCENT WOMEN FEEL INFORMED ABOUT CARDIOVASCULAR DISEASE, THE GREATEST LIFETIME HEALTH RISK TO WOMEN IN THE UNITED STATES
Melphalan Induces Cardiotoxicity through Oxidative Stress in Cardiomyocytes Derived from Human Induced Pluripotent Stem Cells

Rui Liu1, Dong Li1, Fangxu Sun2, Antonio Rampoldi1, Joshua T. Maxwell1, Ronghu Wu2, Peter Fischbach1, Sharon M. Castellino1, Yuhong Du3, Haian Fu3, Anant Mandawat4, and Chunhui Xu1,5

1Department of Pediatrics, Emory University School of Medicine and Children’s Healthcare of Atlanta, Atlanta, GA, USA
2School of Chemistry and Biochemistry and the Petit Institute for Bioengineering and Bioscience, Georgia Institute of Technology, Atlanta, GA, USA
3Emory Chemical Biology Discovery and the Petit Institute for Bioengineering and Bioscience, Emory University School of Medicine, Atlanta, GA, USA
4Department of Medicine and Department of Hematology and Medical Oncology, Emory University School of Medicine; Cardio-Oncology Program, Winship Cancer Institute of Emory University, Atlanta, GA, USA
5Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University, Atlanta, GA, USA

AIM

Treatment-induced cardiotoxicity is a leading noncancer-related cause of acute and late onset morbidity and mortality in cancer patients on antineoplastic drugs such as melphalan—increasing clinical case reports have documented that it could induce cardiotoxicity including severe arrhythmias and heart failure. As the mechanism by which melphalan impairs cardiac cells remains poorly understood, here we report the use of cardiomyocytes derived from human induced pluripotent stem cells (hiPSC-CMs) to investigate the cellular and molecular mechanisms of melphalan-induced cardiotoxicity and to explore potential targeted therapeutics.

METHODS & RESULTS

Figure 1. Schematic diagram showing the experiment workflow.

hiPSC-CMs were harvested to determine CM purity at day 57, and the rest of the cells were cultured until day 20 for subsequent assessments.

Figure 2. Melphalan treatment induces cell loss and apoptosis in hiPSC-CMs. a Representative images of hiPSC-CMs treated with melphalan for 5 days. Scale bar: 40 μm. b Measurement of cell viability by CellTiter-Blue Viability assay. c Quantification of ATP content/ well which indirectly showed viability by CellTiter-Glo 3D Viability assay. d Representative images and quantification of cell apoptosis by CellEvent Caspase-3/7 Green Detection reagent and Hoechst staining. Scale bar: 50 μm. e qRT-PCR analysis showing relative gene expression levels of apoptosis related genes BCL2 and BAX in hiPSC-CMs treated with melphalan for 3 days.

Figure 3. Melphalan treatment of hiPSC-CMs results in Ca2+ handling defect and alters expression of genes encoding calcium channels and sarcomeric proteins. a Representative traces showing intracellular Ca2+ transients in hiPSC-CMs treated with melphalan for 3 days. i, normal Ca2+ transients; ii, vi, abnormal Ca2+ transients. b Stacked bar charts showing percentage of CMs exhibiting normal or abnormal Ca2+ transients under each condition. c qRT-PCR panel showing relative gene expression levels of Ca2+ transporting related genes including RYR2 and CACNA1C, and Ca2+ structure related genes including TNNI1, TNNI2, MYH6/7 and MYL2/7 in hiPSC-CMs treated with melphalan for 3 days.

Figure 4. Melphalan treatment of hiPSC-CMs alters the expression of proteins identified by proteomic analysis. Proteomic analysis of hiPSC-CMs treated with 0 and 20 μM melphalan for 3 days. a Volcano plot illustrating proteins with statistically significant differences in their abundance between control and melphalan treated hiPSC-CMs (P-value < 0.05 and fold change > 1.5). b Venn diagram showing the numbers of differentially expressed proteins identified by proteomics and genes identified by RNA-Seq. c Bar charts showing up- and down-regulated proteins clustered by GO enrichment analysis.

Figure 5. Melphalan treatment causes oxidative stress in hiPSC-CMs. a Representative images and quantification of intracellular ROS production in hiPSC-CMs treated with melphalan for 3 days via carboxy-H2DCFDA and Hoechst staining. Scale bar: 50 μm. b Representative images and quantification of mitochondrial ROS production in hiPSC-CMs treated with melphalan for 3 days via MitoSOX and Hoechst staining. Scale bar: 100 μm. c qRT-PCR analysis showing relative gene expression levels of oxidative stress related genes in melphalan-treated hiPSC-CMs for 3 days.

Figure 6. N-acetyl-L-cysteine (NAC) mitigates the cell loss and mitochondrial ROS production in hiPSC-CMs under melphalan treatment.

a Representative images and measurement of cell viability via CellTiter-Blue Viability Assay in hiPSC-CMs upon melphalan treatment with or without NAC supplementation for 3 days. Scale bar: 40 μm. b Representative images and quantification of intracellular ROS production in hiPSC-CMs upon melphalan treatment with or without NAC supplementation for 3 days via carboxy-H2DCFDA and Hoechst staining. Scale bar: 100 μm. c Representative images and quantification of mitochondrial ROS production in hiPSC-CMs upon melphalan treatment with or without NAC supplementation for 3 days via MitoSOX and Hoechst staining. Scale bar: 100 μm.

Figure 7. NAC ameliorates the alteration of cardiomyocyte beating indexes caused by melphalan.

Analysis of hiPSC-CM contractility upon melphalan treatment with or without NAC supplementation for 3 days. a Representative traces showing the beating velocity recording of hiPSC-CMs. Blue dots denote contraction, and red triangles denote relaxation. b Stacked bar charts showing the percentage of wells of cells with regular or irregular contractility pattern.

Figure 8. NAC attenuates melphalan-induced alteration of hiPSC-CM transcriptome profiles characterized by RNA-Seq analysis.

RNA-Seq analysis of hiPSC-CMs upon 0 and 20 μM melphalan treatment with or without NAC supplementation for 3 days. a Volcano plots presenting the DEGs when comparing any two groups. The up or down-regulated DEGs were identified based on padj < 0.01 and fold change > 2. b Bar charts showing top 20 down-regulated GO terms in melphalan-treated hiPSC-CMs compared with control group, and the enrichment results of these GO terms in Mel+NAC-treated hiPSC-CMs compared with melphalan group. c Chord diagram showing the DEGs of interested GO clusters in melphalan-treated hiPSC-CMs compared with control group, and the relative expression of these genes in Mel+NAC-treated hiPSC-CMs. d Heatmap showing the DEGs involved in GO terms of oxidative stress and cardiac muscle contraction in melphalan- or Mel+NAC-treated hiPSC-CMs compared with control group. padj, adjusted P-value; Control, no melphalan; Mel, 20 μM melphalan; Mel+NAC, 20 μM melphalan with 1 mM NAC.

CONCLUSIONS

- Melphalan treatment of hiPSC-CMs induces oxidative stress, apoptosis and cell death, deranged Ca2+ handling, dysfunctional contractility, and alterations of global transcriptomic and proteomic profiles.
- N-acetyl-L-cysteine can attenuate these deleterious effects of melphalan treatment in hiPSC-CMs, indicating that oxidative stress plays a central role in melphalan-induced cardiotoxicity.

ACKNOWLEDGMENTS

- This study was supported by the Children’s Heart Research and Outcomes Center at Emory University and Children’s Healthcare of Atlanta; the Center for Pediatric Technology at Emory University and Georgia Institute of Technology; Imagine, Innovate and Impact (13) Funds from the Emory School of Medicine and through the Georgia CTSI NIH award [UL1-TR002378]; the Center for Advancement of Science in Space [GA-2017-266]; and the National Institutes of Health [R21AI025723 and R01HL136345].
BACKGROUND

Human induced pluripotent stem cells (hiPSCs) • Are able to differentiate in other cell types • Possess unlimited self-renewal ability

We have previously demonstrated the efficient generation of cardiomyocytes from hiPSCs (hiPSC-CMs) in simulated microgravity (Jha et al., 2016).

We have established a method for cryopreservation and CO2-independent culture of 3D cardiac progenitors from hiPSCs.

This allowed astronauts to thaw the micro-tissues directly onboard at the International Space Station (ISS) to differentiate and characterize cardiomyocytes exposed to real microgravity.

METHODS

Figure 1. Generation of cardiac progenitor spheres. (A) Schematic of cardiac differentiation procedure. (B) Representative picture of the cardiospheres formation in Aggrewell plate.

RESULTS

Figure 2. Evaluation of CO2-independent medium. (A) Purity of cardiomyocytes maintained in different medium with and without CO2. (B) Representative picture of viable cardiomyocytes normalized to control culture in basal medium. (n=3, *P<0.05)

Figure 3. (A) Morphology of cardiac spheres (CS) in CO2 independent medium with and without CO2. (B) Total number of viable cells (Trypan Blue negative) and dead cells (Trypan Blue positive) in CO2 independent medium with and without CO2.

Figure 4. Detection of cardiomyocyte and gap junction markers in cells maintained in CO2 independent medium with and without CO2.

Figure 5. Morphology and Live (green)/Dead (red) assay of cardiac spheres (CS) in basal and CO2 independent medium.

Figure 6. Purity of cardiomyocytes (% α-actinin-positive cells) in CO2 independent medium. (A) Representative images of cardiomyocytes (percentage of α-actinin-positive cells) in CO2 independent medium (n=4). (C) Representative images of cell purity assay.

Figure 7. Effect of sphere size on cryopreservation. (A) Representative figures of Live (green)/Dead (red) assay (B) Average size of cardiac spheres (CS) (n=10) after thawing and after 72h in complete CO2 independent medium. (C) Representative images of cell purity assay (D) Purity of cardiomyocytes (Percentage of NKX2.5 positive cells) (n=4).

Figure 8. Effect of pre-incubation on cryopreservation. (A) Total number of viable cells (Trypan Blue negative) in cardiac spheres (CS) in basal medium with and without serum. (B) Percentage of cells treated with ethanol exhibiting regular Ca2+ transients or spontaneous Ca2+ waves (SCWVs) under each condition.

Figure 9. Characterization of hiPSC-CMs in and CO2 independent medium. (A) Total number of viable cells (Trypan Blue negative). (B) Purity of cardiomyocytes (% NKX2.5 positive cells) (n=4) cardiac spheres (CS) in CO2 independent medium with and without Serum. (C) Percentage of cells treated with ethanol exhibiting regular Ca2+ transients or spontaneous Ca2+ waves (SCWVs) under each condition.

CONCLUSIONS

3D progenitor cardiac spheres can be cryopreserved and thawed directly into suspension culture for further differentiation.

Aliquots of hiPSC-CMs can be tested before the mission to evaluate differentiation efficiency and cell purity.

Cryopreservation will allow astronauts to directly thaw the cardiac spheres onboard ISS.

These cells can be maintained in a CO2-independent medium supplemented with serum and buffering reagents.

A medium that supports cell growth without a CO2 incubator greatly facilitates cell maintenance at the ISS.

ACKNOWLEDGEMENTS

Funding: This study was supported in part by CASIS (GA-2017-286), NIH/NIAAA (R21AA025723), NIH/NHLBI (R01HL136435) and the Center for Pediatric Technology at Emory University/Georgia Institute of Technology.

Reference:
Arginine Dysregulation Correlates with Cardiac Remodeling in Mice with Chronic Kidney Disease

Loretta Reyes, MD1,2, Mike Kelleman, MPH1, Frank Harris2, Lou Ann S. Brown, PhD1, Claudia R. Morris, MD1,2, Pamela D. Winterberg, MD1,2
1Emory University School of Medicine Department of Pediatrics and 2Children’s Healthcare of Atlanta

Background
• Nitrile Oxide (NO) is essential for cardiovascular (CV) homeostasis
• Arginine is the common substrate for NO synthase and arginase enzymes (Figure 1)
• Global Arginine Bioavailability Ratio (GABR) reflects arginine bioavailability in relation to catabolism products
• Low GABR is associated with ↑ risk of adverse CV events & mortality in adults
• Kidneys play a key role in arginine synthesis

Methods
• CKD was established in 129X1/SVJ mice via 5/6th nephrectomy
• Echocardiograms & plasma obtained at 8- & 16-weeks after partial nephrectomy
• Echocardiographic relative wall thickness (RWT) was calculated from M mode measures [(IVS;d+LVPW;d)/LVID;d]; E/A ratio measured from trans-mitral valve Doppler measures
• Plasma arginine, citrulline, ornithine & ADMA (asymmetric dimethylarginine) measured via LC/MS/MS
• Blood pressure measured noninvasively via tail cuff
• In a 2nd experiment, CKD & normal control mice fed Teklad 2018 diet supplemented with arginine (4% w/w) or alanine (nitrogenous control)

Results cont’d

Table 1: ADMA, Arginase Activity and ADMA in mice with and without CKD

<table>
<thead>
<tr>
<th>Measure</th>
<th>8 weeks (n=18)</th>
<th>16 weeks (n=17)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (n=9)</td>
<td>CKD (n=9)</td>
</tr>
<tr>
<td>ADMA (μM/L)</td>
<td>7.1 (7.1-7.1)</td>
<td>7.2 (7.2-7.2)</td>
</tr>
<tr>
<td>Arginase Activity (U/L)</td>
<td>7.3 (3.9-7.3)</td>
<td>7.0 (3.7-7.8)</td>
</tr>
<tr>
<td>GABR = Arg/(Cit+Orn)</td>
<td>0.75</td>
<td>0.61</td>
</tr>
</tbody>
</table>

Values reported as Median (IQR) and compared using Wilcoxon rank-sum tests. Arg=arginine, Orn=ornithine, Cit=citrulline

Figure 2: Low GABR and high ADMA correlate with myocardial changes in mice with CKD

Hypothesis
Dysregulated arginine metabolism in a mouse model of CKD contributes to uremic cardiomyopathy

Objectives
• To evaluate the relationship between GABR & myocardial structure/function in mice with CKD
• To evaluate the effect of arginine supplementation on myocardial structure/function in mice with CKD

Conclusions
• In this pilot study, ADMA and arginase activity were significantly increased in mice with longer duration of CKD
• GABR correlated with worsening structural (RWT) and functional (E/A ratio) myocardial changes.
• ADMA correlated with worsening myocardial function (E/A ratio)
• Arginase activity is lower in arginine supplemented mice
• Arginine supplementation improved SBP at 12 weeks.

Acknowledgements
• Funding: Emory-Children’s-Georgia Tech Pediatric Research Alliance pilot grant 2018 (Heart Research and Outcomes Center), K23 DK111998 (PW) and NIH T32 DK007656 (LR)
• Core services: Children’s Healthcare of Atlanta and Emory University Pediatric Biomarkers Core, Animal Physiology Core and Pediatric Biostatistics Core

Author contact: Loretta Reyes, MD; lreyes7@emory.edu

Figure 1: Arginine metabolic pathway

Figure 3: Effect of arginine supplementation in mice with and without CKD
ARGinine METABOLISM IS DYSREGULATED IN PEDIATRIC CHRONIC KIDNEY DISEASE AND CORRELATES WITH LEFT VENTRICULAR HYPERTROPHY

LORETTA REYES, MD,1,2, PAMELA D. WINTERBERG, MD,1,2, ROSHAN GEORGE, MD, 1,2, FRANK HARRIS,2, MICHAEL KELLEMAN, MPH, LOU ANN BROWN, PhD1 AND CLAUDIA R. MORRIS, MD1,2

1Department of Pediatrics, Emory University School of Medicine, Atlanta, GA; 2Children’s Healthcare of Atlanta, Atlanta, GA

BACKGROUND

Cardiovascular Disease in CKD

- Leading cause of death in Chronic Kidney Disease (CKD) & End Stage Renal Disease (ESRD)
- Left ventricular hypertrophy (LVH) is common in children with CKD/ESRD
  - risk factor for morbidity/mortality in adults
- ~30 fold increased mortality risk in children & young adults with CKD

ARGinine Metabolism and Vascular Health

- Nitric oxide (NO) is essential for CV health
- Kidneys play key role in endogenous arginine synthesis
- Arginine is common substrate for NO synthase & arginase enzymes
- ↓ arginine bioavailability limits NO synthesis

OBJECTIVE

- Evaluate alterations in arginine biosynthesis pathways in children with CKD/ESRD & correlate with measures of LVH

RESULTS

Patient Demographics (n=58)

<table>
<thead>
<tr>
<th>Measure</th>
<th>Normal (n=11)</th>
<th>CKD (n=32)</th>
<th>ESRD (n=15)</th>
<th>P-value (Nml vs CKD)</th>
<th>P-value (Nml vs ESRD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>9.9 (2.5-17.5)</td>
<td>10.5 (4.5-19.5)</td>
<td>11.5 (7.7-16.4)</td>
<td>0.75</td>
<td>0.07</td>
</tr>
<tr>
<td>Male (%)</td>
<td>5 (45%)</td>
<td>12 (85%)</td>
<td>22 (69%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White (%)</td>
<td>4 (36.4%)</td>
<td>5 (38.3%)</td>
<td>8 (53.3%)</td>
<td>0.13</td>
<td></td>
</tr>
<tr>
<td>Black (%)</td>
<td>5 (45.5%)</td>
<td>7 (53.8%)</td>
<td>4 (26.7%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other (%)</td>
<td>2 (17.2%)</td>
<td>0 (0%)</td>
<td>2 (13.3%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total nitrate/nitrite (NOx) measured by Greiss reaction</td>
<td>277 (124.5-460.3)</td>
<td>277 (9.3-251.4)</td>
<td>277 (9.3-251.4)</td>
<td>0.75</td>
<td>0.07</td>
</tr>
<tr>
<td>Arginine activity (unit/L)</td>
<td>7 (5.4-17.1)</td>
<td>7.5 (7.4-17.5)</td>
<td>7.6 (6.6-8.7)</td>
<td>0.76</td>
<td>0.001</td>
</tr>
<tr>
<td>Arginine activity (unit/L)</td>
<td>7 (5.4-17.1)</td>
<td>7.5 (7.4-17.5)</td>
<td>7.6 (6.6-8.7)</td>
<td>0.76</td>
<td>0.001</td>
</tr>
<tr>
<td>Arginine activity (unit/L)</td>
<td>7 (5.4-17.1)</td>
<td>7.5 (7.4-17.5)</td>
<td>7.6 (6.6-8.7)</td>
<td>0.76</td>
<td>0.001</td>
</tr>
<tr>
<td>NOx (μM/L)</td>
<td>28.2 (13.1-22.7)</td>
<td>28.2 (13.1-22.7)</td>
<td>28.2 (13.1-22.7)</td>
<td>0.76</td>
<td>0.001</td>
</tr>
<tr>
<td>ADMA (μM/L)</td>
<td>51.5 (25.6-132.7)</td>
<td>51.5 (25.6-132.7)</td>
<td>51.5 (25.6-132.7)</td>
<td>0.76</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Increased arginase activity, ADMA & NOx in CKD/ESRD

Increased arginase activity in CKD despite decreased concentration

Global Arginine Bioavailability Ratio (GABR)

- Reflects arginine bioavailability in relation to catabolism products
- Associated with ↑ risk of adverse CV events & mortality in adults
  - (Tang et al. 2009, J Am Coll Cardiol.)
  - (Morris et al. 2005, JAMA)

Potential Mechanisms for Arginine Dysregulation in CKD

- de novo arginine synthesis
- ↓ cellular import of arginine
- Diversion of arginine to other metabolic pathways
- Accumulation of NO synthase inhibitors e.g. ADMA

CONCLUSIONS

- ADMA associates with LVH; NOx appears to be protective

Methodology

- Analyzed banked plasma from healthy children (n=11) & children with CKD/ESRD (n=47)
- Arginine, ornithine, citrulline, ADMA measured by LC/MS/MS
- Arginase concentration/activity measured by ELISA/colorimetric assay
- Total nitrate/nitrite (NOx) measured by Greiss reaction
- Clinically acquired echocardiograms in children with CKD/ESRD reviewed for measures of LVH
- LVH defined as height indexed LV mass (LVMI) z-score ≥ 2

Values reported as Median (25th – 75th) & compared using Wilcoxon rank-sum tests using multiple comparisons based on Bonferroni corrections

Values reported as Median (25th – 75th) & compared using Wilcoxon rank-sum tests using multiple comparisons based on Bonferroni corrections

REFERENCES

1. All authors declare no conflicts of interest relevant to this study
2. Funded in part by an Emory Children’s Heart Center Pediatric Research Alliance pilot grant from the Novel Research and Outcomes (NRO) Center (Pfizer), KIB CHY 11484 (Pfizer), and T32 DK07585-10(R)3. Supported by services offered by the Children’s Healthcare of Atlanta and Emory University Pediatric Biomarkers Core and Biostatistics Core.
A Systematic Chart Review of Children Diagnosed with ASD and Presenting Behavioral Health Concerns in the Emergency Department

Paoula Dyanova1,3, Alexis Pavlov1,2,3, Nathan Call1,2,3, & Colin Muething1,2,3
Marcus Autism Center1, Children’s Healthcare of Atlanta2, & Emory University School of Medicine3

Introduction

• Children diagnosed with Autism Spectrum Disorder (ASD) are more likely to be admitted to the emergency department (ED) for behavioral or psychiatric concerns than their typically developing peers (Lytle, Hunt, Moratschek, Hall-Mennes, & Sajatovic, 2018; Straus, Coburn, Maskell, Pappagianopoulos, & Cantrell, 2010).
• These children have higher rates of inpatient psychiatric admissions and often require longer lengths of stay, ultimately incurring significantly larger medical costs (Carbone, Young, Stoddard, Wilkes, & Trasande, 2015).
• They also experience higher rates of sedation and restraint during a hospital admission (Lunsky, et al., 2014).
• The purpose of this study is to further extend the research by identifying common themes among children with ASD who are admitted to the ED for behavioral concerns.

Methods

• Retrospective data were collected from 46 children (55 ED visits) from the first quarter of 2019 (M = 14; range, 8-20)
  1. Documented ASD diagnosis
  2. Admitted to the ED Between 01/2019-03/2019
  3. Chief complaint of behavioral health concern

Results

• 55% African Americans, 40% White, 5% Asian or Pacific Islander
• 83% insured through Medicaid or Medicaid managed care plans
• Prior to the ED admission, 98% were prescribed behavioral medications and 80% of those children were prescribed two or more medications.
• 68% of children had a documented comorbid behavioral or psychiatric disorder (e.g., ADHD, depression, bi-polar disorder).
• Almost half of the sample were admitted for aggressive behavior (AGG) (47%), followed by suicidal ideation (SI) (16%), and Self-injurious behavior (SIB) (15%)
  • 45% were admitted for more than one behavioral complaint
    • I.e., 11% AGG and SIB, 9% AGG and homicidal ideation, 4% AGG and SIB
• Average length of admission was 27 hours.
• 91% of children required some form of behavioral intervention (i.e., mechanical restraint, chemical restraint, or monitoring).
• Approximately 70% of children were admitted to a ED location 0-25 miles from their home and 30% were admitted to an ED within 26-100 miles.
• 55% were transferred to another medical or psychiatric facility at discharge.
• 47% were previously admitted to the ED for a behavioral complaint within the past year of the admission date we reviewed.

Discussion

• The majority of children were non-Hispanic males from low-income households.
• Almost half of children were admitted for aggression and for more than one behavioral concern.
• Medical providers often lack sufficient training related to meeting the unique needs of this population (Straus et al., 2019). This likely impacts the intrusiveness of interventions and length of stay for these children which is reflected in our data.
  • Long admission times ranging from 2 hours up to 117 hours.
  • High rates of intrusive interventions and, in 36% of cases, more than one intervention was required. These data do not account for the frequency of intervention within each admission (e.g., number of restraints within visit).

Impact and Future Directions:

• It is imperative to understand patterns in children diagnosed with ASD who are admitted to the ED and the impact that it has on the healthcare system.
• This information can identify potential predictors to attempt to prevent future hospitalizations.
• More charts should be examined to explore if a unique profile exists which when indicated, could help medical providers better prepare and treat children diagnosed with ASD in the ED for behavioral health concerns.
• Future studies should evaluate how to better serve this population during ED admissions.
  • I.e. Develop or reference coping plans, ask caregivers what is reinforcing for the child, initiate comfort measures at the start of admission, use of tablets, adapt medical procedures to accommodate child.

Retrospective data were collected from 46 children (55 ED visits) from the first quarter of 2019 (M = 14; range, 8-20)
Inclusion criteria:
1. Documented ASD diagnosis
2. Admitted to the ED Between 01/2019-03/2019
3. Chief complaint of behavioral health concern

Figure 1. Percentage of behavioral complaint indicated on the ED charts of children in the study.

Figure 2. Percentage of children requiring intrusive intervention due to challenging behaviors.

Figure 3. Percentage of children and their corresponding diagnoses and prescribed outpatient medications prior to admission. Percentages do not add to 100% due to multiple diagnoses and medications.

Table: Initiation of Restraints and Behavioral Watch

<table>
<thead>
<tr>
<th>Drug Class</th>
<th>Percentage</th>
<th>Diagnosis</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beta/Alpha Blockers</td>
<td>62</td>
<td>ASD</td>
<td>100</td>
</tr>
<tr>
<td>Atypical Antipsychotics</td>
<td>58</td>
<td>ADHD</td>
<td>49</td>
</tr>
<tr>
<td>SSRI</td>
<td>44</td>
<td>Bipolar/Mood Disorder</td>
<td>25</td>
</tr>
<tr>
<td>Anticonvulsant</td>
<td>31</td>
<td>ODD</td>
<td>18</td>
</tr>
<tr>
<td>Stimulant</td>
<td>18</td>
<td>Schizophrenia</td>
<td>13</td>
</tr>
<tr>
<td>Anticholinergics</td>
<td>18</td>
<td>ID/DD</td>
<td>7</td>
</tr>
<tr>
<td>Benzodiazepines</td>
<td>13</td>
<td>Depression</td>
<td>5</td>
</tr>
<tr>
<td>Traditional Antipsychotics</td>
<td>13</td>
<td>OCD</td>
<td>2</td>
</tr>
<tr>
<td>No Medications Indicated</td>
<td>7</td>
<td>Anxiety</td>
<td>2</td>
</tr>
<tr>
<td>TCA’s</td>
<td>4</td>
<td>PTSD</td>
<td>0</td>
</tr>
<tr>
<td>Other (e.g. Vistaril)</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SNRI</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAOI</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lithium</td>
<td>0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1/5/2020
Feasibility of EI providers implementing a parent-mediated intervention: A RCT in autism treatment

Chelsea Rock¹, Eileen Kaiser¹, Naima Bond¹, and Jennifer Stapel-Wax¹²
Marcus Autism Center¹, Emory University School of Medicine², Atlanta, GA

ESI Model for Toddlers
- Early Social Interaction Model (ESI) is a parent-implemented intervention teaching families how to support children’s active engagement in everyday activities.
- The purpose of this study is to train Part C Early Intervention Providers (EIPs) to coach families of toddlers with early signs of ASD.
  - Shown in a RCT previously in parents by FSU.
  - EIPs will have access to an autism specific screening tool (Smart ESAC) that can identify children at risk for ASD.

Can interventionists reach fidelity?

Initial findings show 3 ESI EIPs who are regularly coached and experiencing the model in a group setting may reflect higher fidelity compared to 1 BAU EIP in the control group.

Can interventionists reach fidelity?

Screening: Within 11 months, 13 EIPs participating in Cohorts 1 and 2 identified 20 children under age 2 with signs of autism using the Smart ESAC tool.

Intervention Deployment
- EIPs will receive online training in Autism Navigator and randomize into one of two study arms:
  - Business-as-Usual (BAU): monthly support
  - Early Social Interaction (ESI): weekly supervision
- EIPs will offer study participation to families with children demonstrating red flags for ASD.
- Integrity of implementation collected by study coach every session completed via video.

Reference

Acknowledgements
The authors would like to acknowledge the other study site, Florida State University, and continue to thank all the EIPs and families for participating in the study. The material is based on work supported by the Institute of Education Services conducted at the Marcus Autism Center.
Patterns of Social Visual Engagement in the First Two Years of Life Differentially Predicts Language Abilities in Children with and without Autism Spectrum Disorder
Sanju Koirala, Deniz Parmaksiz, Stella Yuan, Sarah Shultz, Ami Klin, Warren Jones and Laura A. Edwards
Marcus Autism Center, Children’s Healthcare of Atlanta, and Emory University School of Medicine, Atlanta, GA

BACKGROUND
Successful interaction with the social world requires attention towards salient aspect of the social environment. Over the first two-years of life, infants’ show developmental changes in the way they visually engage to talking faces. Typically Developing (TD) infants show an initial period of predominant fixation on a speakers’ eyes followed by a shift in attention to the speakers’ mouth at around 8-10 months, a developmental timepoint when canonical babbling emerges. Similar shift in attention to mouth occurs in the second year of life, when first words are spoken. These shifts in attention between eyes and mouth have been shown to predict language outcome in TD infants. In contrast, infants later diagnosed with autism spectrum disorder (ASD) show atypical patterns of social visual engagement to faces, with early and prolonged declines in eye-looking over the first two years of life. Mouth-looking in infants with ASD increases during the same time, but the relationship between these patterns of early looking and later language abilities is not well understood.

OBJECTIVE
To examine the relationship between social visual engagement at the beginning of the first and second years of life, and language development at the end of the second year, in a cohort of ASD and TD infants.

METHODS
Eye tracking data was collected as a measure of social visual engagement during the first and second years of life while participants watched naturalistic videos of caregivers. Data were quantified as percentage of time spent visually fixated on regions of interest (ROIs): eyes, mouth, body, and objects.

The following two developmental assessments were administered at 24 months:
1. Mullen Scales of Early Learning (MSEL) as measure of language development
2. Autism Diagnostic Observation Schedule (ADOS) to quantify social disability

Pearson’s Correlation tested the association between SVE and language and social disability scores.

CONCLUSION
• In TD infants, eye-looking in the first year of life positively predicts receptive language score at 24 months.
• In ASD infants, early patterns of social visual engagement were unrelated to language outcome at 24 months instead they were predictive of social disability scores at 24 months.

FUTURE DIRECTIONS
• Create longitudinal trajectories of social visual engagement to faces for both ASD and TD groups
• Examine such longitudinal trajectories as the predictors of language outcomes.

REFERENCES

ACKNOWLEDGEMENTS

Table 1. Showing the participants’ sample size, mean age, language and ADOS scores.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Mean age (months)</th>
<th>N</th>
<th>Mean Receptive Language Scores (SD)</th>
<th>Mean Expressive Language Scores (SD)</th>
<th>Mean ADOS scores (total) (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TD</td>
<td>5.14</td>
<td>93</td>
<td>28.10(3.04)</td>
<td>28.16(3.77)</td>
<td>3.13(2.68)</td>
</tr>
<tr>
<td></td>
<td>15.21</td>
<td>80</td>
<td>27.11(2.87)</td>
<td>27.68(5.19)</td>
<td>3.04(2.60)</td>
</tr>
<tr>
<td>ASD</td>
<td>5.12</td>
<td>40</td>
<td>20.35(7.16)</td>
<td>21.3(7.27)</td>
<td>13.53(5.87)</td>
</tr>
<tr>
<td></td>
<td>15.23</td>
<td>45</td>
<td>21.04(7.09)</td>
<td>22.12(7.52)</td>
<td>13.56(5.32)</td>
</tr>
</tbody>
</table>

A. Correlations between percent fixation on speakers’ eyes in the first year of life and receptive language scores at 24 months. Percent fixation on the eyes positively predicts receptive language for typically developing infants but not for infants later diagnosed with ASD (r=0.312, p=0.002).

B. Correlations between percent fixation on speakers’ mouth in the second year of life and expressive language scores at 24 months. Percent fixation on the mouth trended towards positive prediction of expressive language for typically developing infants but not for infants later diagnosed with ASD (r=0.193, p=0.08).
The Adaptive Value of Gaze to the Mouth and the First Word Milestone in Typical Development and in Autism Spectrum Disorder

Elizabeth H. Kushner, Sarah Shultz, Ami Klin, Warren Jones, Laura A. Edwards

Marcus Autism Center, Children’s Healthcare of Atlanta, and Emory University School of Medicine, Atlanta, GA

Results: The Adaptive Value of Visual Fixation Across Word Learning

Methods

Participants were assessed concurrently using:

- Eye-tracking measures of social visual engagement, quantified as percentage of time spent fixated on different facial regions of interest (ROIs).
- ROIs include eyes, mouth, body, and objects. The proportion of time a child spends fixated to each of these regions is recorded. Her.
- Mullen Scales of Early Learning (MSEL) Receptive (RL) and Expressive Language (EL) Scales
- Children were classified into one of four Word Learning groups based on their early word learning status measured from the MSEL Expressive Language Item 11:

<table>
<thead>
<tr>
<th>Mullen Item 11: Says First Words...</th>
<th>Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No Words</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>One Word</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>2-7 Words</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>8+ Words</td>
<td></td>
</tr>
</tbody>
</table>

Results: Fixation Levels Across Word Learning

Children with ASD who had 8 or more words looked significantly less at mouths than TD children (p = 0.049). No significant differences in levels of eye-looking were observed between ASD and TD groups at any stage of language acquisition.

Discussion

For TD children, the adaptive value of mouth-looking changes across stages of word learning: specifically, mouth-looking positively predicts receptive language ability before word onset, then positively predicts expressive language ability during the immediate period following word learning. It is possible that mouth looking serves as a means for scaffolding the initial production of language, helping children understand and then produce words. However, none of these patterns were observed in children with ASD. Future analyses will explore potential explanations for these differences in the adaptive value of mouth looking. We will explore possible sources of heterogeneity in our ASD group and explore these questions within a longitudinally followed cohort.

References


Acknowledgements

This work is support by the Marcus Foundation, Whitehead Foundation, Georgia Research Allance, and National Institutes of Mental Health (P50 MH100029 and 2P50 MH100029-6). Many thanks to all of the children and families, staff members and clinicians who dedicated their time and service to make this work possible.
Autism Spectrum Disorder (ASD) diagnoses are about 4 times more common in males than females. Although research on sex differences in language development in ASD remains limited, females with ASD without intellectual disability have been found to exhibit more visual engagement (SVE) suggest that females with ASD demonstrate increased attention to faces relative to ASD males during parallel play, and point to an association between SVE to faces and expressive language. While typically developing (TD) infants increase attention to mouth over infancy, followed by a return to focus on the eyes by two years, this pattern of SVE over development may hence explain differential language outcomes in ASD.

Examine sex-based differences in the associations between SVE at the beginning of the first and second years of life, and language development and social disability at the end of the second year of life, in a longitudinally-followed cohort of ASD and TD infants.

Eye tracking data collected as measure of SVE during early infancy and toddlerhood while children watched scenes of naturalistic caregiver interactions. Pearson correlations were used to test associations between the cross-sectional SVE measures and scores on the following two developmental assessments, administered at 24 months:

1. Mullen Scales of Early Learning (MSEL) as benchmark of language development
2. Autism Diagnostic Observation Schedule (ADOS) to quantify social disability

Patterns of SVE to the face were unrelated to later language in the ASD group, but (C) eye-looking in infancy negatively predicted ADOS total scores; this association was driven by females with ASD ($r_{female} = -0.6728, p_{female} = 0.0330$; $r_{male} = -0.1163, p_{male} = 0.5406$). (D) In females with ASD only, mouth-looking in infancy positively predicted ADOS total scores ($r_{female} = 0.7784, p_{female} = 0.0080$).

Preliminary results indicate that increased attention to distinct areas of the face during the first two years of life predicts language development in typical development. Notably, sex differences were observed in the strength of the association between SVE and expressive language in toddlerhood. In ASD, visual fixation patterns to the face were related to later social disability in females only. These findings suggest that visual fixation has differential adaptive value in ASD, and by sex. Future work will include the investigation of sex-based trajectory differences in SVE to the face and relationships to later language outcomes.

References:
Background & Aims

- Engagement is critical for social learning – information that does not engage cognition, even when looked at, will go unprocessed and unlearned.
- Despite its importance, the neural mechanisms underlying engagement remain unknown, largely because no studies have successfully quantified what viewers themselves find engaging.
- This study uses a novel measure of engagement – eye-bleeping1,2 – to index a viewer’s own subjective engagement with social stimuli (e.g., faces).

- Successful social adaptive action depends on selectively engaging with things that have the greatest behavioral relevance. Therefore, atypical engagement with the social world can contribute to social disability (e.g., in Autism Spectrum Disorder (ASD)).
- It is unknown whether hypoactivation of social brain regions in response to social stimuli (e.g., faces) in ASD reflects reduced engagement with those social stimuli (a hallmark of ASD) or recruitment of atypical brain circuitry.

Methods: Data Analysis

A. Eye-Fixation Data

To identify moments when viewers in each group looked at the same face onscreen.

- Regions of interest coded for each movie clip frame

B. Eye-Blinking Data

To identify moments when viewers themselves perceived those faces as engaging.

- Method capitalizes on the fact that eyeblinks interrupt the flow of visual information
- Viewers unconsciously time their blinks to avoid missing information perceived to be critical, or ‘highly engaging’1,2
- Moments of statistically significant blink inhibition and increased blinking were identified for each group separately by comparison with permuted data2

C. fMRI Data

To investigate brain activity during events of interest: ‘highly engaging’ and ‘less engaging’ faces

![Figure 1. Simultaneous eye-tracking (A and B) and fMRI (C) data were used to examine brain activity when each group (TD, ASD) looked at faces that they themselves perceived as ‘highly engaging’ or ‘less engaging’.](image)

![Figure 2. Brain regions activated when A) TD children and B) children with ASD view faces perceived by their group as ‘highly engaging’ versus ‘less engaging’. Voxels were thresholded at an entry level of p<2.3 and then assessed at a corrected p<.05 using a Gaussian random field theory approach. Color ranges from z=2.3 to 3.2. A) Preliminary whole-brain analyses reveal increased activation in bilateral occipital cortex, left middle temporal gyrus, bilateral posterior cingulate, left orbitofrontal cortex and inferior frontal gyrus, right angular gyrus, and right fusiform gyrus. B) Preliminary whole-brain analyses reveal increased activation in temporal occipital cortex.](image)

Results

Preliminary results from TD children reveal that even when viewing the same stimulus category (e.g., faces), one’s own engagement with the stimulus modulates brain activation, even in canonical face processing areas like the fusiform gyrus.

Preliminary results from ASD children suggest that previous reports of hypoactivation in ASD were unlikely to be driven solely by attenuated engagement with presented faces. Even when children with ASD look at faces that they perceive as engaging, they process those stimuli using atypical neural circuitry – regions typically involved in object perception.

Conclusions

Aim 1

- Engagement is a strong predictor of learning, and therefore, understanding how engagement influences social brain circuitry both in TD and ASD children can inform future understanding of social disability and targets for social learning interventions.
- Immediate next steps include replicating preliminary findings in a larger sample, performing between-group contrasts, and performing region-of-interest analyses in canonical face-sensitive regions.

Aim 2

Acknowledgements

This work was funded by an Emory University Pediatric Seed Grant and K01MH108741. A huge thank you to all the families and children, staff, and clinicians who dedicated their time and made this project possible.

References

- Marcus Autism Center, Children’s Healthcare of Atlanta, and Emory University School of Medicine, Atlanta, GA.
A Randomized Controlled Trial (RCT) Comparing Parent-Implemented versus Clinician-Implemented Naturalistic Developmental Behavioral Intervention in Infant Siblings Identified as At-Risk for Autism Spectrum Disorder (ASD):

Preliminary Results

Sandra Reed1, Camille Woodard1, Hallie Bedol1, Elizabeth Coe1, and Jennifer Stapel-Wax1,2

Marcus Autism Center1, Emory University School of Medicine2, Atlanta, GA

INTRODUCTION

- Children with ASD demonstrate improved outcomes from early intervention including vastly improved chances of both learning functional language and placement into a typical kindergarten class.
- The median age of diagnosis is 4 years (Maenner, 2020).
- Given that a reliable diagnosis can be made by 24 months of age, earlier intervention that maximizes early social and language development is key to better outcomes. Additionally, McDonald et. al. (2019) provided a familial recurrence rate of 16-36% for the disorder; thus there are many children potentially at risk who can benefit from early intervention.
- There is a need for more research into early intervention, specifically targeting younger siblings of children with ASD. Prior research targeting children under 24 months has focused on parent training and implementation of naturalistic approaches (Bradshaw et. al., 2014).
- The impact of offering parents universal education on developmental milestones will be assessed.
- This review will illustrate the methodology, provide preliminary descriptive results of toddlers eligible for treatment and highlight variables impacting future research.

DESCRIPTION OF RCT

- A 2-stage sequential multiple assignment randomized trial (SMART) design is used to study intervention with the total sample of high and low risk infants and their families.
- At assignment #1 at six months of age, families are randomized to usual care versus an online portal of guides to their infant’s development.
- At assignment #2, the families are re-assigned to usual care versus an online portal of guides to their child’s development or a parent implemented versus clinician implemented intervention for infants found to be at risk for ASD at twelve months of age.

Study Flow: SMART DESIGN

PRELIMINARY RESULTS

- 172 participants have been enrolled to date.
- At six months of age, fifty have been randomized to receive preventative parent education and forty-three to usual care.
- At twelve months of age, thirty toddlers have reached assignment #2 and have been randomized to either usual care or preventative parental education.
- Thirty-eight of the enrolled total sample are considered “high risk” due to a confirmed diagnosis of ASD in an older sibling.
- Sixteen toddlers have been identified with early symptoms of ASD.
- Treatment–eligible participants were randomized with 11 at high risk and 5 at low risk for ASD. Equal numbers of male and females randomized to treatment.

CONCLUSIONS

Future comparisons of the Parent-Implemented versus the Clinician-Implemented NDBI will reveal information on which intervention is most effective for children at-risk. Utilizing the preventative parent education, the impact on the effectiveness of treatment for infants at risk for ASD will allow more data to reveal the importance of parental education in early interventions as well as for typically developing children. Research will also be able to look for trends between sexes and between high and low-risk children. Based on randomizations, the study results will identify a correlation in the Implemented NDBI and scores on the assessments conducted at study end. This will provide evidence on intervention effectiveness for early toddlers showing red flags of ASD.

REFERENCES


ACKNOWLEDGEMENTS

The authors would like to acknowledge the other study site Florida State University, Children’s Healthcare of Atlanta, and thank all families for participating in the study.
Exploring $5\text{HT}_2$ Receptors as Targets for Treating Epilepsy in Fragile X Syndrome: A Preclinical Study of Fmr1 Knock-out Mice

Tanishka S. Saraf, Yiming Chen, Jessica L. Armstrong, Clinton E. Canal

Mercer University College of Pharmacy, Atlanta, Georgia

INTRODUCTION

Epilepsy prevalence is ~12% in children with autism spectrum disorder (ASD) and ~25% in children with fragile X syndrome (FXS), a monogenic, neurodevelopmental disorder characterized by intellectual disabilities, severe anxiety, attention deficit hyperactivity disorder, and sensory hypersensitivity—FXS is also the most known, cause of ASD. Seizure risk increases by three times in individuals with comorbid FXS and autism. Juvenile (P23–P25) Fmr1 knock-out mice—a genetic model of FXS/ASD—have a high prevalence of sound-induced (auditory) seizures, recapitulating the increased incidence of seizures and sensory hypersensitivity in FXS and ASD. Several early studies linked alterations in serotonin (5-hydroxytryptamine, 5-HT) to ASD, and selective serotonin reuptake inhibitors (SSRIs) are among the most commonly prescribed medicines to treat psychiatric symptoms in ASD and FXS. Critically, there are at least 14 genetically-encoded 5-HT receptors (5-HTRs) all indirectly activated by SSRIs—yet detailed prevalence and lethality by 19% and 32%, respectively, however, effects did not reach the predetermined significance level (Fisher’s exact test, **P<0.0001 and **P<0.0005, respectively). Lorcaserin, 3 mg/kg, reduced seizure prevalence and lethality by 19% and 32%, respectively, however, effects did not reach the predetermined significance level (Fisher’s exact test, **P<0.0002 and &P<0.0058, respectively). Lorcaserin exhibited full agonist activity at human 5-HT$_7$Rs and 5-HT$_4$Rs with ~17-fold potency for 5-HT$_7$Rs (Failing et al., 2010; canal. Both: 2016; PMID: 26073920, unpublished data). This project explores the anti-epileptic potential of 5-HT$_2$ receptor agonists in FXS and ASD, beginning with tests of lorcaserin to prevent audiogenic seizures in Fmr1 knock-out mice.

METHODS

**Fmr1 knock-out** (FVB:129P2-Pdebr$^g$ Tyr$^{+/-}$ Fmr1$^{+/+}$$^R_{R1}$KO) and wild-type control (FVB:129P2-Pdebr$^g$ Tyr$^{+/-}$KO) mice were purchased from the Jackson Laboratory to develop a colony. Genotyping of litters by PCR was performed based on established protocols. Audiogenic seizures were assessed by exposing male and female juvenile Fmr1 knock-out mice to a 120 dB alarm (Radiocheck) for 5 min, 30 min after intraperitoneal administration of vehicle (Mibbq water), lorcaserin (AdooQ Bioscience) 1 (L1), 3 (L3), 5.6 (L5.6), or 10 mg/kg (L10), M100907 0.03 mg/kg (M0.03), M0.03 mg/kg (M0.03), or MPEP. Juvenile wild-type mice were treated with vehicle before exposing to the alarm. Responses, including wild running and jumping (WRJ), tonic-clonic seizures (TCS) with and without respiratory arrest, were scored and recorded.

Radioligand saturation binding was performed using cell membranes of brain tissue collected from juvenile male and female wild-type and Fmr1 knock-out mice, as previously described (Canal et al., 2011; PMID: 20165943). [3H]Mesulergine and [3H]Ketanserin (PerkinElmer) with cold ligands to block off-targets were used to label 5-HT$_7$Rs and 5-HT$_4$Rs, respectively.

Adult, male C57BL/6J were used to test in vivo interactions between lorcaserin and racemic (±)-2,5-dimethoxy-4-iodoamphetamine (DOI) specifically effects on the head-twitch response (HTR) and locomotion. 5-HT$_2$ receptor phosphoinositide hydrolysis assays were performed as previously described (Chen et al., 2019; PMID: 30845376) using human 5-HT$_2$A and 5-HT$_2$C receptors transfected in HEK293 cells.

DISCUSSION

Lorcaserin (3 mg/kg) reduced seizure prevalence and lethality in juvenile Fmr1 knock-out mice. However, effects were not statistically significant. Overall, lorcaserin did not significantly prevent or prolong latency to or shorten duration of audiogenic seizures; instead, at doses other than 3 mg/kg, lorcaserin potentiated seizures, displaying mild (but not statistically significant) proconvulsant effects. At 5.6 mg/kg, onset latency to all responses, number and duration of WRJ before TCS increased, thus, prolonging the time spent in seizure avoidance behavior. Coadministration of M0.03 and L5.6, increased seizure prevalence but decreased severity of seizures with a similar pattern of anticonvulsant activity (increasing onset latency) observed at 5.6 mg/kg. Thus, lorcaserin’s 5-HT$_2$-specific effects at higher doses, may involve potential anticonvulsant action. These complex mixed effects of lorcaserin in FXS may involve molecular mechanisms that are distinct from seizure attenuation effects in Dravet and Lennox-Gastaut syndromes. Preliminary results suggest decreases in 5-HT$_7$Rs and 5-HT$_4$Rs in brains of juvenile Fmr1 knock-out mice. These data suggest 5-HT$_7$Rs are altered in Fmr1 knock-out mice, and might be therapeutic targets for FXS or ASD.

Lorcaserin is marketed as a selective 5-HT$_2$C agonist, but its selectivity over 5-HT$_7$ is only ~17-fold. Lorcaserin also has agonist activity at 5-HT$_2$A and 5-HT$_4$Rs, but its selectivity over 5-HT$_2$A is ~17-fold. Lorcaserin also has agonist activity from the clinical dose. At 5.6 mg/kg, it did NOT attenuate the HTR elicited by MPEP (positive control). Lorcaserin dose-dependently increased WRJ, TCS, and HTR frequency (Failing et al., 2010; canal. Both: 2016; PMID: 26073920, unpublished data). This project explores the anti-epileptic potential of 5-HT$_2$ receptor agonists in FXS and ASD, beginning with tests of lorcaserin to prevent audiogenic seizures in Fmr1 knock-out mice.
It would be beneficial to assess if transitioning from an overnight continuous enteral feeding schedule to a daytime bolus feeding schedule promotes an increase in oral intake in children with mild feeding difficulties.

Caitlin Waddle, MS, RDN, LD, Children's Healthcare of Atlanta, Atlanta, GA

Examination of tube feeding schedules and oral intake: A retrospective chart review.

Stimulation of appetite is a critical component of increasing oral intake and weaning tube feeding. Intermittent daytime bolus feeding is often recommended for appetite stimulation as it is thought to be more physiological and preserves hunger and satiety cycles. Overnight feeding is recommended, as well, to enhance appetite, but little research has compared the use of overnight feeding to a daytime bolus schedule in terms of improving oral intake.

Clinical standards for recommended enteral feeding schedules to promote oral intake is not well documented.

Background

- Stimulation of appetite is a critical component of increasing oral intake and weaning tube feeding. Intermittent daytime bolus feeding is often recommended for appetite stimulation as it is thought to be more physiological and preserves hunger and satiety cycles.
- Overnight feeding is recommended, as well, to enhance appetite, but little research has compared the use of overnight feeding to a daytime bolus schedule in terms of improving oral intake.
- Clinical standards for recommended enteral feeding schedules to promote oral intake is not well documented.

Methods

- A retrospective electronic chart review was completed reviewing feeding tube dependent children that presented for a Dietitian evaluation in the Children's Multidisciplinary Feeding Clinic.

Table 1. Enrollment Criteria

<table>
<thead>
<tr>
<th>Inclusion Criteria</th>
<th>Exclusion Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Evaluated by Dietitian in the multidisciplinary feeding program between January 1, 2018 to May 31, 2019.</td>
<td>- Periactin (Cyproheptadine) listed as an active medication.</td>
</tr>
<tr>
<td>- Children receiving a portion of calorie intake via nasogastric or gastrostomy tube.</td>
<td>- Oral motor assessment not clearly indicating if skill deficit was present.</td>
</tr>
<tr>
<td>- Children receiving jejunal tube feeding.</td>
<td>- A child with a feeding tube in place that was not actively in use for a portion of calorie needs.</td>
</tr>
</tbody>
</table>

Results

- Of the 336 patients reviewed, 85 subjects met study criteria. The median age of 3, with a range of 1-15.
- 63.5% (n = 54) of the subjects that presented to the Dietitian for evaluation were consuming 0% of oral intake by mouth at the time of evaluation.

A continuous overnight tube feeding schedule does not appear to promote increased oral intake in comparison to a daytime bolus feeding schedule.

<table>
<thead>
<tr>
<th>N (Row %)</th>
<th>Percent Oral Intake = 0%</th>
<th>Percent Oral Intake &gt; 0%</th>
<th>Oral Intake &gt; 0% OR (95% CI)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral Motor Deficit – None Feed Type</td>
<td>Bolus Feed</td>
<td>5 (38.5%)</td>
<td>8 (61.5%)</td>
<td>1.07 (0.13, 8.79)</td>
</tr>
<tr>
<td></td>
<td>Continuous Feed</td>
<td>2 (40%)</td>
<td>3 (60%)</td>
<td>Reference</td>
</tr>
<tr>
<td>Oral Motor Deficit – Mild Feed Type</td>
<td>Bolus Feed</td>
<td>3 (37.5%)</td>
<td>5 (62.5%)</td>
<td>1.11 (0.11, 10.99)</td>
</tr>
<tr>
<td></td>
<td>Continuous Feed</td>
<td>2 (40%)</td>
<td>3 (60%)</td>
<td></td>
</tr>
<tr>
<td>Oral Motor Deficit – Moderate Feed Type</td>
<td>Bolus Feed</td>
<td>15 (83.3%)</td>
<td>3 (16.7%)</td>
<td>1.00 (0.08, 11.93)</td>
</tr>
<tr>
<td></td>
<td>Continuous Feed</td>
<td>5 (83.3%)</td>
<td>1 (16.7%)</td>
<td></td>
</tr>
<tr>
<td>Oral Motor Deficit – Severe Feed Type</td>
<td>Bolus Feed</td>
<td>18 (75%)</td>
<td>6 (25%)</td>
<td>0.67 (0.10, 4.61)</td>
</tr>
<tr>
<td></td>
<td>Continuous Feed</td>
<td>4 (66.7%)</td>
<td>2 (33.3%)</td>
<td></td>
</tr>
</tbody>
</table>

Discussion

- The patient population may not be generalizable to other tube dependent children and likely have severe feeding problems as parents presented to evaluation seeking an intensive intervention.
- Appetite stimulation, alone, may not be enough to promote oral intake in children with complex feeding disorders and treatment from a multidisciplinary team is recommended.

Future Directions

- It would be beneficial to assess if transitioning from an overnight continuous enteral feeding schedule to a daytime bolus feeding schedule promotes an increase in oral intake in children with mild feeding difficulties.
Social Visual Engagement Patterns among ASD Children with Different Levels of Social Impairment have Differential Adaptive Value in Predicting Early Language Outcomes

Stella Yuan, Sanju Koirala, Deniz Parmaksiz, Sarah Shultz, Ami Klin, Warren Jones, and Laura Edwards
Marcus Autism Center, Children’s Healthcare of Atlanta, and Emory University School of Medicine

Background
Heterogeneity in autism spectrum disorder (ASD) is multi-level, manifesting itself from molecular genetics through brain circuitry to clinical and behavioral endpoints. Failure to capture heterogeneity not only presents obstacles to understanding the biological underpinnings of ASD, but also hinders the shift toward individualized treatment and targeted interventions. Measures that characterize the core clinical features of ASD, the most prominent being lack of engagement toward socially engaging stimuli, could provide a potential target for parsing phenotypic heterogeneity. In a recent eye-tracking study involving school-aged ASD children, Rise et al. found that the adaptive value of looking at specific social scenes differs from child to child, depending on their respective cognitive profiles. Given that ASD is a neurodevelopmental disorder, such findings raise intriguing questions as to whether diverging social visual engagement patterns differentiate ASD subgroups even in the earliest months of life, and whether these divergence reflect or predict language development outcomes by the second year of life.

Objectives

Objective 1: Explore the possibility of categorizing ASD subgroups with distinct clinical, cognitive, behavioral, and developmental profiles
Objective 2: Analyze fixation pattern differences between ASD subgroups, and examine whether differential adaptive values of social visual engagement exist

Participants & Methods

Table 1: Participant information including age at clinical assessment and cognitive ability scores. Group-wise comparison is performed using homoscedastic t-test, with p-value and significance reported

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>N (male, female)</th>
<th>MSEL Assessment</th>
<th>VIQ</th>
<th>NDIQ</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>121 (65, 56)</td>
<td>84.66 ± 11.36</td>
<td>112.94 ± 16.39</td>
<td>111.54 ± 20.36</td>
</tr>
<tr>
<td>48</td>
<td>121 (65, 56)</td>
<td>78.94 ± 11.36</td>
<td>108.95 ± 17.45</td>
<td></td>
</tr>
</tbody>
</table>

Subset ASD subjects: children with ASD are classified into high or low social functioning groups based on their social affect (SA) score from ADOS administered at 24 months of age. Quantitative eye-tracking data: for participants within each ASD subgroup, their eye tracking data were collected longitudinally in the first three years of life, and the proportion of time subjects spent fixating on the respective regions of interest (ROIs) are used to compare and contrast different social visual engagement patterns during early development.

Assess relation to language outcome: for each subgroup, cross-sectional analysis using Pearson’s correlation explores whether fixation on certain ROIs are associated with higher or lower receptive and expressive language outcomes as measured by MSEL administered at age two.

Discussion

- ASD children with lower ADOS SA scores not only looked more at the eyes compared to their high SA score counterpart throughout development, but also showed adaptive values of eye-looking, with the potential of effect of language acquisition scaffolding present in as early as the first few months of life.

These results suggest that the two ASD subgroups diverge in social learning mechanisms during early development, which could result in a snowballing effect that reinforces specific atypicality of the child’s cognitive profile.

- From previous research, typically developing children show an increase in amount of mouth looking in the first two years of life to facilitate language acquisition; however, neither ASD subgroups demonstrated such a mouth-looking-promoted language building experience. Indeed, we even observe that the more severely socially impaired group of children actually spend more time looking at mouth. This leaves us contemplating how fundamentally different learning experiences are constructed in TD compared to ASD children, and warns us how only looking at single data profiles and not taking multitudes of analysis levels into account might result in misleading interpretations.

- Future work should leverage on the longitudinal behavioral trajectories and clinical assessment datasets to continue probing characteristics of ASD subgroups and their effect on language abilities.

References


Acknowledgements

The authors wish to acknowledge the contributions of their team members, including('.', '...'). This research was supported by the Marcus Autism Center at Emory University School of Medicine through a Research Fund Grant. The authors acknowledge the assistance of the Atlanta Autism Research Network (AARNet) and the Children’s Healthcare of Atlanta in recruiting participants. The current study was approved by Emory University Institutional Review Board (IRB) and all participants provided informed consent.