Duke Department of Pediatrics

2018 Research Retreat

Monday, April 9, 2018
Trent Semans Center
1:00 - 7:30 pm

KEYNOTE ADDRESS
From Bench to Population: Leveraging Duke Resources for Your Research

L. Ebony Boulware, MD, MPH
Professor and Chief, Division of General Internal Medicine
Department of Medicine
Director, Clinical and Translational Science Institute
Associate Vice Chancellor for Translational Research
Vice Dean for Translational Research
Duke University School of Medicine

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Duke University Medical Center Department of Pediatrics
2018 Research Retreat - Trent Semans Center
Monday, April 9th 2018

Agenda

10-1pm Poster hanging

1pm-3pm Platform presentations accepted to upcoming PAS meeting Classroom 3 (3rd floor)
1:10pm Welcome and Overview - Moderated by Dr. Brian Smith
1:15pm Dr. Melissa Kay - Toddler dietary intake: Results from a multi-site study of low-income families.
1:30pm Dr. Noelle Younge - Disrupted Maturation of the Microbiota and Metabolome among Extremely Preterm Infants with Postnatal Growth Failure.
1:45pm Dr. Charles Wood - Beliefs Regarding Infant Weight and Future Health Differ By Race/Ethnicity, Education, and Income.
2:00pm Dr. Kristin Weimer - Lactoferrin and protection against postnatal cytomegalovirus infection in premature infants.
2:15pm Dr. Jennifer Varner - Targeted and whole exome sequencing identifies genetic causes of SRNS in 40% of families with autosomal dominant disease.
2:30pm Dr. Samrat Das - Trends in incidence and selected characteristics of pediatric patients with non-accidental trauma in the United States.
2:45pm Dr. Frances Saccoccio - Humoral Immune Correlates of Protection Against Postnatal Cytomegalovirus Acquisition.

3-5:15pm Poster viewing - light food and drinks 6th floor
3:00-5:00 Authors present at posters
5:15pm Poster takedown & make way to Learning Hall

5:25-7:30 Top Abstracts and keynote address Learning Hall (2nd floor)
5:25pm Opening remarks Dr. Ann Reed
William Cleland Professor of Pediatrics & Chair, Department of Pediatrics

5:30pm-6:15pm Keynote Introduction by Dr. Sallie Permar
L. Ebony Boulware, MD, MPH Professor and Chief, Division of General Internal Medicine in the Department of Medicine; Director, Clinical and Translational Science Institute Associate Vice Chancellor for Translational Research; Vice Dean for Translational Science
“From Bench to Population: Leveraging Duke Resources for Your Research”

6:20pm Recognition of Top 5 Basic Science abstracts - Nancie Maclver, MD, PhD
6:25pm Top Basic Research Abstract - Dr. Chengzhi Xie
A combination treatment with histidine-rich peptides and Alglucosidase alfa markedly reduces cytoplasmic glycogen storage in liver of GSD III mice.

6:40pm Recognition of Top 2 QI abstracts - Heather McLean, MD
6:45pm Top QI Abstract - Dr. Amy Lee
Improving Pain Management in Pediatric Patients Admitted for Uncomplicated Vaso-Occlusive Episodes.

7:00pm Recognition of Top 5 Clinical Research abstracts - Rasheed Gbabdegesin, MD, MBBS
7:05pm Top Clinical Research Abstract - Dr. Jennifer D. Varner
Targeted and whole exome sequencing identifies genetic causes of SRNS in 40% of families with autosomal dominant disease.

7:20pm Closing Remarks Dr. Coleen Cunningham
Vice Chair of Research, Department of Pediatrics
Important Research Resources

These are institutional research resources available for your studies. Please visit their tables and find out what they have to offer.

CCRU

DEPRU

DOCR

Histology Core

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myRESEARCHnavigators

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**Thank You**

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#1 Breast Milk-Associated Oxysterols Reverse Perinatal Brain Injury Through Gli-dependent Oligodendrogenesis.

Agnes Chao1*, Pavle Matak1*, James Powers1, Kelly Pegram1, Rebecca Jo1, Colin Hutson1, Laura Dubois2, J. Will Thompson2, Ronald N. Goldberg1, & Eric J. Benner1. 1Division of Neonatology, Department of Pediatrics, Duke University Medical Center, the Jean and George Brumley, Jr. Neonatal-Perinatal Institute, Durham, NC, USA. 2Duke Proteomics and Metabolomics Shared Resource, Duke University Medical Center, Durham, NC 27710 USA. *Equal contribution.

Background: Perinatal white matter injury is the leading cause of neurodevelopmental deficits in survivors of premature birth. Sepsis and inflammation are significant risk factors and there are no treatment options available to mitigate neurologic injury. A significant challenge to the development of novel therapeutic strategies in neonates is the appropriate concern for safety. Here, we identified multiple oxysterols in human maternal breast milk and explored the therapeutic potential of these oxysterols in directing neural stem cells (NSCs) into the oligodendrocyte lineage via the Sonic Hedgehog (Shh) pathway in vitro. Further, we investigated whether breast milk associated oxysterols can rescue perinatal diffuse white matter injury (DWMI) in vivo.

Objective & Hypothesis: We hypothesize that breast milk associated oxysterols target NSCs via the Shh pathway to promote oligodendrogenesis in vitro, and rescue perinatal myelin injury in vivo.

Methods: Oxysterols were identified in breast milk using liquid chromatography tandem mass spectrometry. NSCs were cultured from the SVZ of CD-1 mice, and treated with 20HC, 22HC or 25HC. Tissue was analyzed with immunohistochemistry/ stereology, western blot, and flow cytometry, looking for markers of the oligodendrocyte lineage (CNPase, MBP). Shh pathway activation was established by quantifying upregulation of target genes, Gli1 and Patched1, with western blot analysis and RT-PCR. Gli-dependence was explored using pharmacological inhibition with Gli antagonist, GANT61, and Gli1/Gli2 knockout mice. Perinatal sepsis leading to DWMI was modeled in mice via stoic injection into the peritoneal cavity on postnatal day 5. Mice then received either oxysterols vs. vehicle. Stereology determined oligodendrocyte numbers in the corpus callosum.

Results: 20HC, 22HC and 25HC induce Gli-dependent oligodendrocyte production from NSCs in vitro. Both Gli1 and Gli2 are required for oxysterol-induced oligodendrogenesis. Injured mice that received perinatal treatment with 20HC or 25HC had significantly increased periventricular oligodendrocyte numbers and improved motor function in adulthood, suggesting rescue of perinatal myelin injury.

Conclusions: Following perinatal DWMI, systemic administration of oxysterols rescued periventricular myelin injury and reversed motor deficits in mice. Because oxysterols are found in human maternal breast milk, this approach may be further developed into a novel and safe therapeutic strategy to mitigate perinatal DWMI.

#2 Title: Correction of Respiratory Insufficiency in an ALS Mouse Model Following Intralingual Administration of rAAVrh10-miRSOD1

Authors: Angela L. McCalli, Lori A. Lindz, Katherine A. Johnsonz, Ellyn Andelz, Olivia Stricklinz, Allison Keeler-Klunksz, Christian Mullerz,s, Robert H. Brown Jrs, Teresa E. Leverz,s, Nicole L. Nicholsz, and Mai K. ElMallahz.

Background & Hypothesis: Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disease with no current cure. Patients with ALS die 3-5 years after diagnosis from respiratory failure. However, if the bulbar muscles and motor neurons are affected first, death occurs within 2-3 years. Bulbar involvement results in dysarthria and dysphagia leading to recurrent aspiration, choking and aggravation of respiratory disease. Approximately 2% of ALS cases are linked to gain of function mutations in the gene encoding the Cu/Zn superoxide dismutase (SOD1). Mutant SOD1 is found aggregated in mitochondria disrupting many cellular processes, specifically in motor neurons. Respiratory insufficiency increases over time in ALS patients as their hypoglossal and phrenic motor neurons begin to deteriorate, and innervation of the tongue and diaphragm is lost. Our hypothesis is that reducing the expression of mutant SOD1 in the tongue will reduce lingual pathology and in turn improve nutrition, decrease upper airway pathology and impact respiratory function.

Objective: The objective of this project is to mitigate upper airway pathophysiology in an ALS model, by reducing the expression of mutant SOD1 through an intralingual injection of rAAVrh10 vectors carrying a microRNA against the mutant SOD1. Method: At 8.5 weeks of age, rAAVrh10-miRSOD1 or PBS was intralingually injected into SOD1G93A mice. Non-transgenic littermate control mice were injected with PBS. Beginning at 13 weeks of age respiration of treated SOD1G93A mice and both controls, was regularly monitored via whole body plethysmography under normoxic conditions and with respiratory challenges of hypoxia (11% O2) + hypercapnia (7% CO2). Minute ventilation, a comprehensive analysis of volume inspired per minute, was improved in miRSOD1 treated mice, during the respiratory challenge. Additionally, videofluoroscopic swallow study testing was performed once every two weeks from 3 months of age through end-stage disease. Blinded reviewers analyzed the video for several parameters (i.e. lick and swallow rates). Results: Early results indicate a mitigated dysphagia phenotype in the miRSOD1 treated mice compared to the vehicle treated mice. These functional benefits resulted in increased survival of miRSOD1 treated animals by one week. These physiological improvements are correlated to the presence of rAAV genomes and a reduction in SOD1G93A mRNA. Conclusions: In conclusion, it appears that targeting the tongue has a significant impact on the overall respiratory function of this ALS mouse model and intralingual therapy may be a potential therapeutic target for bulbar insufficiency in ALS patients.
Background & Hypothesis: Transplacental passage of immunoglobulins from mother to fetus during pregnancy provides the neonate with a population of circulating protective antibodies and is critical to neonatal immune protection. This process can be therapeutically exploited to systematically boost the neonatal levels of specific antibodies. Administration of the Tetanus, diphtheria, and pertussis vaccine (TDaP) is now recommended in every pregnancy to increase neonatal levels of pertussis antibodies. We hypothesized that antibody populations resulting from recently administered vaccines have different transfer efficiencies as compared to remotely administered vaccines. We also hypothesized that transplacental transfer efficiency corresponds to the distribution of IgG subclasses within each specific antibody population.

Objective: Determine the concentrations, transfer efficiency, and subclass composition of 12 vaccine-elicited antibodies in maternal-infant serum pairs, and determine impact of remote vs recent TDaP vaccination.

Methods: A total of 58 mother-infant pairs were used. 23 (40%) of the women received Tdap during pregnancy; all women received the seasonal influenza vaccine during pregnancy. Maternal and cord blood samples were collected at the time of delivery. Binding antibody multiplex assays were performed on all samples to detect the following antibodies: hepatitis B, rubella, pertussis toxin, pertactin, pertussis FHA, pertussis Fim 2/3, tetanus, diphtheria, Influenza A/California, Influenza B/Brisbane, Influenza A/Victoria, and Influenza B/Massachusetts. Geometric mean concentrations (or mean fluorescence indices) were calculated for each antibody; the cord-to-maternal transfer ratios were then calculated. Antibody concentrations and transfer ratios for each TDaP antibody were compared between the recent and remotely boosted women. The impact of recent vs remote Tdap on concentrations and transfer ratio of remotely administered vaccines (Hep B and rubella) was also determined.

Results: Both maternal and cord blood levels of all 6 TDaP antibodies were significantly higher after Tdap boosting during pregnancy. For diphtheria, this resulted in significantly more neonates with protective titers against diphtheria. Although there was a trend toward more robust transfer efficiency after recent Tdap administration, it did not reach statistical significance for any TDaP antibody. Transfer efficiency did not correlate with maternal antibody concentration for TDaP, rubella, and Hep B antibodies; however, for influenza antibodies, transfer efficiency was moderately correlated with concentration.

Conclusions: Prenatal Tdap vaccine results in increased levels of vaccine-elicited antibodies, but does not result in a statistically significant increase in transfer efficiency of those antibodies. Subclass assays are ongoing to assess contribution to transfer efficiency.

Background & Hypothesis: Demyelination is common to many devastating CNS diseases, including progressive multiple sclerosis, cerebral palsy and Krabbe disease. While many of these diseases lack effective treatments, mesenchymal stromal cell (MSC) therapy has emerged as a viable approach to treating some demyelinating diseases. MSCs, which benefit many rodent models of demyelinating disease through their immunosuppressive effects, are abundant in umbilical cord tissue. Our hypothesis is that human umbilical cord tissue derived MSCs can limit neuroinflammation by suppressing the activation of T cells in the CNS. Through suppression of neuroinflammation, ongoing demyelination will be halted and reversed.

Objective: In order to test this hypothesis, we assessed the ability of MSCs to prevent clinical disease progression in experimental autoimmune encephalomyelitis (EAE), the mouse model of multiple sclerosis.

Methods: To induce EAE, we immunized mice with MOG peptide, a CNS antigen, as well as complete Freund's adjuvant and pertussis toxin. Within 9-12 days of EAE induction, mice began to exhibit clinical signs of disease, in the form of ascending paralysis. Based on the severity of these clinical signs, we scored mice according to a welldelineated scale of 0-5. This clinical score is a direct reflection of the degree of neuroinflammation. In one experiment, MSCs were injected prior to the onset of EAE disease as a preventative dose, while in a subsequent experiment, they were injected shortly after onset as a therapeutic dose. We continued to monitor their clinical scores after MSC injection.

Results: In both experiments, we found a statistically significant improvement in clinical score in mice that received injections of MSCs, in comparison to mice that did not.

Conclusions: Our results demonstrate that MSCs have a prophylactic as well as therapeutic role in the prevention of EAE disease progression. Further experiments will aim to elucidate the mechanism of MSC-mediated T-cell suppression in EAE and other mouse models of CNS neuroinflammation, with the intention of eventually using these cells to treat pediatric and adult patients with selective demyelinating diseases.
Background & Hypothesis: The vast majority of new HIV infections among children occur via mother-to-child transmission (MTCT), with more than 150,000 pediatric infections occurring annually. Thus, there is a need to understand not only the pathogenesis of adult HIV-1 infection, but also the newborn immune response to infection in order to develop pediatric prevention strategies and achieve an HIV-free generation. Recent studies have indicated that HIV-1 infected infants develop broadly neutralizing antibody (bNAb) responses earlier than adults, and these responses tend to be of greater breadth and potency. Utilizing a rhesus macaque model of simian-human immunodeficiency virus (SHIV) infection, we conducted a longitudinal analysis on the virus-specific humoral immune response in SHIV-infected adult and infant macaques. We hypothesized that infant macaques would develop HIV Env-specific IgG and neutralization responses earlier than adults, and that infant neutralization responses would be of a greater magnitude and breadth.

Objective: To conduct a comparative analysis on the development of the humoral immune response among infant and adult rhesus macaques during SHIV CH505 infection.

Methods: Twelve adult rhesus macaques were infected intravenously, and six infant macaques were infected orally with a SHIV CH505 virus. Plasma viral loads were monitored and plasma gp120- and gp41-specific IgG levels were measured by ELISA through 12-weeks post-infection. In addition, neutralization activity was measured against the autologous CH505 transmitted/founder (T/F) virus and MW965, a clade C tier 1 (easy to neutralize) virus.

Results: Plasma Env-specific IgG responses were detectable in infants and adults by 3 weeks post-infection (wpi), with no significant difference in the magnitude of these responses. Autologous neutralization responses were detected in 75% of adults and 50% of infant macaques at 12-wpi. However, Tier 1 neutralization responses were detected much earlier, by 4-wpi in both groups, and increased through 12-wpi with similar magnitudes.

Conclusions: The humoral immune response to SHIV infection develops similarly among infant and adult macaques, which suggests that the infant B-cell repertoire is equipped to respond to HIV-1 infection and promote the production of functional antibody responses. Additionally, these data highlight the possibility of infant immunization as a strategy for prevention.
Titile and Authors: Targeting fusion-positive rhabdomyosarcoma cancer stem cells by interfering with TAZ/PAX3-FOXO1 transcriptional programming. Breanne A Burgess, Michael D Deel, MD, Corinne M Linardic, MD PhD

Background and Hypothesis: Fusion Positive Rhabdomyosarcoma (FP-RMS) is a pediatric soft-tissue sarcoma derived from cells with skeletal muscle lineage. Although patients initially respond to therapy, most will become refractory or relapse within five years. PAX3-FOXO1 (P3F), a transcription factor that is a fusion product generated by a t(2:13) chromosomal translocation, is the principal oncogenic driver of FP-RMS. However, it is absent of viable drug binding sites. A potential targetable protein, though, is TAZ: a transcriptional co-activator that conveys stem-like properties to adult epithelial cancers, and may regulate P3F transcriptional activity. TAZ is a critical co-activator of wild-type PAX3 mediated transcription, and TEAD1 and AP-1 (common binding partners to TAZ) are among the top enriched transcription factor motifs in P3F binding sites. Our laboratory demonstrated that TAZ is abundant in FP-RMS tumors and promotes proliferation, inhibits apoptosis, and attenuates xenograft tumor growth. We therefore hypothesize that the tumorigenic properties of P3F are dependent upon TAZ through a P3F/TAZ/TEAD1/AP-1 transcriptional complex. Methods: To investigate the functional interaction of TAZ with P3F, we are performing chromatin immunoprecipitation and DNA sequencing (ChiP-Seq) analysis of co-immunoprecipitation (co-IP) of epitope-tagged TAZ and P3F. Using P3F and TEAD reporters, we are determining the transcriptional activity of each in the presence of either wild-type TAZ (WT), constitutively active TAZ (S89A), or TAZ knocked out using CRISPR-Cas9 (KO). To investigate the role of TAZ in stemness, we are culturing FP-RMS cells with either WT, S89A, or KO TAZ as spheres (rhabdospheres), which enriches the cells with stem-like properties. Using qPCR, we are quantifying TAZ expression along with the expression of three pluripotent mesenchymal stem cell markers (Sox2, Oct4, and Nanog). Results: We have demonstrated that TAZ and P3F complex via co-IP in FP-RMS cell lines. We are now performing co-IP coupled mass spectrometry to determine the extent of the TAZ/P3F interaction. Through serial passaging of the rhabdospheres, TAZ expression was enriched along with the stem cell markers Sox2, Oct4 and Nanog. Further, TAZ KO bars the enrichments for the stem cell markers, while TAZ S89A increases expression of these genes. We plan to cross TAZ-floxed mice into our FP-RMS transgenic mouse model to determine if TAZ loss of function inhibits P3F-initiated tumorigenesis. Conclusions: Previous work from our lab showed that TAZ is important in FP-RMS tumor biology. Our current studies expand upon our knowledge about TAZ in FP-RMS by exploring transcriptional regulation and protein-protein interactions in gain- and loss-of-function studies using our new CRISPR model. These studies in concert with our investigation into the TAZ/P3F relationship in our in-vivo mouse model will give us more insight into TAZ as a promising novel target for treating this aggressive sarcoma.

Title and Authors: A combination treatment with histidine-rich peptides and Alglucosidase alfa markedly reduces cytoplasmic glycogen storage in liver of GSD III mice

Authors: Chengzhi Xie, Fengqin Gao, Haiqing Yi, Priya S. Kishnani, and Baodong Sun Background & Hypothesis: Recombinant human acid α-glucosidase (rhGAA, Alglucosidase alfa) is an FDA approved therapy for Pompe disease (glycogen storage disease type II, GSD II), in which glycogen accumulates in the lysosomes rather than in the cytosol of affected cells. Following intravenous administration of rhGAA in patients or mice, most enzyme (>90%) was taken up by liver cells and delivered into lysosomes through M6PR-mediated endocytic pathway. A method that can extend the delivery of rhGAA to the cytosol would be a desirable approach for clearing cytoplasmic glycogen accumulation in other GSDs, like GSDIII. Recently it has been demonstrated that histidine-rich, pH-responsive LAH4 family peptides can promote cytoplasmic delivery of a variety of macromolecular cargos including DNA and protein through the mechanism of endosomal escape. We hypothesize that co-administration of such an endosome-disrupting peptide with rhGAA will facilitate rhGAA delivery to the cytosol and therefore promote the correction of glycogen storage in cytoplasmic GSDs. Objective: We aimed to test our hypothesis using LAH6-L1-80, a member of the LAH4 family peptides, in a mouse model of GSD III. Methods: Ten-week-old GSD III mice were intravenously injected with rhGAA (20 mg/kg, n=5), LAH6-L1-80 (20 mg/kg, n=4), or the combination of the two (20 mg/kg rhGAA + 20 mg/kg LAH6-L1-80, n=8) twice weekly for 4 weeks. Mice were sacrificed 48h after the last injection. Age-matched untreated (UT, n = 6) mice were used as controls. Uptake of rhGAA and glycogen content were analyzed in liver, heart, and skeletal muscle. Results: GAA activity was extremely high in liver but slightly increased in heart of both the rhGAA-treated and the rhGAA+LAH6-L1-80-treated mice. Western blot showed the presence of the 110 kDa full-length rhGAA band (unprocessed cytoplasmic form) in liver lysates of rhGAA+LAH6-L1-80-treated mice, but not the rhGAA-treated mice, which indicates that the LAH6-L1-80 peptide induced rhGAA release from endosomes. The LAH6-L1-80+ rhGAA combination treatment reduced glycogen content only in liver (-48%), and significantly lowered the liver/body weight ratio. In contrast, treatment with rhGAA or LAH6-L1-80 alone had no effect on glycogen storage in any tissues. In addition, LAH6-L1-80 showed no significant toxicity as indicated by the unchanged plasma levels of ALT and AST (liver functions), and BUN and Creatinine (kidney functions). Conclusions: Co-administration of LAH6-L1-80 and rhGAA effectively reduced cytoplasmic glycogen accumulation in liver of GSD III mice. This novel treatment approach has the potential to address the unmet need for treatment of cytoplasmic GSDs.
#9 Determinants of transplacental IgG transfer in HIV-infected pregnant women


**Background & Hypothesis:** Factors that modulate the transplacental transfer of maternal IgG are not fully defined. In HIV-infected women, the transplacental transfer is impaired, offering a unique setting to define clinical, placental, and antibody determinants of IgG transfer. We therefore hypothesize that maternal HIV-disease progression factors, IgG characteristics, and placental factors are important for transplacental IgG transfer.

**Objective:** To define factors that modulate transplacental IgG transfer

**Methods:** We measured HIV and standard vaccine antigen-specific IgG serum concentrations in 167 HIV-infected women and their infants in US and Malawi cohorts by a binding antibody multiplex assay. The measured IgG antibodies were: HIV Env antigens, tetanus toxoid, pertussis toxin, influenza hemagglutinin, rubella virus capsid, hepatitis B surface antigen, respiratory syncytial virus surface antigen, and diphtheria toxin.

Transplacental IgG transfer efficiency was calculated as the infant cord blood IgG concentration over maternal serum levels. We measured HIV Env antigens, tetanus toxoid, pertussis toxin, influenza hemagglutinin, rubella virus capsid, hepatitis B surface antigen, respiratory syncytial virus surface antigen, and diphtheria toxin.

**Results:** From 167 HIV-infected mother infant pairs, 3 patterns of maternal transplacental IgG transfer phenotypes were observed: efficient, variable, and poor IgG transfer. 11 pairs had efficient IgG transfer against most tested antigens, whereas in 82 pairs the IgG transfer was variable from one antigen to another. Finally, 74 pairs had poor IgG transfer across most tested antigens. Maternal plasma viral loads and total serum IgG levels were statistically significantly higher in U.S. HIV-infected women with poor transplacental IgG transfer (p < 0.02, p < 0.0001, respectively). Maternal gp120 and V3-specific IgG serum magnitude responses negatively correlated with transplacental IgG transfer of gp120 and V3-specific IgG, but this trend was not observed for other antigen-specific IgG. Poorly transferred gp120-specific IgG had higher frequencies of Fc region fucosylation (p <0.0001) compared to efficiently transferred pertussis and tetanus toxoid-specific IgG. Interestingly, placental Fc receptor expression levels were variably expressed in women with variable transplacental IgG transfer. Fc receptor neonatal (FcRn) expression ranged from 1-7.1 reads per kilobase million expression levels in HIV-infected women with variable transplacental IgG transfer compared to 2.3-6.5 in women with poor transfer.

**Conclusions:** In HIV-infected women, the transplacental IgG transfer efficiency can have efficient, variable, and poor phenotype. Furthermore, IgG characteristics such as serum magnitude, Fc region glycan profiles, and placental Fc receptor expression levels are all important for transplacental IgG transfer efficiency.

#10 The development of neutralizing antibodies against maternal autologous viruses in HIV-infected pregnant women immunized with an HIV-1 envelope vaccine


**Background:** Despite anti-retroviral therapy and efforts to eliminate pediatric HIV-1, mother-to-child transmission (MTCT) of HIV-1 results in 150,000 new pediatric infections annually. Thus, further preventive strategies are required to achieve an HIV-free generation. Prior work suggests that a maternal vaccine which boosts non-broad, neutralizing antibody (Ab) responses against autologous viruses in pregnant women, the source of vertically transmitted variants, could effectively decrease MTCT. However, it is not yet known whether HIV-1 envelope (Env) vaccines can enhance autologous virus neutralizing Ab responses in HIV-infected pregnant women.

**Objective:** To assess whether immunization of HIV-infected pregnant women with an Env vaccine in the AIDS Vaccine Evaluation Group Protocols 104/102 improved the mother’s ability to neutralize her circulating viruses.

**Methods:** Plasma samples were obtained for 11 vaccinees (n=10 for gp120 Env vaccine, n=1 for gp160 Env vaccine) and 6 placebo-recipients. Using single-genome amplification (SGA), full-length env genes were obtained at a pre-immunization timepoint and after several vaccine boosts for each patient. Variants representing major clusters of the phylogenetic tree were selected and pseudoviruses from pre- and post-immunization timepoints were produced by overlap PCR. The neutralization potency of maternal plasma against each autologous virus across all visit timepoints was assessed in the TZM-bl cell neutralization assay. The mean change in plasma ID50 against autologous viruses in each vaccinee and placebo-control will be compared by the Mann-Whitney U test.

**Results:** For the gp160-vaccinated mother compared to the placebo-control, phylogenetic trees constructed for the env sequences demonstrate a relative narrowing in maternal virus diversity after vaccination. Preliminary neutralization data shows that after gp160 immunization, the neutralization potency of maternal plasma against pre-immunization timepoint viruses increased across timepoints. In addition, the post-immunization virus population is relatively more resistant to neutralization by concurrent plasma samples in the vaccinee compared to the placebo-control.

**Conclusions:** Our results suggest that rgp160 vaccination may limit viral diversity and promote the development of resistant escape variants through enhancement of maternal plasma autologous virus neutralization. In ongoing investigation, we are completing SGA for gp120-vaccinated mothers and additional placebo-controls and will similarly interrogate the kinetics of their autologous NAb responses. This work can be applied to investigations of the effectiveness of next-generation maternal HIV-1 Env vaccines in pregnant women, which would work synergistically with anti-retroviral therapy to further reduce infant HIV infections.
Title & Authors: Characterizing phosphoregulation of the virulence-associated transcription factor CrzA by calcineurin in Aspergillus fumigatus


Background & Hypothesis: Calcium signaling plays a vital role in the growth and pathogenesis of Aspergillus fumigatus. The phosphatase calcineurin regulates the cellular response to calcium in this fungus primarily through activation of the transcription factor CrzA. Dephosphorylation of CrzA by calcineurin enables its translocation into the nucleus and subsequent transcriptional upregulation of key virulence-associated genes. While calcineurin is conserved throughout the eukarya, CrzA is a fungal-specific molecule with only minimal homology to NFAT, its closest mammalian counterpart. As such, CrzA represents a potentially excellent target for the treatment of invasive aspergillosis. However, little is known concerning the mechanisms of CrzA activation.

Objective: To better understand the biochemical mechanisms underlying the control of CrzA by calcineurin.

Methods: We employed liquid chromatography-tandem mass spectroscopy (LC-MS/MS) to identify specific sites of phosphorylation involved in the activation of this transcription factor. Strains expressing GFP-labeled CrzA were generated in both wild-type and calcineurin A deletion backgrounds and labeled CrzA was isolated from each background using a GFP-Trap affinity purification method. Specific clusters of phosphorylated residues were then mutated via site-directed mutagenesis to phosphomimetic amino acids in order to characterize the functional roles of phosphorylation at these sites with regard to nuclear translocation of CrzA, hyphal growth, conidiation and cell wall stress tolerance.

Results: Using this methodology, we characterized twenty such sites, the majority of which were specific to filamentous fungi and located in a serine-rich region N-terminal to the conserved DNA-binding domain of the protein. Mutation of phosphorylated sites had minimal effect on fungal phenotype or CrzA nuclear translocation.

Conclusions: Our findings indicate a more complex regulatory process for CrzA distinct from that identified for mammalian NFAT. These findings provide important new insight into the mechanisms of CrzA activation that will likely constitute essential knowledge for the successful pursuit of new antifungal agents targeting this unique regulator of Aspergillus virulence.

Title & Authors: Humoral Immune Correlates of Protection Against Postnatal Cytomegalovirus Acquisition

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Background: Congenital cytomegalovirus (CMV) is the leading infectious cause of birth defects in the US. Development of an effective CMV vaccine is a public health priority. However, CMV vaccine development is limited by a poor understanding of the immune correlates of protective immunity. Pre-existing CMV immunity is partially protective against congenital CMV as CMV seropositive women transmit CMV to the fetus at a lower rate than CMV transmission during primary infection. The undefined role of CMV-specific IgG in protection against CMV acquisition is a major gap in knowledge needed to guide CMV vaccine development.

Objective: Identification of passively acquired maternal antibodies that contribute to protection against postnatal CMV acquisition is predicted to elucidate immunologic targets for CMV vaccine design. We hypothesize that placental transfer of high-avidity CMV-specific IgG provides protection against postnatal CMV acquisition.

Methods: We analyzed 29 CMV-seropositive Ugandan mothers whose infants were followed weekly for postnatal CMV acquisition using saliva PCR. 12 infants acquired CMV and 17 infants did not acquire CMV in the first 6 months of life. We compared CMV-specific IgG responses at delivery of mothers whose infants acquired CMV to mothers whose infants did not acquire CMV.

Results: We found no difference in CMV-specific IgG binding or avidity to whole virions or gB. CMV-specific IgG measured by binding antibody multiplex assay to multiple antigens (gB, gH/gL, and gH/gL/UL128-131) involved in virus-cell entry and IgG neutralization of CMV entry into epithelial cells were also the same between groups.

Conclusions: These data suggest that maternal CMV-specific IgG at delivery do not predict protection against postnatal CMV acquisition. We are currently evaluating the role of passively acquired maternal CMV-specific IgG in infant sera in postnatal CMV acquisition.
**#13 Development of Broadly Neutralizing Antibodies in HIV-1 Infected Children**

*Shuk Hang (Grace) Li, Maria Dennis, Josh Eudailey, Youyi Fong, Justin Pollara, Kevin Saunders, Celia LaBranche, David Montefiori, Sallie Pernar, and Genevieve Fouda*

**Background:** Mother-to-child transmission of HIV accounts for approximately 150,000 new pediatric infections each year. Broadly neutralizing antibodies (bnAbs) have been shown to prevent virus acquisition in non-human primate models, suggesting that an effective HIV vaccine may require the induction of bnAbs. While only a subset of infected adults develop bnAbs 2 to 3 years post-infection, a previous study demonstrated that 20 of 28 HIV-infected infants achieved cross-clade neutralization, some within 1 year of infection. Thus, it is important to further characterize the development of bnAb responses in HIV-infected children in comparison to HIV-infected adults.

**Objective:** To assess the ontogeny and function of HIV-specific antibodies in a large cohort of HIV-infected children in the absence of ART-induced virologic suppression.

**Methods:** We have obtained plasma from 212 HIV clade B infected children aged 1 to 3 prior to initiation of ART. The neutralizing activity of these samples was assessed using a panel of 10 tier-2 viruses from multiple clades. The neutralization breadth and potency in children was compared to that of chronically infected adults using a published dataset. ADCC activity was measured against target cells infected with an infectious molecular clone virus representing the clade B transmitted/founder isolate HIV.B.WITO. In addition, binding to various HIV-envelope antigens was assessed using a binding antibody multiplex assay (BAMA). Samples from 44 clade B chronically infected adults were tested by the same BAMA panel to compare antibody binding profiles between adults and children.

**Results:** By one year of age, the neutralization breadth and potency of children was comparable to that of adults. Overall, 1 to 3-year-old children exhibited greater neutralization potency against more viruses than chronically infected adults. ADCC antibodies were detected in the majority of infants at 1-year-old, and antibody titers increased over time. In contrast, while the magnitude of most binding antibodies increased from one to two years of age, 2 and 3-year-old children had levels of HIV-specific antibodies that were comparable to that of adults.

**Conclusions:** These preliminary data support previous works indicating that children can develop cross–clade neutralizing antibodies earlier than adults. In future work, we will define the breadth of ADCC mediating antibodies in children in comparison to adults and map the specificity of cross-clade neutralizing antibodies in children. Our ultimate goal is to identify children with broadly neutralizing antibody responses for isolation and characterization of monoclonal antibodies.

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**#14 Deficiency of Shank3 Impaired Excitatory Synapse Function in Nucleus Accumbens**

*Haidun Yan, Xiaoming Wang, Alexandra L. Bey, Yong-Hui Jiang*

**Background & Hypothesis:** Mutations in the SHANK3 gene have been discovered in autism spectrum disorder (ASD), and Shank3 deficiency is known to cause impairments in synaptic transmission. How Shank3 deficiency affects specific neuronal circuits and their cellular mechanisms underlying ASD remains elusive.

**Objective:** To understanding contribution of cortex (PFC) to nucleus accumbens (NAc) circuit underlying ASD mechanism, we identifying how shank3 deletion changes in excitatory synapse transmission in NAc medium spiny neurons (MSNs), and confirm Shank3 deletion induced synapse functional changes are mediated by direct (D1) or indirect (D2) pathway MSNs.

**Methods:** Here we used complete knockout mouse model of the autism-associated Shank3 gene with a deletion of exons 4-22 (Δe4-22), 2) and Cre-knockout mouse model of shank3 deleted in direct or indirect pathway medium spiny neurons (MSNs, D1 or D2 cells). Using whole-cell patch clamp technique, we recorded excitatory post synaptic currents (EPSCs) in NAc core MSNs.

**Results:** We found that both of NMDA-receptor and AMPA-receptor mediated evoked excitatory post synaptic currents (eEPSCs) reduced in neurons from Shank3 conditional knock mouse that compared to Shank 3 control WT mice, and these reductions of synaptic currents were observed in D2 MSNs from D2 shank3 Cre-knock mouse model, not in D1 MSNs.

**Conclusions:** These finding demonstrate that shank3 deletion impaired excitatory synapse transmission in NAc striatal pathway in mouse model of autism and data suggest the indirectly pathway (D2) disruption might play a causative role in the synaptic transmission of shank3 mutant mice, resulting in PFC (prefrontal cortex)-NAc-VTA (ventral tegmental area) circuit abnormalities that contribute to ASD-like behavior.
#15 Title & Authors: Tail Domain of the Aspergillus fumigatus Class V Myosin Orchestrates Septal Localization and Hyphal Growth

Hilary Renshaw, José M. Vargas-Muñiz, Praveen R. Juvvadi, Amber D. Richards, Greg Waitt, Erik J. Soderblom, M. Arthur Moseley, William J. Steinbach

Background & Hypothesis: Polarized hyphal growth and septation facilitate invasion of host tissue by Aspergillus fumigatus. To better understand these fundamental cellular processes, we have chosen to study the class V myosin (MyoE), an actin-based cargo protein. The globular tail domains of class V myosins have been shown to be important for cargo binding and actin cable organization. Additionally, phosphorylation plays a role in class V myosin cargo choice. Our previous studies on the class V myosin, MyoE, in the fungal pathogen A. fumigatus confirmed its requirement for normal morphology and virulence. However, the domains and molecular mechanisms governing MyoE’s function remain unknown. Objective: To determine the function of the MyoE tail domain and phosphorylation of MyoE in hyphal growth and septation

Methods: We generated a series of MyoE tail domain truncation mutants and analyzed for radial growth, conidiation, septation, cell wall components by staining, and hyphal growth by microscopy. We determined the phosphorylation status of MyoE using phosphoenrichment and LC-MS/MS. Phosphorylated residues were analyzed for requirement by substituting serine/threonine for alanine (mimicking a nonphosphorylated state of that residue) and examined for the phenotypes previously described. To determine possible regulators of phosphorylation, we examined the phosphorylation status of MyoE in the absence of the serine/threonine phosphatase, calcineurin.

Results: By analyzing tail mutants we demonstrate that the tail is required for radial growth, conidiation, septation frequency, and MyoE localization at the septum. Furthermore, MyoE is phosphorylated at multiple residues in vivo; however, alanine substitution mutants revealed that no single phosphorylated residue was critical. Importantly, in the absence of the phosphatase calcineurin, an additional residue was phosphorylated in its tail domain. Mutation of this tail residue led to mislocalization of MyoE from the septa.

Conclusions: We have demonstrated the importance of the A. fumigatus MyoE tail domain in the integral processes of hyphal growth and septation. Furthermore, we have shown that phosphorylation plays an important role in localization of MyoE, and that its localization at the septum is microtubule- and actin-independent. Future studies directed towards understanding how MyoE remains stably at the septa and determining MyoE protein-protein interactions would reveal the precise role for MyoE in the regulation septation.

#16 Title: Early Infancy Gut Microbiota Predicts the Quality of Vaccine-Induced Antibody Responses in Rhesus Macaques, Authors: Holly Heimsath, Amir Ardesthi, Olaf Mueller, Bonnie Phillips, Justin Pollara, Joshua Eudailey, Erika Kunz, Genevieve Fouda, Zhengzheng Wei, Laura-Leigh Rowlette, Holly Dressman, Kristina De Paris, Guido Ferrari, Koen Van Rompay, and Sallie R. Permar

Background & Hypothesis: Despite considerable advances made over the past thirty years, we remain without a highly-effective HIV vaccine or novel strategies for pediatric HIV-1 prevention that move beyond ART-based therapy. Evidence is emerging in a number of vaccine settings, including HIV vaccination, that commensal microbiota are linked to vaccine-elicited immune responses. The gut microbiome is most plastic during infancy, with the transition from a relatively sterile environment in utero to one of constant exposure to pathogenic and nonpathogenic microbial organisms. A successful HIV-1 vaccine may need to harness the unique landscape of the pediatric immune system by early immunization with concurrent rational manipulation of the microbiome to enhance vaccine efficacy. Objective: To define the relationship between the developing microbiome in infant rhesus monkeys and the immunologic response following HIV-1 vaccination.

Methods: Four vaccine groups, 1) Conventional, 2) Co-Administration, 3) Protein Only, and 4) Extended Interval, each consisting of 5 infant rhesus monkeys were immunized with varying HIV Envelope (Env) prime/boost modalities and intervals, employing both systemic and mucosal routes of immunization (Phillips et al, Clin Vaccine Immunol. 2017). Env-specific plasma antibody responses were measured by multiple immunological assays. Phylogenetic profiling of infant microbiomes was conducted by extracting 16S ribosomal RNA from stool samples pre- and post-immunization. The variable region 4 (V4) of 16S rRNA was amplified and amplicons sequenced on an Illumina MiSeq platform. 16S rRNA reads were quality filtered, de-multiplexed, and clustered into operational taxonomic units (OTUs) using vsearch. A subsequent diversity analysis was performed with QIIME, LASSO regression, PCA, and other machine based learning algorithms.

Results: Principal component analysis of 16S rRNA relative abundance data showed diversification of the gut microbiota clusters based on age. This age-based diversification held true when a Shannon diversity index was applied. Further analyses were employed using all animals. Comparing stool microbiota abundance to Env-specific immune responses, we found distinct positive correlations of taxa present at birth to variable loop 1/2 binding, and also to that of variable loop 3. Using the LASSO machine learning analysis, the presence of specific species in stool at birth were predictive of >70% of the tier 1 neutralizing antibody responses. In contrast, the presence of other species was negatively associated with gp140 and ADCC responses. When correlating peak antigen specific memory B cells from the colon or 1086.C gp120 stool IgA to taxa found at birth, the model did not fit and correlations could not be determined.

Conclusions/Future Directions: These exploratory data suggest that in infant rhesus macaques, the intestinal microbiota is associated with HIV Env-elicited immune responses that have been previously correlated with lower infection rates (RV144 trial). Subsequent investigations will seek to identify specific taxa that enhance Env-elicited immunity, thus facilitating rational manipulation of the microbiome using probiotics to enhance potentially-protective immune responses following HIV-1 immunization.
#18 A single intravenous injection of an AAV-PHP.B vector encoding human acid α-glucosidase corrects both muscle and brain defects in murine Pompe disease.

Jeong-A Lim, Haiqing Yi, Fengqin Gao, Priya Kishinani, and Baodong Sun

**Background & Hypothesis:** Pompe disease, caused by a deficiency of acid α-glucosidase (GAA), was recently shown to cause central nervous system (CNS) defects in both human patients and GAA-KO mice, in addition to severe cardiac and skeletal muscle myopathy. Enzyme replacement therapy has limited effect on skeletal muscle and the brain due to autophagic accumulation and the blood-brain-barrier (BBB).

**Objective:** The purpose of this study is to improve the therapy in both brain and skeletal muscle using the newly developed AAV-PHP.B vector, which showed high efficiency in transducing CNS.

**Methods:** Two-week-old GAA-KO mice were intravenously injected with the AAV-PHP.B-GAA vector, which contains rhGAA with CB promoter, at a dose of 5x10^{12} vg/kg. Improvement of neurologic and neuromuscular function was evaluated by behavioral tests including cylinder test, beam walking, footprint, and rota-rod to assess motor coordination and balance; von Frey test to detect the sensory defect; novel object recognition test to measure the cognitive defect. All mice were euthanized at 4 months of age for analysis of biochemical and histological corrections. AAV vector biodistribution (copy numbers) was quantified by real-time PCR.

**Results:** In the AAV-treated mice, GAA activity was extremely high in the heart (>26 folds of WT value) and restored to WT levels in the brain (cerebellum and cerebrum cortex), skeletal muscles, and liver. AAV copy numbers were high in the brain, heart, and liver, but low in the skeletal muscles. Consistent with these results, AAV treatment significantly reduced glycogen contents in these tissues: by >90% in the heart, nearly 100% in the heart, and 73-80% in skeletal muscles. Periodic acid–Schiff (PAS) staining of brain sections from untreated GAA-KO mice reveals widespread lysosomal glycogen accumulations in the entire brain. Particularly, white matter and the Purkinje cell layer in the cerebellum, the glomerular layer of the olfactory bulb, and corpus callosum area show extensive glycogen accumulation. PAS-positive glycogen is mostly observed in the glial cells rather than neuronal cells in most brain regions, except the hindbrain (Pons and Medulla) where glycogen accumulates both in neuronal and glial cells. In the AAV-treated mice, no visible glycogen can be found in any regions of the brain.

All the functional tests performed in this study showed significant improvement by the AAV treatment.

**Conclusions:** For the first time, we demonstrated that a single intravenous injection of AAV-PHP.B vector into GAA-KO mice at a young age corrected disease phenotypes in both the brain and muscles. The AAV-PHP.B vector should also be effective for other neuromuscular and neurodegenerative disorders.
**#19 The role of sirtuin 3 on T cell subset metabolism and function**

*Jonathan Warren, Keiko Danzaki, Amanda Nichols, William Eisner, and Nancie MacIver*

**Background & Hypothesis:** Malnutrition suppresses immune function, increases susceptibility to infection, and predisposes to death from infectious diseases, particularly in vulnerable pediatric populations. For many reasons, simply refeeding these patients is not always feasible or sufficient, and immunity can remain impaired for several months following refeeding or treatment of malnutrition. We have evidence to suggest that epigenetic modifications (such as DNA methylation or protein acetylation) may play a role in nutritional regulation of immune dysfunction and prolonged impairment following refeeding. More specifically, we have identified sirtuins, protein deacetylases that mediate the adaptive response to a variety of stresses, including calorie restriction, as a potential link between malnutrition and immune cell response. Sirtuin 3 (SIRT3) is a protein deacetylase and epigenetic regulator within the mitochondria that has been shown to be activated in fasting and calorie restriction. Additionally, SIRT3 is known to promote oxidative metabolism. Whole-body deletion of SIRT3 weakens the suppressive function of T regulatory cells (Treg cells), a T cell subset that relies on oxidative metabolism.

**Objective:** The present study aims to determine the role of SIRT3 in T cell subset function and metabolism using a targeted genetic approach, and to determine the role of SIRT3 in T cell dysfunction in malnutrition.

**Methods:** All studies were performed in mice on a C57BL/6 background. T cell-specific SIRT3 conditional knockout mouse (SIRT3 cKO) and appropriate controls were generated. CD4+ T cells were isolated from total splenocytes and were analyzed as bulk CD4+ T cells or differentiated in vitro into T cell subsets (Th1, Th17, and Treg). For malnutrition studies, mice were fasted 48 hours and compared to ad libitum fed controls.

**Results:** SIRT3 protein was upregulated in Treg cells generated from WT animals. Treg cells from SIRT3 cKO mice displayed higher glucose uptake relative to WT Treg cells in the fed state, but no difference was apparent in fasting. SIRT3 cKO mice also exhibited impaired Treg cell differentiation and impaired suppressive activity in the fed state, but not after fasting.

**Conclusions:** These data uniquely implicate SIRT3 in the regulation of the proliferation and activity of Treg cells, a T cell subset known to rely on oxidative metabolism. Future studies will further characterize mitochondrial capacity and substrate preference in T cell subsets in fed and fasted SIRT3 cKO mice. Results from our studies will help to elucidate mechanisms by which changes in malnutrition alter immune function, and determine why immune dysfunction persists following treatment of malnutrition, so that we may identify interventions to target and augment immunity, prevent and treat infections, and reduce risk and mortality in malnourished individuals.

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**#20 The effect of insulin and insulin-like growth factor-1 on CD4+ T cell metabolism and Function**

*Kaitlin Kiernan, Keiko Danzaki, Amanda Nichols, William Eisner, Nancie J MacIver*

**Background & Hypothesis:** Insulin is secreted from pancreatic β cells in response to increasing blood glucose levels to promote the cellular uptake of glucose into metabolic tissues such as muscle and fat and to maintain normal blood sugars. Insulin levels are also nutritionally regulated and are generally elevated in response to obesity, due largely to the development of insulin resistance of metabolic tissues following weight gain. We have previously reported that nutritionally-regulated hormones, such as leptin, can influence immune cell function and metabolism. Because insulin receptor expression is upregulated following T cell activation, we proposed that insulin may also have a role in mediating T cell glucose uptake and metabolism to fuel T cell activation. Moreover, T cell metabolism and function are intimately linked and changes in cellular metabolism can alter T cell differentiation/function and vice versa. We therefore propose to study how insulin and related family members, including insulin-like growth factor 1 (IGF-1), regulate T cell metabolism, differentiation and function and thereby influence immunity.

**Objective:** To determine how insulin and IGF-1 influence both T cell metabolism and T cell differentiation/cytokine production.

**Methods:** We analyzed the response of activated CD4+ T cells to treatment with insulin and/or IGF-1 *in vitro* using both radioactive and flow cytometry based glucose uptake assays, as well as qPCR to determine gene expression changes.

**Results:** We found that activated CD4+ T cells cultured with insulin or IGF-1 had increased glucose metabolism *in vitro*, as indicated by increased glucose transporter 1 (Glut1) expression and increased glucose uptake. The greatest effect of insulin on T cell glucose uptake comes at 24 hours of insulin treatment, while IGF-1 exerts its greatest effect on T cell glucose uptake at 6 hours of treatment. Analysis of mRNA from CD4+ T cell subsets showed that each functionally distinct subset of CD4+ T cell has a specific expression level of insulin receptor and IGF-1 receptor, indicating some specificity in responsiveness of T helper subsets to insulin and IGF-1.

**Conclusions:** Overall, insulin and IGF-1 promote increased T cell glucose uptake and glycolytic metabolism. Future studies will determine the role of insulin and/or IGF-1 signaling in T cell immune responses to infection and in the context of obesity, where systemic levels of insulin and IGF-1 are elevated. We have T cell specific insulin receptor conditional knockout mice, and we are generating T cell specific IGF-1 receptor conditional knockout mice, to further understand the effect of insulin and IGF-1 signaling on T cell metabolism and function.
Anencephaly-associated de novo RHOA GTPase mutation decreases activity

Kyle N. Erwin*, Aintzane Urbizu-Serrano*, Karen Soldano, Melanie Garrett, Allison Ashley-Koch‡, Mary R. Hutson‡

Background & Hypothesis: Anencephaly is a severe neural tube birth defect (NTD) characterized by missing parts of the brain and skull. The prevalence in the US is 3 in 10,000 pregnancies or 1,206 cases per year. Nongenetic risk factors of anencephaly have been identified, however the genetic basis is little understood. We have identified a novel de novo RHOA mutation in a proband which is absent in either of the parents. The mutant residue is located in a novel uncharacterized domain of RHOA and is highly conserved throughout evolution across species and several GTPase families including Ran, Rac, Ras, and Rho. The RHOA pathway is critical to neural tube closure and perturbations of the pathway cause neural tube defects in animal models. We hypothesize that the mutant RHOA protein exhibits altered activity contributing to the formation of anencephaly.

Objective: To determine the activity of a novel de novo mutant RHOA protein and determine its role in causing cranial neural tube defects in an animal model.

Methods: In vitro methods to probe RHOA activity include luciferase assays, actin morphology, and active-RHOA ELISA. An ex ovo chick embryo culture method is employed allowing facile investigation of neural tube defects. Embryos are cultured in multiwell plates allowing both genetic (neural tube electroporation of the mutant RHOA and other variants), and pharmacological approaches.

Results: Our in vitro data from cell assays indicates the RHOA mutant exhibits decreased activity. We have developed an ex ovo chick embryo culture method suitable for studying neural tube defects. Ex ovo chick embryos treated with a pharmacological inhibitor of RHOA results in NTD’s in up to 100% of embryos depending on dose.

Conclusions: A novel de novo RHOA mutation identified in an anencephalic patient exhibits decreased activity in vitro. An ex ovo chick embryo culture method has been optimized for the investigation of NTDs and has been used to validate RHOA requirement in neural tube closure. Further characterization of the mechanism by which the mutant RHOA exhibits reduced activity will help determine how neural tube defects arise genetically and broadly inform small GTPase biology as the mutant residue is highly conserved among several small GTPase families.

The Gut Microbiota of Healthy Infants in the Community is a Reservoir for ESBL and Carbapenemase Producing Bacteria.

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Background & Hypothesis: The recent rapid rise of Extended-spectrum Beta Lactamase producing Gram Negative bacteria (ESBL-GBN) has seriously threatened the treatment of common infectious diseases. Neonates have an immature immune system and a delay in appropriate treatment due to ESBL-GBN sepsis can be fatal. Additionally ESBL E. coli such as the strain ST131 are known to be persistent gut and vaginal colonizers. In animal models, these strains out-compete colonization with drug-sensitive, commensal E. coli. Gut colonization with ESBL-GBN in infants may therefore have a profound impact on their microbiome and increase their risk of sepsis.

Objective: To estimate the prevalence of gut colonization with ESBL-GBN in high incidence setting and determine phenotypic differences between these strains and commensal E. coli.

Methods: Stool samples were collected from 100 healthy infants living in a Pakistani suburban community between the ages of 5-7 months. Samples were plated on MacConkey agar to select for Gram negative bacteria. Isolates were screened for resistance against several antimicrobial classes. Molecular testing of the stool samples was done using primers targeting conserved regions of resistance genes.

Results: Forty-eight percent of the infants were positive for ESBL producing Gram negative bacteria, the majority of which were E. coli, and 7.5% were positive for carbapenemase producers, all of which belonged to Klebsiella spp. Molecular testing showed that 85% of the infant stools were positive for TEM beta-lactamase gene, 68% for the CTX-M beta-lactamase gene and 33% for the KPC carbapenemase gene. ESBL producing E. coli were also found to be better growers compared to commensal E. coli in nutrient restricted growth medium when grown as individual strains. In in vitro competition experiments some of these strains can also out-compete commensals in enriched media.

Conclusions: Our studies have revealed that the resistome of otherwise healthy infants may be a major reservoir of antibiotic genes in the community.
#23 Bezafibrate induces autophagy and improves hepatic lipid metabolism in glycogen storage disease type Ia,
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**Background & Hypothesis:** Glucose-6-phosphatase (G6Pase-α) deficiency, also known as von Gierke's Disease or GSD Ia, is characterized by decreased ability of the liver to convert glucose-6-phosphate to glucose leading to glycogen accumulation and hepatosteatosis. Long-term complications of GSD Ia include hepatic adenomas and carcinomas, in association with the suppression of autophagy in the liver. Addressing hepatocellular abnormalities could prevent negative long-term outcomes of the disease.

**Objective:** Determine if the drug bezafibrate induces autophagy in the mouse and dog models of GSD Ia and whether induction of autophagy can alleviate hepatocellular abnormalities in GSD Ia.

**Methods:** GSD Ia mice and dogs were treated with bezafibrate and hepatic glycogen and triglyceride concentrations were measured. Western blotting was performed to investigate pathways affected by the treatment. The effect of acid α-glucosidase (GAA) treatment, with and without bezafibrate, was also studied to investigate synergistic effects.

**Results:** Liver triglyceride and glycogen concentration decreased upon bezafibrate treatment and the autophagy deficit seen in previous GSD Ia models was attenuated. Protein markers for fatty acid oxidation showed increased expression, and fatty acid synthase expression (associated with lipogenesis) was decreased in G6pc-/- mice treated with bezafibrate. Introducing GAA treatment in combination with bezafibrate further decreased glycogen and triglyceride levels in the liver.

**Conclusions:** Based on these results, we determined that bezafibrate induces autophagy in the liver while increasing fatty acid oxidation and decreasing lipogenesis. It represents a potential therapy for combating the hepatocellular abnormalities in GSD Ia and preventing negative long-term complications associated with the disease.

#24 Impact of passive administration of a broadly neutralizing antibody, CH31, on active HIV-1 envelope vaccine responses in infant rhesus monkeys
Maria Dennis, Josh Eudailey, Ken Cronin, Justin Pollara, Munir Alam, Shuanna Shen Koen K.A. Van Rompay, Kristina De Paris, and Sallie Permar

**Background and Hypothesis**
In 2016, there were 1.8 million new HIV-1 infections worldwide; 160,000 of those infections were in children less than 15 years of age. Mother to child transmission (MTCT) remains the primary mode of infection in children. Thus, prevention of MTCT is of high priority, especially in resource-limited countries where mother’s adherence to therapy is challenging. Development of an effective infant vaccine is one solution to prevent future pediatric infections of HIV-1 via MTCT. Including a passive broadly neutralizing antibody (bnAb) simultaneously with an active vaccine is one strategy to maintain high protective antibody levels while the infant generates a response to an active vaccine. We hypothesize that this passive bnAb administration will have a limited effect on, or potentially enhance, the vaccine-elicited antibody responses.

**Objective**
Achieve high levels of protective antibodies with a passively administered broadly neutralizing antibody while minimally impacting active vaccine responses.

**Methods**
The experimental groups contain 5 rhesus monkeys each and received either an HIV Env protein only or an MVA-HIV Env construct plus Env protein immunization. Each group also received a bnAb, CH31, at week 0 of life. Two other groups serve as the controls with respect to each immunization strategy, but did not receive the bnAb. Env-specific antibody levels and characteristics were assessed using ELISA, a multiplex binding assay, TZM-bl cell based neutralization assay, and ADCC.

**Results**
Peak vaccine-elicited antibody levels in infants were achieved 2 weeks after the 2nd immunization and were similar among all groups, with or without CH31. The magnitude of these antibodies were maintained throughout the study, a total of 34 weeks. Peak neutralization titers and ADCC granzyme B activity were also similar among infant vaccinees with or without CH31. The Env protein group without CH31 achieved the highest peak infected cell binding responses against 1 of the 3 viruses tested; yet similar binding was achieved in the remaining 2 viruses. Vaccine-elicited antibodies specific for V3 and C5 epitopes of HIV-1 were of the highest magnitude, with similar epitope-specificity profiles among all groups, measured by conformational and linear epitope mapping.

**Conclusion**
There were no significant differences in infant Env vaccine-elicited antibody responses between infants that did and did not receive a passive bnAb immunization.
**#25 Tet1 mediated transcriptional regulation of the oxytocin receptor gene in the mouse brain**

*Aaron J Towers, Martine W Tremblay, Alex L Bey, Lara J Duffney, Xiaoming Wang, Wenhai Zhang, Wei Xie, Yong-hui Jiang*

**Background & Hypothesis**: The ten-eleven translocation (TET) family of proteins has been shown to demethylate 5-methylcytosine (5mC) by stepwise oxidation to 5-hydroxymethylcytosine (5hmC), 5-formylcytosine, and 5-carboxycytosine. Regulation of this demethylation process is particularly important in the postnatal brain, where 5hmC levels are highest and accumulate with age.

**Objective**: To examine how TET1 and 5hmC regulate transcription of the oxytocin receptor gene (Oxtr), a gene implicated in social behaviors and maternal care, and which is associated with autism spectrum disorders.

**Methods**: Tet1 knock-out mice were generated by deleting coding exon 4 of the Tet1 gene, resulting in a premature stop codon and null allele. We performed a battery of behavioral testing and molecular analysis on the mice.

**Results**: Genome wide RNA-sequencing analysis uncovered a set of neuronal genes including Oxtr which are differentially expressed in the brains of Tet1 knock-out mice. We used qPCR to validate that Oxtr is downregulated in the hippocampus. Whole-genome bisulfite sequencing revealed over 600 differentially methylated regions between Tet1 knock-out and Tet1 WT mice. Targeted bisulfite sequencing of Oxtr revealed a novel intragenic CpG island which is hypermethylated in Tet1 knock-out mice. Further characterization of this region showed that hypermethylation occurred during early embryonic development and persisted throughout adulthood. In addition, we have revealed multiple novel splice variants of Oxtr. These variants are selectively reduced in the brain of Tet1 knock-out mice. Tet1 knock-out mice also resulted in abnormal behaviors such as decreased sociability, increased aggression, and reduced maternal care, phenotypes which are consistent with established behavior ofOXTR deficient mice.

**Conclusions**: Our results show epigenetic regulation of Oxtr expression by DNA methylation and TET1. These data also show a role for Tet1 in social behavior. Together, these data offer novel insights into epigenetic regulation of Oxtr and its role in neuropsychiatric disorders.

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**#26 Isolation & characterization of vaccine-elicited HIV env-specific monoclonal antibodies in infant rhesus monkeys**


**Background & Hypothesis**: A pediatric HIV-1 vaccine is critically needed to eradicate the approximately 160,000 pediatric HIV-1 infections that continue to occur annually. Previous studies have demonstrated that specific antibody binding levels and functional activities correlate with reduced risk of infection. Understanding how best to elicit these specific responses in the setting of vaccination is critical to designing an effective pediatric vaccine.

**Objective**: To define the immunogenetic and functional characteristics of monoclonal antibodies (mAbs) isolated from HIV Env-specific memory B cells of infant rhesus macaques (RMs) immunized with distinct vaccine regimens.

**Methods**: Groups of five infant RMs were immunized with: 1) **Protein Only**: HIV Env C.1086 gp120 protein administered intramuscularly (IM) with a squalene adjuvant and intranasally (IN) with the TLR7/8 agonist R848; 2) **Co-Administration** of MVA-C.1086 gp120 & HIV Env C.1086 gp120 (IM/IN); 3) **Extended Interval**, similar to Co-Administration but over an extended time span; 4) 3M-IDRI: HIV Env C.1086 & TV1 gp120 proteins administered IM with the TLR7/8 agonist 3M-IDRI adjuvant. The magnitude of vaccine-elicited antibodies was measured in plasma using a Binding Antibody Multiplex Assay. HIV Env-specific memory B cells were sorted by flow cytometry using tissues or PBMCs collected at necropsy. Variable heavy & light immunoglobulin genes were amplified by nested PCR & used for production of mAbs which were screened by ELISA for binding to C.1086 gp120. Epitope specificity & ability to bind to infected cells of env-reactive mAbs was assessed.

**Results**: All vaccine regimens induced robust HIV Env-specific antibodies. A total of 40 Env-reactive mAbs from memory B cells isolated from the spleen, Submandibular LN, Axillary LN, Submental LN, Retropharyngeal LN and PBMCs demonstrated strong binding to C.1086 gp120. These mAbs were directed against several epitopes including Variable loops 1/2 (V1V2), variable loop 3 (V3) and the CD4 binding site. Interestingly one CD4 binding site mAb was able to bind to HIV-infected cells. Overall the magnitude of plasma binding was higher in the 3M-IDRI group, suggesting this TLR agonist adjuvant enhanced the immune response to vaccination. mAbs isolated from the 3M-IDRI group (n=17) had lower HCDR3 lengths as compared to the other animal groups (n=23) (median 11.7 vs 16.7; p<0.0001, Mann Whitney U test). Similarly, the median Somatic Hypermutation (SHM) rate of the 3M-IDRI group (n=17) was statistically significantly lower than that of the other animal groups (n=23) (median 2.4% vs 5.1% p = 0.0014, Mann Whitney U test).

**Conclusions**: Our preliminary results suggest that although 3M-052 adjuvanted HIV Env in 3M-IDRI induced a higher magnitude antibody response, these antibodies do not exhibit increased affinity maturation compared to those elicited by the other vaccine regimen. Further characterization of the properties of these mAbs is ongoing.
#27 Structure of Aspergillus fumigatus Calcineurin-FK506-FKB12 Complex Reveals Critical FKB12-Binding Residues Distinct from Human FKB12 and the Potential for Development of Non-immunosuppressive FK506 Analogs as Antifungal Therapeutics. Praveen R Juvvadi1, Benjamin G Bobay2, Sophie M Gobeil3, Ron A Venters2, Len D Spicer3, David Fox Ill4, Joseph Heitman5, William J Steinbach1,5*. 1Pediatric Infectious Diseases, Department of Pediatrics, Duke University Medical Center. 2Duke University NMR Center. 3Department of Biochemistry, Duke University. 4Beryllium Discovery, Bainbridge Island, WA 98110. 5Department of Molecular Genetics & Microbiology, Duke University Medical Center, Durham NC, USA.

Background: Calcineurin orchestrates growth and virulence in major pathogenic fungi including Aspergillus fumigatus responsible for life-threatening infections worldwide. Cellular functions of calcineurin make it an attractive antifungal target but the immunosuppressive effects of the calcineurin inhibitors, FK506 and CsA, make it difficult to exploit the antifungal potential due to conservation of calcineurin in the host and the fungal pathogen. 

Objective: To gain molecular understanding of calcineurin-immunophilin-immunosuppressor complexes to facilitate the design of novel non-immunosuppressive CsA and FK506 analogs for fungal-specific targeting.

Methods: We solved the crystal structure of calcineurin-FK506-FKB12 complex in A. fumigatus and used site-directed mutagenesis to construct mutations in the CnaA catalytic subunit of calcineurin and FKB12. To identify differences between the A. fumigatus FKB12 and Human FKB12, we heterologously expressed and mutated Human FKB12 in A. fumigatus. FKB12 constructs were GFP tagged to visualize the binding of FKB12 to calcineurin in the presence of FK506 in vivo by fluorescence microscopy. Molecular modeling and NMR analysis along with molecular dynamic simulations substantiated structural and mutagenesis studies.

Results: Human FKB12 bound to A. fumigatus calcineurin in the presence of FK506 but did not inhibit the function of A. fumigatus calcineurin. However, mutation of specific residues in the 40s and 80s loop in human FK506 induced FK506 sensitivity. Important A. fumigatus FKB12 residues in the 40s and 80s loop required for the inhibition of calcineurin were identified. NMR studies on FK506-FKB12-binding, and molecular dynamic simulations of the A. fumigatus calcineurin-FK506-FKB12 complex based on the crystal structures, revealed that a key Phe residue (F88) in 80s loop of A. fumigatus FKB12 that is not conserved in human FKB12 is essential for binding and inhibiting fungal calcineurin.

Conclusions: Our study for the first time provides the structural basis for the mechanism of inhibition of A. fumigatus calcineurin by FK506-FKB12 complex. The identification of specific differences between human FK506 and A. fumigatus FKB12 with respect to calcineurin inhibition broadens insight into developing novel non-immunosuppressive calcineurin inhibitors for effective antifungal targeting.

#28 Determinants of Tenascin-C and HIV-1 envelope binding and neutralization

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Background & Hypothesis: Inefficient transmission of HIV-1 from mother to child via breastfeeding suggests that naturally occurring antiviral factors in breast milk may prevent mucosal infection in the majority of HIV-1 exposed breastfed infants. Tenascin-C (TNC) is a large, hexameric extracellular matrix glycoprotein that we have previously demonstrated to be a broad-spectrum HIV-1 neutralizing factor. However, the mechanism of the interaction between TNC and the HIV-1 Env has been largely uncharacterized.

Objective: Our objective is to identify the domains of TNC involved in HIV-1 Env binding, to identify amino acid residues of the HIV-1 Env V3 loop that are determinants of TNC binding and neutralization, and to characterize the role of TNC glycosylations to its virus neutralizing activity.

Methods: Binding between TNC proteins and HIV Env proteins was assessed by Enzyme-Linked Immunosorbant Assay (ELISA) and Surface Plasmon Resonance (SPR). Alanine-mutant HIV-1 viruses were generated by site-directed mutagenesis and pseudoviruses were produced by subsequent transfection of the mutated Env plasmids. TNC-mediated neutralization of HIV-1 was assessed in a TZM-bl reporter cell assay. TNC glycan profiles were characterized with high-resolution liquid chromatography/mass spectrometry.

Results: The FN-III and fbg domains of TNC were each capable of binding MN.3 gp120 and the V3 scaffold protein MN.V3 gp70. TNC constructs lacking these domains were unable to bind either Env protein. Alanine mutation analysis revealed that the TNC-Env binding and neutralization is dependent on positions 321/322 in the Env V3 loop. TNC produced in various cell lines demonstrated variable neutralization potency, which did not correlate with the glycosylation profile of each variant. However, enzymatic deglycosylation of TNC abrogated neutralization activity.

Conclusions: Our findings suggest that neutralization of HIV-1 by TNC is mediated by a binding interaction involving V3 region residues and the TNC FN-III and fbg domains. Furthermore, this interaction is dependent on TNC glycosylation.
#29 Determining the Developmental Role of Shank3 in the Pathogenesis of Autism Spectrum Disorders

**Authors:** Samuel W. Hulbert, Alexandra L. Bey, Xiaoming Wang, Mary Lin, Lara J. Duffney, Martine W. Tremblay, Yong-hui Jiang

**Background & Hypothesis:** Approximately 1 in 68 children in the United States are diagnosed with Autism Spectrum Disorders (ASDs). The high prevalence and cost of ASDs, along with the fact that there are currently no FDA-approved medications to help ease the core features of the disorders make determining the underlying pathophysiology and developing safe, cost-effective interventions an issue of great importance from a public health perspective. Loss-of-function and likely-gene-disrupting mutations implicated in neuronal synapse function have been identified in patients with ASDs. However, it is unclear how and when during development synaptic dysfunction causes an individual to develop an ASD and this information is likely critical for optimal treatment. One of the most consistent findings from human genetics studies of ASDs is mutations in and, most often, deletions of SHANK3. The Shank3 complete knockout (Shank3e4-22-/-) mice display multiple behavioral phenotypes that are robust, consistent, and resemble the core features of ASD (e.g. repetitive self-grooming) as well as intellectual disability (e.g. impaired instrumental learning). The central hypothesis of this project is that there is a critical period in development for the pathogenesis of ASDs.

**Objective:** 1) identify the developmental time period for Shank3 function that produces the behavioral phenotypes that present in the Shank3e4-22-/- mice and 2) determine the mechanistic role of Shank3 during development in vivo.

**Methods:** I have optimized a system to deplete Shank3 expression at multiple time points in development in mice after crossing the Jiang lab’s Shank3e4-22loxP/loxP and Shank3e4-22loxP/- mice to the ubiquitously expressed tamoxifen-inducible Cre line, CAGGS-CreER.

**Results and Conclusions:** Mice with Shank3 deleted in adulthood do not present with the same behavioral phenotypes as the conventional mutant mice, thereby supporting an important developmental role for Shank3. Future work will be geared towards disrupting Shank3 expression during development and determining what processes are disrupted.

#30 Transplacental transfer of IgG in the setting of Zika virus infection during pregnancy

_T Singh*, CA Lopez*, C Giuberti, H Itell, H Heimsath, R Dietze, HM Lazear, SR Permar (* equal contribution)

**Background & Hypothesis:** As many as 10% of Zika virus (ZIKV) exposed pregnancies result in congenital ZIKV infection and fetal neurological disease, posing a significant public health burden. Placental transfer of maternal antibodies may be key to preventing neonatal infections and potentially reducing severe fetal outcomes. While Zika virus is known to be able to infect the placenta, it is not known whether maternal Zika infection affects transplacental IgG transfer to the fetus, as with maternal HIV-1 and malaria infections. **Objective:** Assess whether ZIKV infection during pregnancy disrupts transplacental IgG transfer.

**Methods:** We enrolled a cohort of 26 pregnant women in Brazil with Zika-like symptoms (ie. rash, fever, conjunctivitis, and arthritis). The magnitude of antibody response to flaviviruses and vaccine antigens was measured in maternal plasma and infant cord blood from delivery. Focus reduction neutralization test was used to determine neutralizing titer to ZIKV and Dengue (DENV) serotypes 1-4.

**Results:** In this cohort, 9 were confirmed positive for ZIKV infection by RT-PCR diagnosis, one had prolonged viremia, and one had a microcephalic infant. The magnitude of IgG binding responses to ZIKV, and DENV-2 were not significantly different between maternal plasma and infant cord blood pairs from delivery, with or without Zika infection during pregnancy (Wilcoxon Signed Rank Test). All Zika-positive patients had neutralizing titers to ZIKV at delivery, regardless of trimester of infection (Mean ZIKV Neut50 Titer: 5397). ZIKV and DENV 1-4 specific neutralizing titers were directly correlated between infant cord blood and maternal plasma (ZIKV: r>0.97, p<0.0001; Spearman Correlation). 85% of our cohort consisted of patients with neutralizing titers to DENV, suggesting prior DENV immunity. No significant differences were observed in transplacental IgG transfer efficiency of antibodies against common vaccine antigens (HepB, Tetanus, RSV, Pertussis, HiB, Diptheria, Rubella) in Zika-positive and Zika-negative patients (Mean percent vaccine-specific IgG transfer: Zika+ = 107%; Zika- = 118%, ns.; Mann Whitney test).

**Conclusions:** Thus, we conclude that maternal ZIKV infection does not affect efficient maternal-fetal transfer of Zika specific functional and vaccine-elicited antibodies. Therefore, ZIKV infection during pregnancy does not appear to affect maternal-fetal IgG transfer, including the transfer of anti-ZIKV antibodies.
#31 Characterization of Asparagine Synthetase Deficiency Mouse

**Xiaodi Yao, David B Goldstein and Yong-Hui Jiang**

**Background & Hypothesis:** Asparagine Synthetase Deficiency (ASNSD) is a recently identified rare autosome recessive neurological disease characterized by severe microcephaly, developmental delay, intellectual disability, cerebral atrophy and intractable seizures. ASNSD patients carry compound heterozygous or homozygous mutations in the ASNS gene encoding asparagine synthetase (ASNS). ASNS is believed to catalyze the biosynthesis of asparagine (ASN), a non-essential amino acid in humans, from aspartate and glutamine in an ATP-dependent manner. However, very few investigations have followed this lead and the exact physiological relevance related to the function of ASNS in ASN synthesis remains elusive. Remarkably, nothing is known about the pathogenesis of ASNSD and the function of ASNS in brain development and there is no treatment available.

**Objective:** The current research is to determine the specific role of ASNS in brain development and delineate the mechanism underlying the neuropathogenesis of ASNSD using Asns mutant mice.

**Methods:** We generated Asns conventional knockout (KO) mouse and brain specific conditional KO mouse to investigate the consequence of Asns loss in brain development.

**Results:** Conventional Asns-KO results in perinatal lethality and significantly reduced brain mass. Brain-specific Asns-KO mice by Nestin-Cre (AsnsNestin-/-) nicely recapitulates core features of ASNSD. AsnsNestin-/- mice have smaller brain mass, growth retardation and high penetrance for spontaneous seizures and lethality at postnatal days 15-22 (P15-22). Surprisingly, I observed a drastic reduction of parvalbumin positive interneurons in the neocortex of P14 Asns Nestin-/- mice but not somatostatin or calretinin expressing inhibitory neurons.

**Conclusions:** Our results demonstrate that ASNS plays critical roles in early brain development. The phenotypes observed in Asns KO mice mimics clinical features of ASNSD patients suggesting that Asns KO mouse is a good model to study the mechanisms underlying neurological impairment caused by ASNSD and to test potential strategies for interventions.

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#32 Transcriptomic analysis reveals differentiated gene expression and elevated PKA signaling pathway in Shank3 complete knockout mice.

**Xiaoming Wang, Ping Wang, Yong-Hui Jiang**

**Background & Hypothesis:** The SHANK3 gene encodes a major scaffolding protein which localizes at postsynaptic terminals of excitatory synapses and plays critical roles in neuronal communication and brain development. Mutations on SHANK3 have been well-documented in patients with autism spectrum disorders (ASD). Recently we reported a Shank3 complete knockout mice where almost entire coding region of Shank3 is deleted and all shank3 protein isoforms are disrupted. These mice displayed strong ASD-like phenotypes including impaired social behaviors, repetitive grooming, and decreased reward-motivated behaviors. However, the molecular basis and signaling pathway involving these abnormal behaviors remain elusive.

**Objective:** To explore the molecular basis and signaling pathway that underlie the impaired behaviors in Shank3 mutant mice.

**Methods:** RNA seq data using striatal tissue from four pairs of Shank3 knockout mice and littermates were analyzed. DESeq2 was used to determine differentially expressed genes (DEGs). DAVID was used for Gene Ontology (GO) analysis with 12,843 expressed protein-coding genes as background. Ingenuity pathway analysis (IPA) was used for canonical pathway analysis and disease association. Protein interaction network was analyzed using STRING.

**Results:** Transcriptomic analysis revealed 881 altered genes in Shank3 mutant mice of which 541 are up-regulated and 340 are down-regulated. Upregulated genes are enriched in synaptic transmission, calcium ion binding, and syntaxin binding. Downregulated genes are enriched in GTPase regulator activity. Upstream regulator analysis uncovered an elevated PKA-Creb signaling pathway which was confirmed by western blot.

**Conclusions:** Altered gene expression and elevated PKA-Creb signaling pathway may involve in the pathogenesis of SHANK3-causing ASD.
**#33 T cell oxidative metabolism, a potential target for restoring immune function in obesity**

Yazan Alwarawrah, Amanda Nichols, William Eisner, William Green, Melinda Beck, and Nancie MacIver

**Background & Hypothesis:** Obesity is associated with low grade systemic inflammation and changes in the adipose tissue immune cell populations. These changes in immune cells are responsible for many of the pathologies accompanying obesity such as insulin resistance and type 2 diabetes. At the same time obesity is also associated with a reduction in protective immunity. T cells play an important role in this obesity-associated immune response, and changes in T cell metabolism can influence function. We hypothesize that obesity is associated with changes in T cell metabolism that alters T cell function and can be targeted to restore immune function in obesity.

**Objective:** To determine the changes that occur to T cells populations and metabolism during obesity and reveal metabolic pathways that can be targeted to restore T cell function.

**Methods:** The stromal vascular fraction was isolated from visceral adipose tissue of C57BL/6 mice fed high fat (DIO mice) or regular chow diet (control mice). These cells were analyzed for T cell populations and metabolic markers by flow cytometry. From the same mice, CD4+ T cells were isolated from spleen and analyzed for metabolic activity using extracellular flux assays and intracellular flow cytometry. Human CD4+ T cells isolated from obese patients and obese patients on metformin were analyzed by extracellular flux assays. Metformin was tested for its effects on isolated CD4+ T cell metabolism and activation.

**Results:** Proportions of regulatory T cells (Treg) were found to decrease in the adipose tissue of DIO mice while CD8+ T cells were found to increase, indicating an inflammatory environment in the adipose tissue of the DIO mice. CD4+ T cells isolated from the spleen of DIO mice were found to have high oxygen consumption rate (OCR) which was consistent with human data where CD4+ T cells from obese patients had higher OCR than normal weight individuals. CD4+ T cells from obese subjects on metformin were found to have lower OCR than obese subjects not on metformin, similar to levels of lean control subjects. Treatment of isolated mouse CD4+ T cells with metformin was found to inhibit OCR and alter the expression of several activation markers.

**Conclusions:** During obesity, T cell populations are altered with reduced Treg cells and increased CD8+ T cells, and CD4+ T cell metabolism is characterized by an increase in oxidative metabolism. Treatment with metformin can reverse elevated T cell OCR in obesity and may be an effective candidate to reverse T cell metabolic dysfunction and thereby restore T cell immunity in obesity.

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**#34 Reactivation of Prader-Willi Syndrome Genes by epigenetic editing.**

Yuna Kim, Josh Black, Yan Xiong, Charles Gersbach, Jian Jin, and Yong-hui Jiang

The importance of epigenetic regulation in human development and diseases is well established, and there is increasing enthusiasm for the development of epigenetic therapies to correct deficient gene expressions in clinical settings. Genomic imprinting is a classical paradigm of epigenetic regulation in mammals including human. Imprinted genes express in parental origin-specific patterns that are required for embryo growth, development and behavior control. This parental specific expression is mediated by modifications on DNA and chromatin. Our recent high throughput screening identified G9a histone methyltransferase and methylation of histone H3K9 as a candidate therapeutic target for the treatment of Prader-Willi syndrome (PWS), a neurodevelopment disorder. We hypothesize that G9a-mediated histone methylation functions to maintain imprinted expressions of PWS genes. New technologies for chromatin profiling and modulating epigenetic landscapes using CRISPR provide tools to test this hypothesis and characterize the relationship between DNA methylation and histone modifications. We will focus on a sequence element called the PWS imprinting center (PWS-IC) that exhibits differential DNA methylation and histone modifications. We will apply CRISPR editing tools dCAS9-Tet1 and -LSD1 to reverse the differential modifications at the PWS-IC, which we hypothesize induces the reactivation of PWS genes. We will deeply characterize the chromatin effect of the targeted editing both in human PWS cells and in vivo mouse models of PWS. In summary, we will investigate novel mechanisms of genomic imprinting and how they might be modulated therapeutically.
#35 Title & Authors: An immune tolerance approach using methotrexate in the naïve setting of patients treated with a therapeutic protein: experience in infantile-onset Pompe disease

Ankit K. Desai, MBBS, Zoheb B. Kazi, MBBS, Priya S. Kishnani, MD

**Background & Hypothesis:** Pompe disease is an autosomal recessive lysosomal storage disorder in which deficiency of the lysosomal enzyme acid α-glucosidase (GAA) results in a build-up of glycogen in cardiac, skeletal, and smooth muscle of affected individuals. Enzyme replacement therapy (ERT) with alglucosidase alfa has improved clinical outcomes and prolonged survival in infantile-onset Pompe disease (IOPD). High and sustained antibody titers (HSAT; ≥51,200 or sustained intermediate titers (SIT; ≥12,800 and <51,200 within 12 months on ERT) develop in the majority of cross-reactive immunological material (CRIM)-negative and ~40% of CRIM-positive (CP) patients with IOPD, leading to a poor response to ERT. ≥12,800 and <51,200 within 12 months on ERT) develop in the majority of cross-reactive immunological material (CRIM)-negative and ~40% of CRIM-positive (CP) patients with IOPD, leading to a poor response to ERT.

**Objective:** Assess the efficacy and safety of ITI protocol with only low-dose methotrexate in CP IOPD patients.

**Methods:** Patients with a confirmed diagnosis of Pompe disease and who were CRIM-positive and naïve to ERT were enrolled in the study. Methotrexate was administered on three consecutive days/cycle; with the first three ERT infusions for a total of three cycles. Safety was assessed by measuring ANC, ALT, and AST around the time of methotrexate administration. Based on antibody titers, patients were classified as HSAT, SIT or low titer (LT; ≤6400 in the first 12 months). We performed a retrospective chart review of 37 CP patients with classic IOPD, who received ERT without immunomodulation, to serve as comparator group. These 37 patients with CP IOPD were classified into HSAT, SIT, and LT and compared with cohort of IOPD patients who received methotrexate.

**Results:** We identified 14 patients with IOPD, including one CRIM-negative patient, who received methotrexate ITI protocol and had longitudinal follow-up >6 months. In methotrexate ITI group, 12/14 (85.8%) patients maintained LT and 2/14 (14.2%) patients developed SIT. Median peak titer was 3,200 (50-51,200) at a median time since ERT of 21 weeks (4-94 weeks). Median last titer was 150 (0-12,800) at a median time since ERT of 71 weeks (33-122 weeks). In the comparator group, five (13.5%), seven (18.9%), and 25 (67.6%) developed HSAT, SIT, and LT respectively with median peak titers of 204,800 (51,200-409,600), 25,600 (12,800-51,200), and 800 (200-12,800) respectively. Decrease in ANC (<750 cells/mm3) was seen in two patients and increase in AST and/or ALT (>3 times their baseline) was noted in three patients.

**Conclusions:** Overall, the methotrexate ITI protocol was safely tolerated. No patients developed HSAT in methotrexate ITI group compared to five patients in ERT monotherapy group. These data suggest that low-dose methotrexate minimizes immune response or achieve immune tolerance in CP IOPD patients. Further study is needed to evaluate safety and efficacy in a larger cohort and monitor long-term outcomes of methotrexate ITI.

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#36 Title & Authors: Identification of Clinical Phenotypes within Autoimmune Encephalitis among Children and Adolescents

Ashley V. Adams; William Gallentine, DO; Heather Van Mater, MD; GenaLynne Mooneyham, MD

**Background & Hypothesis:** Autoimmune Encephalitis (AE) describes a spectrum of illness characterized by inflammation of the central nervous system due to the production of anti-neuronal antibodies. Within AE there may be a wide range of symptoms to include personality changes, psychosis, seizures, movement disorders, cognitive decline, memory deficits, sleep disruption, and autonomic instability. Recognition of clinical symptoms by pediatricians and psychiatrists is paramount given that early treatment has been demonstrated to be a predictor of positive outcome. However, there is little data on the specific symptoms within the domain of psychiatric disturbance that are most commonly exhibited among children within each antibody subtype. We hypothesize that differences exist among the presenting psychiatric symptoms of three common antibody mediated subtypes of AE: anti-NMDA, anti-VG KC, and anti-GAD65.

**Methods:** A total of 171 charts were reviewed from patients identified as having been evaluated for AE through Duke’s Autoimmune Brain Disorders Program or the consult/liaison child and adolescent psychiatry team. A total of 11 patients were identified as having been diagnosed with NMDA Ab+ AE, 5 patients with VGKC Ab+ AE, and 5 patients with GAD65 Ab+ AE. Data including demographic information and psychiatric, neurologic, and rheumatologic symptoms present at the onset of illness were extracted from each chart. The Liverpool Outcome Score was used as an evaluation of symptom severity at the peak of symptoms prior to initiation of treatment.

**Results:** Patients with NMDA (100%) and GAD65 (80%) antibodies were more likely to have language impairment than patients with VGKC antibodies (60%). All patients experienced cognitive impairment independent of antibody type. Features of delirium, catatonia, and movement disorders were more often seen in patients with NMDA antibodies, while anxiety was more commonly seen in patients with GAD65 antibodies. Aggression was seen in at least 60% of NMDA and VGKC patients, but was absent in all GAD65 patients. The average time to diagnosis for NMDA, VGKC, and GAD65 was 0.80, 12.40, and 20.30 months, respectively. The average Liverpool Outcome Scores for NMDA, VGKC, and GAD65 at symptom onset were 56.8, 65.4, and 65.0, respectively.

**Conclusions:** Both similarities and differences in the symptomatic presentation among the three most common types of seropositive AE exist. The relative delayed time to diagnosis for patients with VGKC and GAD65 AE should prompt providers to test for these antibodies in patients who fit the clinical phenotype for AE.
#37 Title & Authors: Liver Symptoms persist in Glycogen Storage Disease type III: need for a therapeutic approach  
Carine Halaby, Jariya Upadia, Stephanie Austin, Alisha Mavis, Priya Kishnani.

**Background & Hypothesis:** Glycogen storage disease type III (GSD III) is an inherited metabolic disease characterized by deficiency of amylo-1,6-glucosidase, 4-alpha-glucantransferase enzyme (AGL; glycogen debranching enzyme). A deficiency of this enzyme leads to the accumulation of limit dextrin-like molecules due to incomplete breakdown of glycogen. There are two subtypes GSD IIIa (85% of cases) which results in glycogen accumulation in liver and muscle GSD IIib (15% of cases), which results in liver involvement only. During childhood, individuals with GSD IIIa and IIib manifest with liver symptoms notably hepatomegaly, hypoglycemia and marked elevation of liver transaminases. As patients reach adolescence, muscle symptoms become more prominent. At this time, the only available treatment for GSD III is symptomatic. A diet including cornstarch and increased protein is used to help with hypoglycemia and providing alternate fuel via intact neoglucogenesis. However, long term hepatic symptoms, such as cirrhosis, continue to appear despite these interventions.

**Objective:** In preparation for the development of a definitive therapy for GSDIII, such as enzyme replacement therapy, the natural history needs further characterization to accurately describe the endpoint of this clinical trial. As Duke serves as a tertiary care center for GSD, a natural history study was conducted using our Duke cohort, to better describe the hepatic manifestations in patients with GSDIII.

**Methods:** We included pediatric patients (defined as age between 0 to 21yo and 11 months) with GSD III consented to our GSD III Natural History study. Electronic and paper charts were reviewed for clinical, biochemical, molecular genetics, radiological and histological profiles. In addition, an electronic literature search of PubMed was performed for current and past findings of GSD III for years 1983-2017.

**Results:** Twenty six pediatrics patients were enrolled in this study (mean age of 13.7 yrs (range 2- 21 yrs). We analyzed their most recent labs and studies. The mean alanine aminotransferase (ALT) value was 225.4 U/L (range 34-1048 U/L, normal to 19 times above the upper limit of normal [ULN]) and the mean aspartate aminotransferase (AST) was 219.4 U/L (range 30-1095 U/L, normal to 27 times above the ULN). Gamma glutamyltransferase (GGT) and urine Hex 4 values were also found to be abnormal in many patients. Thirteen liver biopsy reports were available: 8 reports from 8 patients (median age 1yr, range 1- 7yo) describe swollen hepatocytes, with evidence of glycogen accumulation in cytoplasm, periportal bridging fibrosis and early nodule.

**Conclusions:** Dietary interventions have improved the life expectancy of individuals with GSD III, making long-term hepatic findings better recognized, albeit unnoticed until later as muscle symptoms prevail. A definitive therapy, one that would prevent the progressive buildup of glycogen in various organs with time, is needed.

#38 Title & Authors: Characterization of pediatric onset common variable immunodeficiency (CVID) in a large cohort  
Baloh CH, Reddy A, Buckley R & Lugar PL

**Background & Hypothesis:** CVID is the most common treatable primary immune deficiency in children and adults. Current literature has yielded mixed results in characterizing pediatric onset CVID. No studies have fully explored mortality risk factors in pediatric onset CVID. This leaves limited data to guide pediatricians as they diagnose and follow patients with this disease.

**Objective:** The objective of the study was to better define CVID in patients with disease onset at a pediatric age. Additionally, we sought to define subgroups of disease and use these to determine which patients need closer monitoring for serious complications such as lung disease and mortality. This is the largest single institution cohort of pediatric CVID to be reported upon.

**Methods:** Retrospective chart review of 204 subjects with CVID at a single institution, of whom 91 had disease onset at a pediatric age. Clinical and laboratory data were collected. Odds ratios and Fisher tests were utilized to examine trends. This study was IRB approved.

**Results:** The clinical features and laboratory results for subjects with pediatric onset CVID are similar to those who had adult onset CVID. However, the majority of the deceased subjects (13/18) were at a pediatric age at CVID symptom onset. These subjects had a lower age at mortality (31 years of age compared to 72), multiple comorbidities, and often depression. The most common cause of death was infection. Lung disease (OR 5, p<0.05) and infection with severe/opportunistic organisms (OR 9, p<0.05) are directly related to increased mortality. Delay in diagnosis of CVID is also correlated with mortality. Delay in diagnosis was also correlated with significantly increased odds of leukemia/lymphoma development (OR 10, p<0.05), requiring surgery (OR 5, p=0.05), having a total of 4 out of 4 CVID comorbidities (OR 7, p<0.05), and increase likelihood of infection with MRSA, VRE, and pseudomonas (OR 3, p<0.05). Intermediary markers correlating with mortality include anemia (OR 16, p=0.05), GERD (OR 6, p<0.05), and depression (OR 6, p<0.05).

**Conclusions:** There are many similarities between pediatric and adult onset CVID, however, the mortality of pediatric CVID in our cohort is striking. This is the first study to identify specific factors correlated with mortality in pediatric onset CVID to guide pediatricians and subspecialists in managing these immune deficient patients.
#39 Title: PURA (purine-rich element binding protein A) Syndrome - A Case Report.

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Abstract: Burst suppression is an electroencephalographic (EEG) pattern characterized by a brief interval of relatively high voltage activity with intervening periods of generalized suppression. It is commonly seen consequent to anoxic brain injury, deep coma states, encephalopathies or hypothermia. This pattern is differentiated from trace discontinua because of longer interburst interval and higher amplitude during bursts. Myotonic discharges (M.D.) seen in an electromyographic (EMG) study are the abnormal wave of excitation due to the firing of repetitive action potentials. It is characterized by waveforms with alternating frequency and amplitude. This unique pattern is seen in muscular dystrophies, muscle channelopathies, toxic or inflammatory myopathies. We report a case of PURA syndrome with significant EEG findings of burst suppression and EMG pattern of myotonic discharges which has not been described in literature before.

#40 Title & Authors: Beliefs Regarding Infant Weight and Future Health Differ By Race/Ethnicity, Education, and Income

Background & Hypothesis: Obesity starts early in life, with significant disparities by race/ethnicity. Prior work shows that parents of older children are concerned about childhood obesity particularly when related to health, yet we know little about parents’ perception of health risk in infancy.

Objective: To determine whether parents associate infancy weight status to health risk and whether the parents’ medical history or demographics affect the association.

Methods: Cross-sectional analysis of 860 2-month old infant-parent dyads enrolled in the Greenlight Intervention Study. We examined characteristics related to 2 statements: “I am worried that if my infant weighs too much [too little], he/she will not be healthy.” Responses were dichotomized as agree (from strongly agree and agree) or disagree (from strongly disagree or disagree). We hypothesized that parent cardiovascular (CV) risk (hypertension, hyperlipidemia, diabetes, stroke, and heart disease) or overweight/obesity (OW/OB) would be associated with beliefs that their infant’s weight would affect the infant’s health. We also examined how beliefs were associated with parent race/ethnicity, education, and household income. We performed chi-square tests and unadjusted logistic regression to examine the relationship between parental factors and beliefs about weight and health risk.

Results: At the 2-month visit, 62% of parents believed that their infant would not be healthy if they weighed too much, while 76% believed their infant would not be healthy if they weighed too little. Only 47% of white parents believed that weighing too much would affect health, while 62% of non-Hispanic black, and 73% of Hispanic parents believed this (overall chi-square p=0.001). Hispanic parents had over 3 times the odds of believing that an infant weighing too much would not be healthy compared to non-Hispanic whites (OR 3.1; 95% CI 2.1-4.5, p<0.001). Beliefs differed by education and household income, with fewer parents believing infant weight affected health as education levels and income increased. There were no significant relationships between beliefs about infant weight and parent CV risk or parental OW/OB

Conclusions: Parent CV risk or OW/OB are not related to the perception of infant weight and health at 2 months. However, we found that Hispanic and non-Hispanic black parents more frequently agreed that if their infant weighed too much it would affect their infant’s health. Further understanding of these beliefs may provide a window of opportunity to prevent obesity and chronic disease.
#41 Title & Authors: Association of Neonatal Morbidities with Growth Outcomes in Very Low Birth Weight Infants at School Age.


Background & Hypothesis: Outcomes for premature infants, including neurodevelopmental, pulmonary, and cardiovascular function, are associated with growth during the neonatal intensive care unit (NICU) stay. Small for gestational age or NICU growth-restricted premature infants are more likely to have growth deficits at school age and beyond. However, the strength of association between common NICU comorbidities and long-term growth and health outcomes is unclear.

Objective: Evaluate associations among NICU morbidities and growth outcomes at school age in infants born at very low birth weight (VLBW, <1500 g birth weight).

Methods: We identified all VLBW infants born between 2001-2012 within the Duke Health Network and hospitalized in the Duke Intensive Care Nursery (ICN). Infants who no longer remained within the Duke Health Network at school age (5-7 years old), either by mortality or loss to follow-up, were excluded. Common NICU morbidities including intraventricular hemorrhage (IVH) grade > 2, surgical or medical necrotizing enterocolitis (NEC), positive cerebrospinal fluid (CSF) cultures, culture-proven sepsis, and chronic lung disease (CLD) defined from oxygen challenge test or radiographic evidence on chest x-ray at 36 weeks post-menstrual age, were recorded, along with height for age and weight for age data at birth and school age. The association between morbidities and height and weight growth failure (weight or height for age <3rd percentile) at school age were assessed with logistic regression analyses adjusted for birth weight.

Results: 294 infants were included with a median gestational age of 25 weeks (range; 22-33) and median birth weight of 783 g (410-1465). Surgical NEC was associated with having school age weight or height <3rd percentile, adjusting for birth weight (OR 3.2; 95% CI (1.1, 9.0) and 3.4; (1.2, 9.9), respectively). Sepsis was also nominally associated with school age weight <3rd percentile (OR 0.5; 95%CI (0.2, 1.1)) and IVH grade > 2 was nominally associated with school age height <3rd percentile (OR 2.1; 95% CI (0.9, 4.8)) while adjusting for birth weight.

Conclusions: Surgical NEC was the only NICU morbidity found to be significantly associated with growth failure at school age after adjustment for birth weight. While avoiding significant morbidities of prematurity may improve growth and health outcomes, continued efforts to optimize nutrition, both in the NICU and in later years, are needed.

#42 Title & Authors: Phase I/II Clinical Trial of Clenbuterol in Pompe Disease Patients Stably Treated with ERT.

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Background & Hypothesis: Clenbuterol enhanced the receptor mediated uptake of recombinant human acid -α-glucosidase (rhGAA) during enzyme replacement therapy (ERT) in mice Pompe disease. We hypothesized that clenbuterol administration would enhance the response to ERT in patients who were stably treated with ERT.

Objective: To investigate whether adjunctive clenbuterol therapy could improve the therapeutic effects of recombinant human acid -α-glucosidase (rhGAA) during enzyme replacement therapy (ERT) in Pompe disease.

Methods: This was a 52 week Phase I/II double-blind, randomized, placebo-controlled study of adjunctive clenbuterol in (20 mcg Spiropent tablets) in patients with LOPD. All subjects were evaluated at Baseline and Week 6 to establish a baseline for motor function testing. At Week 6, subjects were randomized 3:2 to clenbuterol or placebo, and evaluated for safety and efficacy during the Week 12 and 18 visits.

Results: One significant adverse event unrelated to clenbuterol occurred, as well as transient minor adverse events and mild transient elevation of creatine kinase. From an efficacy perspective, at Week 52, the mean 6 minute walk test increased 16 meters (p=0.08) and 3% with regard to predicted performance (p=0.03), and the predicted maximum inspiratory pressure increased 8% (p=0.003) for the clenbuterol treated group, which included stably treated patients on ERT for >38 months. Exploratory endpoints improved including the Quick Motor Function Test score that increased 7 points (p=0.007); and the time for the Gait, Stairs, Gower, Chair test that decreased -2 points (p=0.004). Biopsy of the vastus lateralis demonstrated 50% lower glycogen content and improved histology at Week 52. RNASeq from muscle samples revealed a subset of 44 genes that were significantly altered both in the comparison of Pompe patient muscle with normal muscle, and in the comparison of Pompe patient muscle before and after clenbuterol treatment. Clenbuterol exposure directed gene expression toward more normal levels for 38 of these 44 overlapping genes. Pathway analysis revealed that clenbuterol treatment was associated with the inhibition of myogenesis, which may have been contributed by the downregulation of Wnt pathway as well as upregulation of myostatin.

Conclusions: This study revealed initial safety and efficacy for adjunctive clenbuterol therapy in patients with LOPD who are stably treated with ERT, long after any additional benefits would be expected from ERT alone.
**#43 Sildenafil Exposure in the Neonatal Intensive Care Unit**

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**Background & Hypothesis:** Pulmonary hypertension causes substantial morbidity and mortality in infants. To improve outcomes of these infants, providers are increasingly turning to drugs approved to treat pulmonary hypertension, such as sildenafil. Though FDA-approved for the treatment of pulmonary arterial hypertension in adults, sildenafil is not approved for use in infants. In this study, we hypothesize that sildenafil use has been increasing over time.

**Objective:** We aim to describe the frequency of use of sildenafil in a large number of neonatal intensive care units (NICU), the change of prescribing patterns from 2001-2016, the diagnoses and interventions associated with sildenafil use, and the occurrence of in-hospital morbidity and mortality.

**Methods:** We identified all infants >23 weeks gestational age discharged from 349 NICUs managed by the Pediatrix Medical Group from 2001-2016 who were exposed to sildenafil in the first 180 postnatal days. We categorized infants into two groups based on gestational age - <32 weeks and >32 weeks. We used contingency table analysis to examine the associations based on gestational age group.

**Results:** Sildenafil was administered to 1336/1,161,808 infants (0.11%); none received sildenafil in 2001 vs 151/90,544 (0.17%) in 2016. Among infants < 32 weeks gestational age exposed to sildenafil, 666/704 (95%) had bronchopulmonary dysplasia (BPD). Among infants > 32 weeks gestational age exposed to sildenafil, 248/455 (55%) were diagnosed with BPD and 76/552 (14%) with meconium aspiration. Overall, in infants exposed to sildenafil, 209/921 (23%) died prior to discharge.

**Conclusions:** Use of sildenafil has increased more than five-fold since 2001.Exposed infants were commonly diagnosed with BPD. Further studies evaluating dosing, safety, and efficacy of sildenafil are needed.

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**#44 Systemic and mucosal levels of lactoferrin in preterm infants supplemented with bovine lactoferrin**

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**Background:** Postnatal infections continue to cause high morbidity and mortality in very low birth weight (VLBW) infants despite advances in neonatal care. Lactoferrin (LF), the major whey protein in human milk, has been explored as an intervention to combat this burden but studies to date have demonstrated variable efficacy with different dosing and products. LF absorption has not yet been examined in these studies and may give insight on dosing and efficacy.

**Objective:** To assess the absorption of three different bovine lactoferrin (bLF) doses and to evaluate the effect of bLF and human lactoferrin (hLF) levels on postnatal infections.

**Methods:** 31 preterm infants in the United States with birth weights <1500 grams were enrolled into one of three bLF supplementation groups: 100 (n=10), 200 (n=10), or 300 mg/kg/day (n=11). Infants received treatment for 30 days. Infant saliva and blood, and maternal breast milk specimens were collected before treatment initiation (baseline), at 22-days post initiation (D22), and 7 days after the last dose (D37). hLF and bLF levels in rehydrated saliva swabs, plasma, and breast milk from all time points were measured by commercial ELISA kits. Baseline and D37 saliva specimens were screened by qPCR to determine postnatal cytomegalovirus (CMV) acquisition. Lab personnel were blinded to treatment group.

**Results:** By D22, bLF levels reached a range of 0.007-3.27 μg/mL in saliva and 0.007-0.03 μg/mL in plasma, whereas the concurrent ranges in hLF levels were 0.63-370 μg/mL and 0.36-4.07 μg/mL in these compartments, respectively. The D22 bLF levels demonstrated a limited increase from baseline detection, and, by D37, all saliva and plasma had undetectable levels of bLF. We did not observe differences in LF levels based on birth weight, gestational age, dosage group, or infection status. One infant in each group developed a bloodstream infection, all due to Gram-positive organisms and presenting on study days 9, 11, and 14 respectively by group, and 2 infants (twins) of 8 in the 200 mg/kg group acquired postnatal CMV.

**Conclusions:** bLF dosing differences did not significantly impact bLF uptake over the 30-day study period and bLF was quickly cleared within one week of treatment cessation. Saliva LF concentrations were higher than plasma levels, inferring LF’s role at mucosal surfaces. Future studies of GI tract LF levels and excretion in the urinary system may further guide optimal supplemental LF dosing to reduce severe infections in preterm infants.
**#45 Title & Authors:** The utility of urine GAGs as a follow-up and confirmatory test in Newborn screening of Mucopolysaccharidosis I

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**Background:** MPS I is a lysosomal storage disease caused by a deficiency of α-L-iduronidase (IDUA), resulting in dermatan sulfate (DS) and heparan sulfate (HS) accumulation in tissues and body fluids. Affected individuals are often asymptomatic at birth, and those with a severe phenotype usually present within the first year of life with nonspecific features. Hematopoietic stem cell transplantation (HSCT) improves survival and is most effective if performed early in the disease course. MPS I was added to the Recommended Uniform Screening Panel in February 2016. A newborn screening pilot study for MPS I was conducted in North Carolina based on the IDUA activity on dried blood spots (DBS) and Sanger sequencing of IDUA gene as a second tier test, with urine GAGs analysis, blood IDUA activity, and targeted mutation testing as confirmatory tests. In this work we compare urinary GAGs concentrations in 18 infants with true and false positive newborn screen results with age-matched control ranges.

**Methods:** Urine samples and calibrators (25 μL) were dried under nitrogen and the residues treated with 0.2 mL of 3M-HCl in methanol for 75 min at 65°C. After evaporation of the reagent, the methanolysate was reconstituted in mobile phase, mixed with a deuterium-labeled chondroitin sulfate (CS), DS and HS methylated dimers in H2O (25 μL). Samples were analyzed using an Acquity®-Xevo-TQ® ultra-performance liquid chromatography system equipped with tandem mass spectrometer (Waters Corporation), with separation on an Amide UPLC column and detection by selected reaction monitoring.

**Results:** Out of 63,545 DBS specimens screened for IDUA activity, the NBS pilot study identified one infant who was confirmed to be affected with the severe form of MPS I based on homozygosity for a known pathogenic variant in the IDUA gene. Eighteen other infants were followed up because of low IDUA activity as a precaution, even though they were deemed low risk based on the sequencing results which identified one or more pseudodeficiency alleles ± heterozygous pathogenic variants (false positive cohort). The infant affected with MPS I had significantly elevated urine DS, and HS on confirmatory testing. In contrast DS and HS were not elevated in the false positive cohort. Blood IDUA activity was low or low-normal for infants in this group.

**Conclusions:** Urinary GAGs were elevated only in the infant with MPS I and not in the cohort with pseudodeficiency alleles, heterozygous pathogenic variants, and variants of unknown significance. The pilot study provided further evidence that urine GAGs were sensitive biomarkers for MPS I.

**#46 Title & Authors:** Plasma lyso-Gb3 as a diagnostic marker for Fabry disease

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**Background:** Fabry disease is an X-linked lysosomal storage disorder caused by a deficiency of alpha-galactosidase A (alpha-GAL) encoded by the GLA gene. A majority of females who are heterozygous for a pathogenic variant in the GLA gene manifest symptoms of the disease. While the measurement of alpha-GAL activity in blood or other tissues is a sensitive test for diagnosing affected males, it does not reliably detect heterozygotes. Plasma lyso-globotriaosylceramide (lyso-Gb3) has recently been recognized as a sensitive biomarker in males and females with Fabry disease and hence has diagnostic utility. We evaluated algorithms for diagnosing males and females with Fabry disease using alpha-GAL activity, GLA gene sequencing, and plasma lyso-Gb3.

**Methods:** Alpha-GAL activity in DBS was determined with a fluorimetric method using 4-MU-α-galactopyranoside as substrate and a fluorimeter. GLA gene sequencing was performed using full gene Sanger sequencing. Plasma lyso-Gb3 was extracted through a MCX cartridge and analyzed by using ultra-performance liquid chromatography autosampler equipped with tandem mass spectrometer (Waters Corporation).

**Results:** Blood samples from 159 males and 289 females referred for Fabry disease testing because of a positive family history, and/or one or more combination of symptoms suggestive of Fabry disease. In general, plasma lyso-Gb3 showed good concordance with alpha-GAL activity in males and GLA sequencing results in females. Plasma lyso-Gb3 was elevated in all males with deficient alpha-GAL activity, almost all females with GLA pathogenic variants, in 3/8 patients with a VOUS. Plasma lyso-Gb3 was not elevated in patients with normal enzyme activity and negative sequencing results. Plasma lyso-Gb3 was elevated in males, but not females, with p.Arg112His and p.Arg363His variants associated with an attenuated phenotype; it was not elevated in any patient (6 males, 6 females) with the p.Ala143Thr variant.

**Conclusions:** In summary alpha-GAL activity had the highest sensitivity as an initial test for diagnosing male patients; reflexing to gene sequencing and plasma lyso-Gb3 was useful for confirmation. Females with Fabry disease were most effectively diagnosed by plasma lyso-Gb3 measurements combined with gene sequencing. Plasma lyso-Gb3 is helpful when gene testing is inconclusive or fails to detect pathogenic variants.
#47 Title & Authors: Expansion of the Phenotype Associated with EFL1-Related Shwachman-Diamond Syndrome: Identification of a Patient with Short Stature, Metaphyseal Abnormalities and Thrombocytopenia


**Introduction:** Shwachman-Diamond syndrome (SDS) is an autosomal recessive ribosomopathy characterized by exocrine pancreatic dysfunction, hematologic abnormalities, short stature and skeletal abnormalities. Approximately 90% of patients with SDS have pathogenic variants in SBDS. Another gene, EFL1, interacts with SBDS during ribosome assembly and has recently been associated with an SDS-like phenotype in six patients. Here we present a patient with a homozygous missense variant in EFL1 expanding the reported phenotypic spectrum.

**Case report:** The patient is a 14-year-old Caucasian female enrolled in the Undiagnosed Diseases Network (UDN) with a previous diagnosis of spondylometaphyseal dysplasia without molecular confirmation. The patient first presented to genetics at 24 months due to poor growth (length and weight well below the 5th percentile). Skeletal survey at age 6 demonstrated metaphyseal dysplasia, later classified as spondylometaphyseal dysplasia, corner fracture type. The patient developed thrombocytopenia at age 6 with a chronic course (range 45-136 x10^9/L) since that time. At age 9 she presented to hematology with pancytopenia associated with fever. Bone marrow biopsy showed lower cellularity than expected for age. Repeat bone marrow biopsy at age 14 demonstrated a hypocellular bone marrow with trilineage hematopoiesis and no other specific abnormalities. She had not had intractable diarrhea or other symptoms of pancreatic insufficiency. Research trio whole exome sequencing (WES) at age 10 was non-diagnostic. On reanalyses of the WES data through the UDN, a novel homozygous missense variant (c.379 A>G; p.T127A) in EFL1 was interpreted as likely pathogenic due to bioinformatics metrics and the recent case series report with phenotypic overlap with SDS.

**Conclusion:** Pathogenic variants in the EFL1 gene have been associated with severe symptoms that overlap with SDS. We report an older patient with a milder general phenotype associated with EFL1-related SDS. Pancytopenia, neutropenia and pancreatic insufficiency may not be universally present or may be mild. metaphyseal abnormalities can be more severe than those typically observed in patients with SDS. Alth number of reported patients is limited, variants in EFL1 appear to cause a broad spectrum of symptoms.

#48 Title & Authors: Food Protein-Induced Enterocolitis Syndrome (FPIES) is Associated with Increased Risk of Feeding Disorders.

**J. D. Squire, A.P. Stallings**

**Background & Hypothesis:** Food protein-induced enterocolitis syndrome (FPIES) is a non-IgE cell-mediated food allergy that may be T cell mediated. Awareness of FPIES in general pediatrics is low, therefore diagnosis and management of FPIES is often delayed, which can lead to adverse outcomes for patients including feeding disorders. We hypothesize that FPIES patients have increased incidence of feeding disorders compared to other typically developing children.

**Objective:** To examine the incidence of feeding and speech disorders in FPIES patients compared to the general population.

**Methods:** FPIES patients were identified using DEDUCE search for a single Duke Pediatric Allergy Clinic site. Families were contacted by phone and asked to complete a 31 question phone survey. Sixty-three patients with FPIES were identified, and 43 patients completed the survey.

**Results:** Fifty-one percent (22/43) of patients were reported to have a feeding problem or disorder. Forty-one percent (18/43) of patients were reported to have food refusal, 9% (4/43) reported having trouble swallowing, and 20% (9/43) reported a combination of other problems (sensory issues, bottle aversion, and difficulty chewing). Forty-nine percent reported no feeding problems. Twenty-three percent (10/43) of patients with a feeding disorder reported referral to feeding therapy. Fourteen percent (6/43) of patients were referred for speech therapy.

**Conclusions:** The incidence of feeding disorders in typically developing children is estimated at close to 25%. Our findings support the hypothesis that FPIES patients are at increased risk of feeding disorders as 50% of parents reporting a feeding disorder. This is likely due to delays in diagnosis, resulting in repeated reactions of delayed vomiting and subsequent food aversion. Fourteen percent of FPIES patient were referred to speech therapy, in contrast to a reported 5-8% prevalence of speech and language impairments in preschool aged children. Our study demonstrates the need to monitor children with FPIES closely for feeding and speech disorders as they may be at higher risk compared to other children.
#50 CLINICAL COURSE AND OUTCOME IN ADULTS WITH PROPIONIC ACIDEMIA: CASE SERIES

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Abstract

Background: The majority of patients with propionic academia present early in life with metabolic decompensation. The spectrum can range from neonatal-onset to late-onset disease. Long-term complications are common. Despite the advent of NBS the outcome is still unfavorable in early treated patients. We report the clinical course and outcome in a retrospective series of four adult patients with propionic academia.

Study Design/methods: Retrospective case series. Four adult patients between the ages of 23 to 32 years with propionic academia who have been treated at Duke University Hospital from 1992 to now.

Results: Three patients presented with acute metabolic crisis in the neonatal period between the ages of 2 weeks to 5 weeks. One patient presented with hypotonia and failure to thrive at 2 weeks of age. All of them have intellectual disability ranging from moderate to severe and behavioral problems. Psychiatric problems are seen in all patients including anxiety, depression, emotional lability, suicidal ideation, OCD, and ADHD. Abnormal cardiac function was seen in 2 patients, one was found to have mild left ventricular dysfunction at age 26. Another patient was diagnosed with cardiomyopathy at age 24 years. One patient had elevated creatinine and microalbuminuria at 21 years of age. She progressed to chronic kidney disease stage requiring hemodialysis at age 24. This patient died at age 25 due to progressive renal failure. Other long-term complications seen in our cases include feeding difficulty (4/4), pancreatitis (2/4), and irregular menstruation (3/3), sensorineural hearing loss (1/4), abnormal MRI brain (2/4), deep venous thrombosis of unclear etiology (1/4), scoliosis (1/4), osteoporosis (1/4), and Raynaud’s like symptoms (2/4).

Conclusion: Our data show intellectual outcome is still poor among adult patients who were diagnosed and treated early in life. Psychiatric problems are common among adult survivors. Common long-term complications of propionic academia seen in our cases include cardiomyopathy, renal failure, and abnormal brain MRI. Less common complications in our cases are sensorineural hearing loss and osteoporosis. DVT is a unique finding noted in one case. There was no identified etiology. This could be coincidental finding or related to propionic academia. Patient registries and natural history studies are required to provide a better understanding of long term complications patients with propionic academia.
Title & Authors: Novel phenotype of ATP1A3 mutation starting in infancy

Jason Richards, Marie McDonald, Allyn McConkie-Rosell, Vandana Shashi, Mohammad Mikati

Background & Hypothesis: ATP1A3 mutations present as AHC, RDP, EE or as other syndromes. Here we present a patient with a unique combination of EE, RDP and AHC features.

Objective: Report a 32-month-old boy demonstrating the novel phenotype of manifestations of each of severe Epileptic Encephalopathy (EE), Rapid Onset Dystonia Parkinsonism (RDP), and Alternating Hemiplegia of Childhood (AHC).

Methods: Case Report

Results: Patient began having secondary generalized tonic-clonic seizures at age 2 months. These were resistant to seven medications (occurring daily to weekly). Between the ages of 4-29 months he had 11 admissions for generalized tonic-clonic status epilepticus (SE) episodes. Video-EEG: seizures originated from the central, left and right temporal lobes. Interictal EEG: generalized slowing, bilateral temporal focal and sharp waves. Between 20-22 months, he had distinct episodes of unilateral and bilateral plegias independent of any seizure activity. These occurred 2-3/week, each lasted 1-2 hours and resolved during sleep. After a febrile illness (age 29 months) and an accompanying SE he regressed. His exam, which previously manifested diffuse low axial tone, ability to elevate arms and legs and to focus on faces, transformed into diffuse severe continuous increased tone with rigidity of all body, no visual fixation, with superimposed severely painful attacks of dystonia of the whole body (2-3 minutes each, 4-6/day) with no concurrent EEG changes. MRI: diffuse atrophy. Extensive workup and WES: novel de novo P.V589F ATP1A3 mutation (c. 1765 G>T), in a conserved position. In silico analysis predicted it to be probably damaging to protein structure.

Conclusions: Our patient manifests a novel phenotype with overlaps with prior syndromes caused by a novel ATP1A3 mutation.

Title: Targeted and whole exome sequencing identifies genetic causes of SRNS in 40% of families with autosomal dominant disease.

Authors: Jennifer D. Varner, Megan Chryst-Stangl, Ayo Matory, Brandon Lane, Guanghong Wu, Gentzon Hall, Rasheed Gbadegesin.

Background: Approximately 20% of children with nephrotic syndrome are resistant to steroid treatment. The most common cause of steroid-resistant nephrotic syndrome (SRNS) is focal segmental glomerulosclerosis (FSGS). FSGS is a leading cause of end-stage kidney disease worldwide and is characterized by nephrotic syndrome and focal scarring of glomerular tuft. Advances in genomics have led to the identification of genes that cause SRNS and a better understanding of its pathogenesis. However, there are no clinical guidelines for genetic screening for SRNS.

Objective: Our objective was to use next generation sequencing to determine the prevalence of mutations in autosomal dominant SRNS genes in a large cohort of patients with familial and sporadic SRNS and to identify clinical predictors of mutation.

Methods: Our multi-ethnic cohort consisted of 497 individuals in 173 families with SRNS, of whom 76 families (43.9%) had familial disease and 97 (56.1%) had sporadic disease. The cohort was screened for mutations in autosomal dominant genes by WES and targeted sequencing of known SRNS genes (INF2, COL4A3, COL4A4, WT1, TRPC6, ACTN4, ANLN, ARHGAP24, CD2AP, and LMX1B). All sequences were confirmed by Sanger sequencing and evaluated for mutations using SequencherTM software.

Results: We identified disease causing variants in 29/76 (39.5%) families with familial SRNS (presumed autosomal dominant) and 4/97 (4.1%) individuals with sporadic SRNS. Mutations in INF2 were the most common cause of SRNS in this cohort and were responsible for 37.5% of all mutations detected. Mutations in INF2, COL4A3 and COL4A4 were responsible for over 50% of all mutations detected. 34.4% of all mutations detected were novel and were absent from all public databases. Truncating/loss of function mutations in some of these genes were associated with early onset disease compared with missense mutations. Family history of SRNS was predictive of identifying a genetic mutation (positive history versus no family history χ2 p<0.00001).

Conclusions: Mutations in known SRNS genes were responsible for disease in about 40% of all families with hereditary SRNS in this cohort, compared to only 4% of patients with sporadic SRNS. Family history is the single most important clinical predictor of genetic mutations in autosomal dominant disease. These data support the need for routine genetic testing in patients with positive family history of SRNS but not in those with sporadic disease.
#53 Title & Authors: Pediatric Intensivists’ Perceptions of a Clinically Meaningful Improvement in Ventilator-Free Days

Jennifer I Sherwin, Ira M Cheifetz, Lisa A Asaro, David Wypij, Amy Cassidy, Martin C J Kneyber, Martha A Q Curley

Background & Hypothesis: Ventilator-free days (VFD), defined as days alive and free from mechanical ventilation, is a common primary endpoint in pediatric acute respiratory distress syndrome (PARDS) clinical trials. While common, little is known about what practicing clinicians believe to be a clinically meaningful improvement in VFDs; specifically, the number of days considered important enough by clinicians to change their practice. The proposed survey question. Countries represented included the United States, Canada, The Netherlands, Australia, and Singapore. The mean response was 1.9 (SD 0.8) VFDs with a range of 1-4 days and a median of 2 days (IQR 1-2).

Conclusions: Based on our survey results, the consensus among pediatric intensivists is that a 2-day increase in VFDs between intervention groups would indicate a clinically meaningful improvement. These data will inform the design of PROSpect and may help guide investigators when designing clinical trials in PARDS.

#54 Vancomycin Levels in Children Supported by a Ventricular Assist Device

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Background & Hypothesis: Vancomycin is commonly used for peri-operative prophylaxis in pediatric patients undergoing ventricular assist device (VAD) implantation. Achieving therapeutic concentrations is essential to therapeutic success, while reducing the risk of toxicities. Because vancomycin undergoes renal clearance and is associated with renal dysfunction, the relationship between vancomycin dosing and renal function is complex. Here, we evaluated the performance of standard vancomycin dosing regimens in achieving therapeutic concentrations in children supported with VADs. We hypothesized that standard dosing regimens will not result in optimal vancomycin concentrations in the population.

Objective: To determine the prevalence of vancomycin doses outside of the therapeutic range in patients with VADs and assess for association of non-therapeutic levels with renal dysfunction.

Methods: We conducted a single-center retrospective cohort study of 21 pediatric patients (<18 years of age) undergoing VAD implantation between 2013-2016. Initial vancomycin dose was 15-20mg/kg, and subsequent doses were adjusted based on our pharmacist driven therapeutic drug monitoring protocol, with trough levels obtained prior to the 4th dose, or 8-12 hours post-dose in patients with renal dysfunction. Target levels were 10-20 μg/ml. We used multivariable linear regression with robust variance estimators to evaluate the association between vancomycin levels, serum creatinine, and vancomycin dose. We considered p<0.05 statistically significant. This study was approved by the local institutional review board.

Results: All 21 patients received at least one dose of vancomycin at a median dose of 18mg/kg (25th, 75th percentile 15, 20). A total of 218 plasma levels were recorded, with a median of 8 (6,14) per patient. The median serum creatinine and estimated glomerular filtration rate, calculated using the Schwartz equation, were 0.9 mg/dL (0.6, 1.6) and 63 ml/min/1.73m2 (31, 104) respectively. Of the 218 levels, 37 (17%) were subtherapeutic (<10 μg/ml) and 61 (28%) were supratherapeutic (>20 μg/ml). As expected, both dose (slope=0.56, 95% confidence interval (CI):0.22, 0.91) and serum creatinine (slope=2.45, 95%CI: 0.49, 4.40) were associated with vancomycin levels.

Conclusions: Pediatric patients with VADs commonly receive vancomycin doses associated with supratherapeutic levels when managed by standard therapeutic drug monitoring. While dose and renal dysfunction remain associated with vancomycin levels, these variables alone appear insufficient to guide dosing in this population, underscoring the need for population specific PK studies.
#55 Title: Initial response to corticosteroid therapy and native kidney biopsy findings is a predictor of disease recurrence following kidney transplantation: A MWPNC study.

Authors: Jonathan H. Pelletier, Karan R. Kumar, Rachel Engen, Adam Bensimhon, Michelle Rheault, Connie Haney, Caroline Straatmann, Cynthia Silva, T. Keefe Davis, Scott Wenderfer, Keisha Gibson, David Selewski, John Barcia, Patricia Weng, Tarak Srivastava, Christoph Licht, Natasha Jawa, Shashi Nagaraj, John Foreman, Delbert Wigfall, Annabelle Chua, Eileen Chambers, Christoph P. Hornik, Laurence A Greenbaum, Rasheed Gbadegesin

**Background:** Steroid resistant nephrotic syndrome (SRNS) is a leading cause of end-stage renal disease (ESRD) in children. Focal segmental glomerulosclerosis (FSGS) is the most common pathologic variant of SRNS. Disease recurrence is common, and is the single most important cause of renal allograft loss. Previous studies have not consistently identified risk factors that are associated with recurrence of disease or response of recurrence to plasmapheresis (PLEX) with intensive immunosuppression protocol.

**Objective:** We sought to determine risk factors associated with the recurrence of disease in renal allografts and factors that predict response to PLEX and intensive immunosuppression following recurrence.

**Methods:** Multicenter retrospective review of kidney transplants performed for SRNS in MWPNC participating centers between 1/2006-12/2015. Data were collected on patient’s demographics, clinical course, and biopsy findings. Patients with primary SRNS (PSRNS) were defined as those initially resistant to corticosteroid therapy at diagnosis, and patients with late SRNS (LSRNS) were defined as those initially responsive to steroids who subsequently developed steroid resistance. We performed multivariable logistic regression (adjusting for sex, race, initial histology, SRNS type, time to ESRD) with random effects for site to determine risk factors associated with SRNS recurrence. The sample size precluded analysis of factors predicting response to PLEX.

**Results:** We identified 154 patients that met inclusion criteria, with 65 (42%) having recurrence of SRNS. We found that initial histology of native kidney biopsy (FSGS vs. minimal change disease; OR 10.51, CI 2.00-55.24, p=0.005), initial steroid responsiveness (PSRNS vs. LSRNS OR 3.83, CI 1.09-13.47, p=0.04), were associated with increased odds of SRNS recurrence in renal allografts.

**Conclusions:** Pediatric patients with minimal change disease histology and LSRNS are at significantly higher risk of disease recurrence following kidney transplantation. These findings may be useful for designing studies to test strategies for preventing recurrence.

#56 Title: Association between hypoglycemia and bacteremia after the first week of life

Authors: Karan R. Kumar, MD, Daniel K. Benjamin, PhD, Somam J. Shah, Rawan M. Fayyad, Toby M. Turla, Laura M. O’Sullivan, Beatrix Wallace, Rachel G. Greenberg, MD, MB, MHS, Kanecia O. Zimmerman, MD, MPH, Reese H. Clark, MD and Christoph P. Hornik, MD, MPH

**Background:** Sepsis is a leading cause of mortality and morbidity in infants. Timely diagnosis is associated with improved outcomes, but remains challenging. In small studies, hypoglycemia has been reported as an early sign of sepsis in infants.

**Objective:** We used a large multicenter electronic database to evaluate the association between hypoglycemia and bacteremia after the first week of life in infants admitted to the neonatal intensive care unit (NICU).

**Methods:** We included all infants admitted to 301 NICUs managed by the Pediatrix Medical Group from 1997 to 2015 with ≥1 blood culture collected between day of life 7 to 120 and ≥1 serum glucose value recorded on the day of or day prior to the culture. We defined bacteremia as a positive blood culture excluding organisms considered contaminants. We used the lowest obtained glucose value and defined hypoglycemia according to 3 definitions: (1) <70 mg/dL, adapted from the American Academy of Pediatrics; (2) <60 mg/dL, adapted from the Pediatric Endocrine Society; and (3) <47 mg/dL, based on neurometabolic studies of hypoglycemia. We examined the association between each definition of hypoglycemia and bacteremia using multivariable logistic regression adjusted for gestational age, small and large for gestational age status, diagnoses and medications associated with hypoglycemia, necrotizing enterocolitis, feeding status, mechanical ventilation, inotropic support, and inborn status. We performed a subgroup analysis on all models stratified by organism class: gram positive, gram negative, and fungal.

**Results:** We identified 50,382 infants with 106,881 cultures. Of those, 9,565 (9%) cultures were positive in 8,515 infants (17%). Hypoglycemia, according to all 3 definitions, was associated with decreased odds of bacteremia from gram-positive organisms. Conversely, hypoglycemia was associated with increased odds of bacteremia from gram-negative organisms for cut-off values <60 mg/dL and <47 mg/dL.

**Conclusions:** In our cohort, hypoglycemia was associated with decreased odds of bacteremia from gram-positive organisms, but increased odds of bacteremia from gram-negative organisms when using a lower cut-off value. Contamination of cultures by gram positive skin flora and residual bias associated with serum glucose monitoring may contribute to these findings. Further studies of hypoglycemia as a diagnostic marker for sepsis, differentiated by organism class, in infants are needed.
#57 Title & Authors: Examining Noncardiac Surgical Procedures in Neonatal and Pediatric Patients on ECMO
Stephanie P. Schwartz, MD, Karan R. Kumar, MD, Julia Salinaro, Desiree Bonadonna, BSE CPP LP, Christoph P. Hornik, MD, MPH, David A. Turner, MD, Andrew J. Lodge, MD

Background & Hypothesis: Extracorporeal membrane oxygenation (ECMO) is frequently used in neonates and pediatric patients with cardiac and respiratory failure. As the complexity and scope of critically ill patients requiring ECMO continues to widen, the number of noncardiac surgical procedures (NCSP) performed on these patients will likely increase. Data on NCSP in this population and the link to morbidity and mortality has not been well documented. This study examined the NCSP required for pediatric patients on ECMO and determined which variables impact outcome. We hypothesized that morbidity and mortality would be increased in patients supported with ECMO requiring NCSP when compared to those who do not require surgical procedures.

Objective: 1. Determine which types of NCSP are required for neonatal and pediatric patients on ECMO. 2. Determine which variables affect outcome (primary outcome = mortality).

Methods: Retrospective review of all neonatal and pediatric patients (<18 years) requiring ECMO at a single tertiary care center from 2013-2015. Multivariate logistic regression was used to examine the association between NCSP and mortality.

Results: 98 patients required ECMO during the study period. 36 patients (37%) required 89 NCSP. The median number of NCSP was 1 for neonates, 2 for infants and 2 for children. There were 65 (73%) thoracic, 14 (16%) general surgery, and 7 (8%) vascular procedures with bronchoscopy (44%), chest tubes (15%), tracheostomy (7%), and central line placement (4%) being most common. In-hospital mortality did not differ between patients with and without NCSP (36% vs 45%, p= 0.39). In adjusted analysis, requiring at least 1 NCSP did not increase mortality (OR 0.45, 95% CI: 0.14-1.45, p= 0.18). This was also true in subgroup analysis examining thoracic, general surgery, vascular, and the five most common NCSP. Patients requiring NCSP had longer median length of stay (54 vs 37 days, p= 0.01) and ICU length of stay (52 vs 31 days, p< 0.01). Patients requiring NCSP had more bleeding complications (50% vs 23%, p= 0.01) but did not require more total blood products per day on ECMO (38 vs 24ml/kg/day on ecmo, p= 0.21). Patients requiring NCSP had statistically higher incidence of acute kidney injury (56% vs 26%, p< 0.01) but similar need for renal replacement therapy (70% vs 50%, p= 0.22).

Conclusions: NCSP are common in pediatric patients supported with ECMO. NCSP on pediatric patients supported with ECMO increased morbidity and hospital length of stay but did not increase mortality.

#58 My Patient Doesn’t Have That! When Genetic Results and Clinical Presentation are Discordant
Kelly Schoch, Jennifer Sullivan, Nicholas Stong, David Goldstein, Marie McDonald, Allyn McConkie-Rosell, Kristi Milowic, Undiagnosed Diseases Network, Yong-hui Jiang, Vandana Shashi

Although next generation sequencing of the exome is increasingly and effectively used in clinical genetics practice, challenges can occur in the interpretation of results and the consequent counseling for patients/families. Since whole exome sequencing (WES) extends beyond targeted molecular testing clinicians can be presented with bioinformatically compelling genetic results that may not reflect the patient’s clinical presentation. Here we present three cases in which bioinformatic investigations strongly support a genetic diagnosis, but there is poor phenotypic fit. Case 1: 4 year-old female with a de novo missense ‘likely pathogenic’ variant in SETBP1 (c.2612T>G, p. I871S) reported on WES, who has mild developmental delays, short stature, hypotonia and minor dysmorphic features, but lacking the congenital /severe neurological, organ and bone problems characteristically seen in Schinzel-Giedion syndrome (SGS). Case 2: 8 year-old boy with a ‘pathogenic’ variant in exon 10 of OFD1 (c.967delA, p.S323AfsX2) with normal intelligence and none of the facial, renal, skeletal, oral or ophthalmologic features of OFD1-related syndromes. Although the frameshift variant was predicted to result in a loss of function (LOF) consistent with known disease mechanism for OFD1 further networking and exon-specific analyses suggested that predicted LOF variants in exon 10 are not actually pathogenic, likely due to an isoform that skips exon 10. Case 3: 8 month-old male with a ‘pathogenic’ variant in MYBP1 (c.788t>G, p. L263R) detected on research reanalyses of WES data, who has severe hypotonia, tremors and developmental delay but lacks the joint contractures seen in the arthrogryposis syndromes caused by MYBP1 (MIM 614335, 614915). Further networking led to the identification of three other individuals with similar variants also in the M-motif of the MYBP1 protein with hypotonia and tremors but without contractures, supporting an expanded phenotype for this disorder. Each of these cases highlights the importance of closely reconsidering the clinical phenotype when interpreting a laboratory report. Due diligence on the part of the clinical team may entail further multi-system phenotyping, consideration of other diagnostic avenues such as research studies utilizing alternative bioinformatic pipelines, and networking within the genetics community (e.g. Genematcher, PhenomeCentral) to identify other patients with similar variant/phenotype discordance.
#59 Title & Authors: Probiotic Use and Safety in the Neonatal Intensive Care Unit: A Multicenter Cohort Study
KD Gray, J Messina, C Cortina, S Gbadegesin, M Foster, M Fowler, T Owens, RH Clark, DK Benjamin, K Zimmerman, RG Greenberg

**Background & Hypothesis:** Premature infants in the neonatal intensive care unit (NICU) are at high risk of abnormal gut bacterial colonization, which is associated with short- and long-term morbidities, including necrotizing enterocolitis (NEC). Probiotics have been used to improve optimal bacterial colonization, but they are not approved by the Food and Drug Administration (FDA) and may be associated with adverse outcomes.

**Objective:** The purpose of this study was to determine the change in frequency of probiotic administration to preterm infants in a multicenter cohort of neonatal intensive care units (NICUs) and the association between probiotic administration and select adverse outcomes, including NEC, bacteraemia, and meningitis.

**Methods:** We performed a multicenter, retrospective cohort study of infants 23-29 weeks gestational age admitted to NICUs in the Pediatrix Medical Group from 1997 to 2015. Infants were excluded if they died or were discharged within 3 days of birth. We evaluated the type of probiotics given and the incidence of exposure to probiotics over time and by site among sites who admitted >100 infants during the study period. We compared demographic and clinical characteristics between infants who received probiotics and those who did not. We compared the proportion of infants with medical or surgical NEC, bacteraemia, or meningitis in each group using Fisher’s exact test. We performed multivariable logistic regression to evaluate the association between probiotics exposure and the select adverse outcomes after adjustment for demographic and clinical characteristics.

**Results:** 110,626 infants were included. Probiotic use increased over the study period (Figure 1). There was a wide variation in probiotic use among NICUs, ranging from 0 to 650 per 1000 infants (Figure 2). Infants exposed to probiotics were more likely to have lower gestational age and birth weight (Table 1). Lactobacillus was the most commonly used probiotic (72%). On adjusted analysis, probiotics administration was not associated with NEC (adjusted odds ratio [95% confidence interval] 0.89 [0.79-1.01] or meningitis (1.57 [0.98-2.53]). Probiotics were associated with decreased odds of bacteremia (0.90 [0.82-0.99]).

**Conclusions:** Probiotic use has increased over time and varies substantially among NICUs. Prospective, randomized, controlled studies of specific products are needed to further investigate the safety and efficacy of probiotics in VLBW infants.

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#60 Title & Authors: Factors for Breastfeeding Success in the First Hospitalization.
KD Gray, E Erickson, C Wood, D Tanaka

**Background & Hypothesis:** Breast-feeding remains an important goal, as it improves maternal infant bonding, and reduces cost and incidence of several childhood illnesses. Current rates fall behind Healthy People 2020 goals, and meeting these goals depends on the successful initiation within the first days of life. Several factors have an association in the initiation and success of breast-feeding including race, socioeconomic status, education, and family support.

**Objective:** Describe the demographics of maternal-infant dyads successful with exclusive breastfeeding throughout the first hospitalization. Understand the influence of donor breast milk and lactation consultations on successful exclusive breastfeeding at hospital discharge.

**Methods:** This was a retrospective cohort study of all infants 37-41 weeks admitted to the full term nursery service from January 1, 2014 to December 31, 2016 at Duke University hospital and Duke Regional hospital. Descriptive data included demographics, payer, and race/ethnicity compared by chi squared and ANOVA analysis prior to interventions. Multivariable regressions compared the influences of donor breast milk and lactation consultation on exclusive-breastfeeding success adjusting for timing of interactions and demographics. Exclusive breast-feeding included those infants receiving only maternal and/or donor breast milk throughout the hospitalization.

**Results:** 7,370 infants were in the cohort. The average exclusive breast-feeding success rate was 55% among all infants. Infants more likely to be successful with exclusive breast-feeding prior to interventions were >39 weeks gestation, non-Hispanic or Latino, and using managed care or third party payer sources (Table 1). Mother-infant pairs seen by lactation consultants were more likely to be successful at 76%, but those seen by lactation consultants were less likely to be Medicaid patients(38%, 935/2479, p<0.01). The combination of donor breast milk and lactation consultation improved exclusive-breastfeeding success through discharge more than donor breast milk alone (61% vs. 52%, p <0.01, Figure 1).

**Conclusions:** Success for exclusive breast-feeding in the first hospitalization are strongly dependent on race and payer. Combined resources such as lactation consultants and donor breast milk can improve breast-feeding success, but those most at risk for failure are least likely to utilize lactation consultation.
#61 Title & Authors: Defect In Chromatin Modifications and Autism Spectrum Disorder (ASD)- Report of a novel and de novo pathogenic mutation of chromatin-modifying enzyme MSL2 in ASD and review of the literature.

Kim Ng, Kacie Riley, Yong-hui Jiang. Division of Medical Genetics.

**Background & Hypothesis:** MSL2 (male-specific lethal 2) is a subunit of a protein complex that plays a major role in histone acetylation and ubiquitination in *Drosophila*. However, direct studies in human MSL2 are few; and the gene is poorly characterized. To date, MSL2 gene has not been reported to be in association with any specific human disease. Recent genomics studies have uncovered numerous rare and de novo pathogenic mutations in more than 20 genes encoding the proteins of epigenetic machinery in autism spectrum disorder (ASD). These discoveries provide the strong support for the role of epigenetic dysregulations in the etiology of ASD. Here, we report a patient with a novel pathogenic mutation in MSL2 in ASD from whole exome sequencing study and hence expanded the list of genes of epigenetic modifying enzymes in ASD.

**Objective:** To understand the role of epigenetic modifications in neurodevelopmental disorders.

**Methods:** A clinical whole exome sequencing study was performed in a subject in the genetic clinic and a retrospective clinical chart review and literature review was conducted.

**Results:** The subject is currently a 6-year-old girl with dysmorphic face, ASD, developmental delay, and other behavioral problems including anxiety and self-injurious behavior. The pregnancy was complicated by intrahepatic cholestasis of pregnancy and Venlafexine exposure in the first trimester. The delivery was not complicated. Carotid deficiency was revealed at the newborn period but repeat measurements at a later age was normal. Perinatal course was complicated by hypotonia and feeding difficulties. Family history was significant for learning difficulties in the father and maternal half-brother. Her current weight is 18.9 kg (47th percentile), height 118.2 cm (17th percentile) and head circumference is 49.6 cm (96th percentile). Her motor development was delayed. The diagnosis of ASD was confirmed with ADOS-2. Her standard score on WPPSI-IV was 75 and at a very low functional range (5th percentile). The performance on Visuomotor Precision Time was average (score=3) but on the Visuomotor Precision Combined, it was extremely poor (score=3). The score for Visual Perception was 45 (.02th percentile), for the Motor Coordination was 63(1st percentile) and Visual Motor Integration was 77(6th percentile). The score on PPVT-IV test was 80(9th percentile). Clinical WES identified an indel of c.796-796delCT (NM_018133.3) that is predicted to result in a frameshift and truncated protein (p.L266Vfs5) that is mostly likely associated with loss-of-function of the protein. The mutation is de novo and has not been seen in any reference dataset. MSL2 has not been associated with any human diseases.

**Conclusions:** The de novo and novel mutation in MSL2 is mostly likely pathogenic. This is the first report of a variant in the MSL2 gene that is implicated in ASD and added to the list of genes of chromatin modifier in ASD.

#62 Title & Authors: Postnatal Cytomegalovirus Infection in Very Low Birth Weight Infants and its Association with Hearing Loss

Kristin Weimer, MD, PhD, Matthew S. Kelly, MD, MPH, Sallie Permar, MD, PhD, Reese H. Clark MD, and Rachel G. Greenberg, MD, MB, MHS

**Background & Hypothesis:** Cytomegalovirus (CMV) is the most common perinatal infection worldwide and the leading cause of infant brain damage and sensorineural hearing loss. While previous studies have suggested that postnatal CMV infection can lead to long-term morbidity in very low birth weight (VLBW) infants, including bronchopulmonary dysplasia (BPD) and neurodevelopmental impairment, the impact on infant hearing and other long-term effects have not been studied. Defining the full scope of morbidity and long-term complications attributed to postnatal CMV infection will define the problem and help determine if treatment can reduce complications in this population.

**Objective:** To evaluate risk of failed hearing screen, necrotizing enterocolitis (NEC), and BPD in VLBW infants with postnatal CMV infection.

**Methods:** We performed a multicenter, retrospective cohort study of infants from over 300 NICUs treated at Pediatrix Medical Group between 2002-2016. We included infants <1500 g, hospitalized on day of life (DOL) 21, with hearing screen results after postmenstrual age 34 weeks and with a diagnosis of postnatal CMV. We excluded infants with evidence of CMV infection prior to DOL 21. Infants with postnatal CMV were matched 1:1 to infants without a diagnosis of postnatal CMV using propensity scores, and Poisson regression was used to examine the effect of postnatal CMV on risk of failed hearing screen, NEC, and BPD.

**Results:** 304 infants with postnatal CMV were identified, and 278/304 (91%) were matched to 278 infants without postnatal CMV (Table 1). Hearing screen failure occurred in 46/278 (17%) of infants with postnatal CMV and 29/278 (10%) of those without postnatal CMV. Postnatal CMV was associated with increased risk of failed hearing screen (risk ratio 1.59, [95% confidence interval 1.04–2.41]) and BPD (1.18 [1.08-1.29]) but was not associated with increased risk of NEC (0.83 [0.29-2.44]).

**Conclusions:** Postnatal CMV infection is associated with an increased risk of failed hearing screen in VLBW infants. We also confirmed the previous finding of an increased risk of BPD in infants with postnatal CMV. Further prospective studies are needed to determine the full impact of postnatal CMV infection and to determine if treatment reduces morbidity.
**#63 Title & Authors:** "Lactoferrin and protection against postnatal cytomegalovirus infection in premature infants"

**Kristin Weimer, MD, PhD, Stevie Rowe, MD, Margarita Bidegain, MD, MHS-CL and Sallie Permar, MD, PhD**

**Background & Hypothesis:** Very low birth weight (VLBW, < 1500 g) preterm infants are at risk for life-threatening infections while in the NICU. Breast milk protects against infections and is recommended for all preterm infants, but carries the risk of infection by cytomegalovirus (CMV) shed in mother's milk. Postnatal CMV in VLBW infants can result in a severe sepsis-like illness. A potential solution is to harness the antimicrobial properties of human milk to protect against CMV acquisition. Lactoferrin is a breast milk and saliva protein with potent activity against CMV. Establishing a role for lactoferrin in prevention of postnatal CMV would implicate its use as a prophylactic agent against postnatal CMV infection. We hypothesize that high CMV load in breast milk will increase the risk of postnatal CMV transmission, while a high concentration of lactoferrin in breast milk and saliva will decrease the risk of postnatal CMV transmission.

**Objective:** To determine the incidence of postnatal CMV in VLBW infants in the Duke NICU and the relationship between breast milk and saliva lactoferrin concentration, breast milk CMV load, and acquisition of postnatal CMV.

**Methods:** 100 VLBW, maternal breast milk fed infants in the Duke NICU and their lactating mothers were enrolled and followed for 3 months, or until discharge. Breast milk and infant saliva samples were collected biweekly. Maternal CMV status was determined by CMV IgG ELISA on breast milk. CMV was detected in infant saliva and breast milk by qPCR. Breast milk and saliva lactoferrin concentration were determined using commercial ELISA kits.

**Results:** 36 mothers (36/60, 60%) are seropositive for CMV. CMV was detected in at least one milk sample from 20/33 mothers (61%, 17-330,000 copies/mL). 5 infants tested positive for postnatal CMV (5/40, 13%) and 5 for congenital CMV (5/40, 13%). In an in vitro CMV neutralization assay, the ID90 of recombinant human lactoferrin against CMV was 787 ng/mL. Mean lactoferrin levels in breast milk and saliva samples tested were 1.5 mg/mL and 29 ng/mL, respectively.

**Conclusions:** In preliminary data, 60% of VLBW infants are at risk for postnatal CMV via breast milk and transmission occurred in at least 5 infants. Levels of lactoferrin in the milk tested should be sufficient to neutralize CMV, but may differ between CMV transmitting and non-transmitting mothers. The ID90 of lactoferrin against CMV is > 1000-fold more than the concentration in saliva, suggesting that exogenous lactoferrin could provide additional protection against CMV acquisition.

**#64 Title:** Corticobasal Syndrome in an Adult Male Gaucher Disease Type 1 Patient: Expansion of the Understanding of the Neurological Spectrum

**Authors:** Kunal C. Potnis, Stephanie DeArmey, Lauren B. Flueckinger, Roy Alcalay, Priya S. Kishnani

**Background:** Gaucher disease (GD) is an autosomal recessive disease that results from a deficiency of the enzyme β-glucocerebrosidase. Individuals with GD typically present with hepatosplenomegaly, pancytopenia, and skeletal abnormalities. Traditionally GD is divided into three types: a non-neuronopathic form (type 1) and two neuronopathic forms (types 2 and 3). Although type 1 is considered non-neuronopathic, peripheral neuropathy and Parkinson's disease are known to occur in some patients with type 1 disease. Here we present a patient with GD type 1 who was later diagnosed with corticobasal syndrome (CBS), a form of atypical parkinsonism.

**Case Report:** The patient is a 61-year-old Caucasian male diagnosed with GD type 1 via bone marrow aspirate at age 14 years. He had a deficiency of β-glucocerebrosidase. He exhibited hepatosplenomegaly and experienced bone crises as a teenager. A common mutation panel revealed one copy of the N409S mutation in the GBA gene. The patient first received treatment with intravenous enzyme replacement therapy at age 49. He began receiving oral eliglustat at age 55 via a clinical trial, which stabilized his systemic Gaucher symptoms. Cognitive decline was reported by the patient and his spouse at age 57, characterized by long-term memory impairment and slurring of speech. Brain MRI at this time displayed evidence of cortical atrophy considered advanced for his age; neuropsychological testing revealed non-amnestic mild cognitive impairment. Given that eliglustat does not cross the blood-brain barrier, the likelihood of the drug affecting cognitive functioning was deemed very low, and the patient expressed a desire to continue on oral treatment.

At age 59, he had symptoms of dementia, apraxia, and vertical gaze palsy, and a diagnosis of atypical parkinsonism was considered. Brain MRI obtained at this time exhibited generalized cerebral and cerebellar volume loss with associated ventriculomegaly. Stereoeagnosis, gait impairment, and shuffling steps were also documented. In addition to experiencing difficulty sleeping and urinary incontinence, the patient also displayed signs of emotional lability via aggression and violent outbursts. A diagnosis of CBS was made by a neurologist.

**Conclusions:** The increased risk of Parkinson's symptoms among carriers and those affected with Gaucher disease is well documented in the literature. Our case highlights the need to consider forms of atypical parkinsonism such as CBS in the management of patients with GD type 1. At least 60 cases of atypical parkinsonism in patients with GD type 1 have been reported in the literature; of these, it appears there has been only one other case of CBS reported. Further assessment of patients with GD type 1 should be completed to identify symptoms indicative of neurological involvement.
**#65 Title & Authors: Effect of Preprandial Ghrelin on Glucose Tolerance**

Laura Page MD & Jenny Tong MD MPH

**Background & Hypothesis:** Released mainly by the stomach, the peptide hormone ghrelin rises before meals and falls after eating, driving hunger and feeding behavior. This pattern is aberrant in type 2 diabetes (T2D), as ghrelin levels are chronically low with diminished postprandial suppression. In insulin resistant mice, increasing preprandial ghrelin levels improves glucose tolerance by promoting release of the incretin hormone glucagon-like peptide-1 (GLP-1). We hypothesized that restoring the preprandial ghrelin spike in subjects with T2D would similarly improve postprandial glucose tolerance through increased GLP-1 release.

**Objective:** To determine the effect of preprandial ghrelin on postprandial glucose tolerance and GLP-1 secretion in subjects with T2D.

**Methods:** Men and women aged 18 - 70 years with a BMI of 25 - 45 kg/m2 and T2D controlled by lifestyle and/or oral medications (HbA1c ≤ 8.5%) were recruited for the study. Participants arrived at the study center four hours after consuming a standardized meal at home. A bolus injection of ghrelin or saline was given 60 minutes prior to the meal tolerance test (MTT) on two separate occasions in a random order, separated by at least one week. Blood samples were collected at 10 - 30 minute intervals during the MTT.

**Results:** Ten participants, 60% female, with a median age of 60 years (IQR: 51.3 - 61), median BMI of 34.5 kg/m2 (IQR: 31.6 - 36.4), and median HbA1c of 7% (IQR: 6.5 - 8.1) completed the study. Glucose tolerance, measured as incremental AUC, was decreased by preprandial ghrelin treatment compared to saline (Ghrelin: 17746, Saline: 13967, p-value: 0.002). Analysis of the effect of ghrelin on postprandial GLP-1 levels in progress.

**Conclusions:** Elevating ghrelin preprandially worsens glucose tolerance in individuals with T2D. Our analysis on the effect of preprandial ghrelin on GLP-1 secretion is pending. However, if preprandial ghrelin increases GLP-1 release in individuals with T2D as previously hypothesized, the increase is not enough to overcome the insulin resistance induced by supraphysiologic ghrelin. In summary, elevating systemic levels of ghrelin is unlikely to improve glucose tolerance in T2D and conversely, ghrelin antagonism may benefit glucose control in this population.

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**#66 Title & Authors: PERSISTENT B CELL DEFICIENCY IN HIV-EXPOSED UNINFECTED INFANTS FOLLOWING IMMUNIZATION**

Sadder, L., Yin, L., Chang, K-F., Fischer, B., Goodenow, M., Sleasman, J.

**Background:** HIV-exposed uninfected (EU) infants are candidates for HIV vaccine trials. However, it is unclear if EU infants have similar immune responses when compared to unexposed healthy infants (HI).

**Methods:** Longitudinal assessment of vaccine responses and plasma biomarkers associated with B cell responses in a cohort of 77 breastfed (BF) or formula fed (FF) HI were compared to 45 EU infants. Comparisons among groups for vaccination titers to *B. pertussis*, *H. flu*, Hep B, and tetanus measured in cord blood samples (CB), 6, and 12 months of age by ELISA. Plasma biomarkers included APRIL, BAFF, sCD163, sCD40L, and IL-10 measured by multiplex ELISA and IgA levels by nephelometry. Statistical comparisons among and within groups used a t test. Regression analysis assessed relationship between vaccine responses and plasma cytokine levels in HI and EU infants.

**Results:** Post vaccination titers to *B. pertussis* and tetanus were lower at birth in EU infants as compared to HI (2394 and 6019 U/ml, p= 0.0006, and 99.8 and 383.8 p=0.004, respectively). However, EU infants had higher tetanus titers at 6 and 12 months and higher pertussis titers at 12 months. In contrast, Hep B titers were significantly lower in EU infants compared to HI. Within all groups, CB levels of APRIL, BAFF, sCD40L, and IL-10 were elevated and declined at 6 and 12 months. BAFF levels were lower in EU infants compared to HI in CB, 6 and 12 months (1626, 342, 305 pg/ml vs 7599, 1045, 862 pg/ml, p<0.05). Linear regression analysis showed a negative correlation between CB and 6 month *B. pertussis* titers in HI (rho= -0.75, p=0.0009) but not in EU infants. There was a strong positive correlation between both APRIL and sCD40L to *B. pertussis*, tetanus, *H. flu*, and Hep B titers at 12 months in HI (rho > 0.51, p <0.05). However, with the exception of tetanus (rho= 0.73, p= 0.01) this correlation was not evident in EU infants.

**Conclusions:** Lower CB vaccine titers in EU infants reflects HIV-associated maternal immune suppression. However, while EU infants’ vaccine responses are more robust for tetanus and *B. pertussis*, subtle immune defects were revealed in pro-inflammatory pathways involving BAFF, APRIL and sCD40L. These results indicate persistent B cell developmental defects in EU infants, which may have implications for HIV vaccine trials in this population.
Adverse Events and Outcomes Associated with Opioid and Benzodiazepine Use in Encephalopathic Neonates During Therapeutic Hypothermia

Melissa C. Kay, PhD, MPH, MS, RD, H. Shonna Yin, MD, MS, Kori B. Flower, MD, MS, MPH, Russell L. Rothman, MD, MPP, Lee M. Sanders, MD, MPH, Alan M. Delamater, PhD, Sophie Ravanbakht, BA, Heidi J. Silver, PhD, RD, Eliana M. Perrin, MD, MPH

Background & Hypothesis: Animal data suggest that benefits of therapeutic hypothermia (TH) for hypoxic-ischemic encephalopathy (HIE) are influenced by concurrent sedation and analgesia. We previously reported wide center variation and increasing prevalence of opioid use during TH for HIE in a large consortium of neonatal intensive care units (NICUs). How these practice variations impact infant outcomes is unknown.

Objective: Compare characteristics, prevalence of adverse events, and hospital outcomes of neonates exposed to opioids and/or benzodiazepines during TH to those who were not exposed.

Methods: We identified neonates ≥ 35 weeks gestational age with HIE who underwent TH at Pediatrix Medical Group NICUs between 2007 and 2015. Neonates were categorized into four groups based on exposure to opioids (fentanyl, morphine) and benzodiazepines (lorazepam, midazolam, diazepam) during TH: unexposed (UE), opioids alone (OA), benzodiazepines alone (BA), and both opioids and benzodiazepines (O+B). We compared characteristics, adverse events (mode and duration of respiratory support, hypotension, and anticonvulsant use), and hospital outcomes at discharge (death and gastrostomy tube use) between the UE group and each of the exposure groups. Sensitivity analyses excluded neonates who died during TH.

Results: 2621 neonates underwent TH in 125 Pediatrix NICUs; 714 UE, 625 OA, 230 BA, and 1052 O+B.

Neonatal characteristics were not significantly different between groups. OA and O+B neonates had longer durations of mechanical ventilation than UE neonates. OA, BA, and O+B neonates had more days of non-invasive respiratory support than UE neonates. Anticonvulsant use was most prevalent in the BA group. The majority of neonates in each group were treated with phenobarbital. OA and O+B neonates were more likely to experience hypotension when compared to UE neonates. Eleven percent of neonates treated with TH died prior to discharge. More neonates in the UE group died than in any other group. Gastrostomy tube use did not vary between groups.

Conclusions: Use of opioids and benzodiazepines during TH was associated with increased respiratory and cardiovascular support. It is unclear how co-morbidities, severity of encephalopathy, routine sedation practices, and center level variance impact this association. Prospective studies are needed to assess if exposure to sedatives and analgesics during TH is an independent predictor of the intensity and duration of physiologic support.

Toddler dietary intake: Results from a multi-site study of low-income families.

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Background & Hypothesis: Dietary patterns established during infancy and toddlerhood tend to persist in childhood. The development of food preferences early in childhood and the prevalence of childhood obesity point to a need for studies examining diet quality during the first 2 years of life, particularly for populations at risk for obesity.

Objective: To evaluate the diet quality of a racially and ethnically diverse sample of 2-year-olds using the Healthy Eating Index (HEI)-2010.

Methods: We used 24-hour dietary recall data collected from 2-year olds (22.5-29.1 months) participating in Greenlight, a low-literacy, cluster randomized trial to prevent childhood obesity at 4 pediatric resident clinics. The scores for each of the 12 components that make up the HEI are derived from recommendations from the USDA Food Patterns. The maximum score of 100 represents compliance with the Dietary Guidelines for Americans. Scores are summarized using medians and interquartile ranges (IQR).

Results: Dietary recalls were collected from 263 caregivers of young children across 4 sites (55.1% Latino, 26.2% Black, non-Latino, 14.5% White, non-Latino, 4.2% Other). The median HEI-2010 score (63.9 IQR 15.1) was significantly (p<0.001) lower than the maximum score of 100. Although component median scores for fruit, dairy and total protein foods achieved the maximum score, median scores for vegetables (1.9 IQR 2.0), greens and beans (0.0 IQR 3.0), whole grains (3.9 IQR 6.5), seafood and plant proteins (0.0 IQR 4.8), unsaturated fatty acids (0.8 IQR 3.2) and sodium (5.5 IQR 5.8) represented inadequate intake. Three percent of toddlers had good diets (HEI>80) and 13% had poor diets (HEI<51).

Conclusions: Despite consuming adequate amounts of fruit, dairy and protein foods, toddlers in this diverse sample are consuming low quality diets as measured by the HEI. Low HEI score is driven largely by inadequate amounts of vegetables, seafood and plant proteins, whole grains, unsaturated fatty acids, and sodium. Further research should work to improve the quality of meals provided to children at increased risk for obesity, which may promote preference for a healthier diet.
Endocrine and metabolic responses to high-intensity aerobic exercise in adolescence

Kelly Mason1, William E. Kraus2,3, Johanna Johnson2, Michael J. Muehlbauer3, and Michael Freemark1,3

Divisions of Pediatric Endocrinology1 and Adult Cardiology2, Duke Center for Living, and the Duke Molecular Physiology Institute1,2,3

Background: Exercise promotes cardiac growth and function to meet the demands of cardiac output at peaks of physical performance. The mechanisms controlling the cardiovascular adaptation to physical activity are poorly understood, but several lines of evidence implicate a central role for growth hormone (GH). GH-deficient adults have reductions in VO2max that are reversed by GH therapy. Moreover, GH-dependent IGF-1 increases cardiac contractility and stroke volume; conversely, IGF-1 receptor knockout mice fail to develop exercise-induced cardiac hypertrophy.

Hypothesis and Objectives: We hypothesized that: (a) exercise-induced increases in GH stimulate increases in free fatty acids (FFA) and ketones, providing energy for increasing cardiac output; (b) the increase in GH is driven by an increase in the gastric hormone ghrelin, a GH secretagogue. To test these hypotheses we measured GH, ghrelin, FFA, ketones, and various other hormones, metabolites and growth factors prior to and after an acute bout of intense exercise in adolescent student-athletes.

Methods: 11 healthy high-school cross-country runners, ages 14-18, underwent a maximal cardiopulmonary exercise test. Blood samples were collected prior to (baseline) and 30 minutes after onset of exercise.

Results: Following intense exercise there were striking increases in cortisol (+42%, p<0.001) and GH (+433%, p<0.001), in association with large (+50-200%, all p<0.01) increases in glycerol, beta-hydroxybutyrate, ketones, and acetyl(C2) carnitine. Insulin levels remained low and glucose levels were minimally (+14%, p<0.05) increased. These findings suggest that up-regulation of cardiac output depends on GH and/or catecholamineinduced lipolysis, fatty acid oxidation, and ketogenesis. In contrast to GH, ghrelin concentrations fell (-18%, p=0.001) during exercise; thus, the rise in GH with intense physical activity was not mediated by an increase in ghrelin. The major correlate of post-exercise GH was the baseline level of insulin [r = (-)0.75, p=0.0009]. With intense activity, there were increases in pyruvate and glutamate, facilitating mitochondrial energy production, and a rise in lactate and alanine, which in combination with glycerol promote gluconeogenesis.

Conclusions: We postulate that a striking rise in GH provides substrates for cardiac energy consumption during intense physical exercise; the rise in GH correlates inversely with baseline insulin but is not induced by ghrelin.

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Neonatal Hypoglycemia: Rapid Assessment Using a Novel Microfluidic Platform.

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Background & Hypothesis: Hypoglycemia is the most common metabolic disorder in newborns and predisposes to seizures and permanent brain injury. Comprehensive evaluation of neonatal hypoglycemia is essential to guide definitive diagnostic tests and effective medical interventions. However, the recommended comprehensive laboratory evaluations cumulatively require large volumes (>3mL) of blood, making diagnostic assessment challenging in newborn, particularly preterm, infants. Moreover, turnaround times for currently available diagnostic assays may exceed 48-72 hours, leading to critical treatment delays.

Objective: To develop a comprehensive, near-patient panel of tests on a digital microfluidic platform for rapid (30-45 min) assessment of severe, persistent hypoglycemic disorders in newborns using microliter volumes of whole blood.

Methods: We developed a panel of 6 rapid assays on a digital microfluidic platform that can simultaneously measure insulin, cortisol, growth hormone (GH), glucose, β-hydroxybutyrate (βOHB) and free fatty acids (FFA) in a small (<50 μl) sample of whole blood and provide diagnostic-equivalent results in less than 45 minutes.

Results: We demonstrate high reliability and precision of each assay on the microfluidic cartridge. Analytes are quantitated in the ranges required to ascertain the root cause of the hypoglycemia (insulin 0.5-30 μIU/ml; cortisol 1-60 μg/dl; GH 0.25-13.5 ng/ml; glucose 10-500 mg/dl; βOHB 0.3-3 mM; FFA 0.1-3 mM); this will facilitate targeted treatment. We are now validating the analytical performance of these assays on the platform and combining all assays to operate simultaneously on the same cartridge. Results obtained with the microfluidic platform will be compared to values obtained in the Duke Clinical Laboratory.

Conclusions: This novel panel of 6 assays on a near patient digital microfluidic platform provides a minimallyinvasive, inexpensive diagnostic tool for rapid, efficient, and accurate assessment of the etiology of severe hypoglycemia in newborns.

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#71 Title: Does obesity increase respiratory tract infections (RTI) and respiratory sequelae in patients with asthma?
Authors: Monica Tang, Rob Henderson, Janet Holbrook, and Jason Lang.

**Background:** Since respiratory tract infections (RTIs) precede most exacerbations, better understanding of the risk factors of RTIs and RTI-associated exacerbations in patients with asthma is a pressing public health need [1-3]. Obesity in patients with asthma is associated with exacerbations and higher asthma-associated healthcare utilization [4-9].

**Objective:** We aimed to determine if obesity increases the risk of self-reported RTIs and related sequelae among adults and children with asthma.

**Methods:** RTIs and RTI-related morbidity from five large asthma trials were analyzed for associations with body habitus, defined as normal weight, overweight and obese based on age-appropriate body mass index (BMI) or BMI-percentile conventions. The primary outcome was rate of visits with RTIs, documented using standardized clinic visit interviews. Secondary asthma outcomes included oral corticosteroids, healthcare contact, and hospitalization (total and RTI-associated for each), and upper respiratory infection (URI) severity (documented using standardized clinic visit interviews as mild, moderate, or severe). We used negative binomial regression on count data, linear regression on continuous data, and chi square testing on categorical data to analyze the effect of body habitus on the primary and secondary outcomes with SAS version 9.4 (SAS Institute Inc., Cary, NC, USA).

**Results:** Body habitus did not affect the rate of visits with respiratory tract infections in children or adults (p=0.6305 and p=0.6100 respectively). In children, body habitus also did not affect the percent of visits with total or RTI-associated exacerbations or URI severity. In adults, obese subjects were more than twice as likely to have visits with exacerbations requiring systemic steroids than lean subjects (rate ratio: 2.0, 95% CI: 1.2 - 3.3, p=0.016). Among adults with a RTI, obese subjects had significantly greater rates of visits with exacerbations requiring systemic steroids (rate ratio= 2.6, 95% CI 1.4-4.9, p=0.004) and asthma-related healthcare contact (rate ratio=1.8, 95% CI 1.2-2.7, p=0.011). Among adults with a URI, body habitus was associated with greater URI severity (p=0.0210).

**Conclusions:** Obesity in asthma does not increase the risk of acquiring RTIs. In adults, but not children, obesity increases the severity of URIs and leads to more RTI-associated asthma exacerbations.

#72 Safety and Efficacy of a higher dose of Alglucosidase Alfa in Early-Onset Forms of Pompe disease

Mrudu Herbert, Laura E. Case, Mugdha Rainkar, Stephanie L. Austin, Lauren Bailey, Stephanie DeArney, Crista Walters, Sarah P. Young, Priya S. Kishnani

**Background & Hypothesis:** Pompe disease is caused by a deficiency of the lysosomal enzyme acid alpha glucosidase (GAA) resulting in glycogen storage within tissues. Enzyme replacement therapy (ERT) with recombinant human GAA (rhGAA) was approved at 20 mg/kg biweekly and has been most beneficial in improving survival and stabilizing or improving the skeletal muscle function in patients with IOPD and late-onset Pompe disease (LOPD). However, it is insufficient over the long term to halt the progression of myopathy in patients. This study is the first systematic report which provides evidence for effectiveness of alternative dosing in Pompe disease based on evolving clinical evidence.

**Methods:** Subjects with Pompe disease who had been treated on a standard ERT dose of 20 mg/kg biweekly and subsequently had a dose increase because of a decline in motor function were recruited to this study. LOPD patients were included if they had symptom onset before age 5 years. Clinical assessments and laboratory evaluations were performed at regular follow-up visits. Motor function was assessed by the six-minute walk test (6MWT) and the Gross Motor Function Measure-88 (GMFM); biochemical markers studied included CK, AST, ALT, and urinary glucose tetrasaccharide (Glc4).

**Results:** Ten subjects with Pompe disease (classic IPD=6, LOPD=4) were included in the study. The median age of the cohort was 13 years (range 8-16 years). Median age at treatment initiation with rhGAA was 0.25 years (range 0.04-0.25 years) in IPD and 3.8 years (range 1.25 -13 years). Safety outcomes: No new infusion-associated reactions were reported at higher doses. Efficacy outcomes: With dose escalation, all but two patients were able to ambulate independently. Improvement in 6MWT distance percent predicted and GMFM scores were reported in all patients at higher doses. Statistically significant reductions in ALT, AST, CK and urine glucose tetrasaccharide (Glc4) were found at higher doses of ERT when compared to the standard dose (p<0.05).

**Conclusions:** In patients with Pompe disease, a higher rhGAA dose of 40 mg/kg weekly or biweekly was found to be safe and resulted in improvements in endurance, strength, mobility, and biochemical markers within12 months. A higher dosing regimen is beneficial for patients with a clinical decline or plateau on a standard dose provided they are immune tolerant. As IOPD survivors are growing older and early-onset LOPD patients reach adolescence and puberty, a well-appreciated challenging scenario in the treatment and management of these patients, a more aggressive treatment with higher doses of rhGAA may be warranted.
Cognition and brain involvement in infantile Pompe disease

Mrudu Herbert, Gail A. Spiridigliozzi, Steven Chen, Mihaela Stefanescu, Stephanie L. Austin, James M. Provenzale, Priya S. Kishnani

Background & Hypothesis: There is limited understanding of central nervous system (CNS) involvement in Pompe disease, a lysosomal storage disorder caused by deficiency of acid-alpha glucosidase. With the availability of enzyme replacement therapy (ERT) for Pompe disease, patients with infantile Pompe disease (IPD) are surviving longer, making an assessment of CNS involvement possible as well as timely. Furthermore, such an assessment is vital to understand disease progression in the CNS, facilitate anticipatory surveillance and institute appropriate rehabilitation measures. This research is the first attempt to link neuroimaging findings in IPD patients with cognitive outcomes.

Objective: This study aims to 1) use neuroimaging to determine the nature of brain involvement in IPD and 2) explore the relationship between cognitive functioning and brain involvement.

Methods: Patients between the age of 5-18 years, with a confirmed diagnosis of Pompe disease and on long-term ERT were included in the study. Brain MRIs were analyzed by a radiologist using T2/FLAIR for white matter changes, myelination changes, neuronal integrity, anatomical abnormalities, and any other significant anomalies. Cognitive assessments were typically performed at same visit by an experienced psychologist; assessed verbal and nonverbal intelligence, memory, language, academic skills, and visual-motor integration. Battery included Wechsler scale and/or Leiter-3 and Peabody Picture Vocabulary Test-4.

Results: Nine patients who met inclusion criteria were recruited into the study; 8 classic IPD, 1 atypical IPD. Nine patients underwent MRI, of which eight completed cognitive testing using age-appropriate scales. Median time between imaging and cognitive testing was 17.5 days (range 1-132 days). MRI images were normal in 4 patients, showed mild hyperintense white matter (WM) lesions in the centrum semiovale were found in 3 individuals and two patients had abnormal periventricular and frontal white matter signal and/or diffuse abnormality of supratentorial white matter. Mean scores for the Wechsler Full Scale IQ Verbal Comprehension Index and Visual-Spatial Index were lower in those with an abnormal MRI compared to those with normal brain in this cohort.

Conclusions: Varying degrees of white matter involvement is present in long-term survivors with IPD. There is evidence that some IPD long-term survivors exhibit a learning disability rather than a decline in overall cognition or an intellectual disability. The association between MRI findings and CNS functioning remains unclear at this point. Longitudinal follow-up of these patients is required to uncover the long-term evolution of white matter changes and presence of any progressive cognitive involvement in this cohort.

Title & Authors: Disrupted Maturation of the Microbiota and Metabolome among Extremely Preterm Infants with Postnatal Growth Failure

NE Younge, CB Newgard, CM Cotten, RN Goldberg, MJ Muehlbauer, JR Bain, RD Stevens, TM O'Connell, JF Rawls, PC Seed, PL Ashley

Background & Hypothesis: Postnatal growth failure is common among extremely preterm infants (EPT; gestational age <28 weeks) and is a risk factor for adverse neurodevelopmental outcomes. We hypothesized that the developing intestinal microbiota influences host metabolism and postnatal growth.

Objective: To compare the fecal microbiota and serum metabolome of EPT infants with postnatal growth failure and appropriate growth.

Methods: Stool and serum samples were collected from EPT infants in the first week of life (wks 0) and weekly once the infants reached full enteral feedings (wks 1-9). Postnatal growth failure was defined as weight below the third percentile at 40 weeks’ postmenstrual age. Fecal 16S rRNA gene sequencing and serum targeted and non-targeted mass spectrometry assays quantified the microbiota and metabolome, respectively.

Results: Infants with growth failure (n=36) had persistently lower diversity of the intestinal microbiota than infants with appropriate growth (n=22) over the course of the study (p=0.002). The microbiota of the growth failure infants was characterized by a high relative abundance of Staphylococcaceae in the early weeks (wks 0-5, p=0.03), followed by a persistent predominance of Enterobacteriaceae (wks 2-9, p<0.01). Infants with appropriate growth had high relative abundance of Veillonellaceae (wks 2-9, p<0.01), Streptococcaceae (wks 3-9, p<0.01), and Peptostreptococcaceae (wks 2-7, p<0.01). We observed similar results in a matched subset of infants (n=38) with similar gestational age, morbidities, diet, and antibiotic exposures. Infants with growth failure had high relative abundance of multiple metabolites including short and medium-chain acylcarnitines, fatty acids, and the ketone body beta-hydroxybutyric acid. We found significant correlations between growth failure-associated microbial clusters and fatty acid oxidation and lipid metabolism pathways. Machine learning algorithms for normal maturation of the microbiota and metabolome composed of 21 bacterial taxa and 9 metabolites revealed a pattern of delayed maturation of the microbiota and metabolome in EPT infants with postnatal growth failure.

Conclusions: EPT infants with postnatal growth failure had disrupted maturation of the microbiota and metabolome characterized by relatively low microbial diversity, enrichment with pathogenic taxa, a paucity of strictly anaerobic bacterial taxa, and a metabolic signature of increased lipolysis and fatty acid oxidation.
#75 Title & Authors: Adaptive changes in tissue cortisol metabolism in obese and diabetic teenagers

Pinar Gumus Balikcioglu, MD, Metin Balikcioglu, PhD, Stuart Alan Chalew, MD and Michael Freemark, MD

Background: White adipose tissue (WAT) expresses the enzyme 11Beta-Hydroxy Steroid Dehydrogenase Type 1 (11β-HSD1), which converts inactive cortisone to biologically active cortisol. Transgenic mice overexpressing 11β-HSD1 in WAT develop obesity, insulin resistance, and glucose intolerance. Some investigations in obese and diabetic adults showed increased 11β-HSD1 activity in WAT, but expression of 11β-HSD1 in obese or diabetic adolescents has not been studied previously.

Objective: We hypothesized that tissue cortisol metabolism would be dysregulated in obese and diabetic teenagers. To that end, we analyzed 24-hour urine samples for metabolites reflecting whole body 11β-HSD1 activity and the activity of 5α reductase, which converts cortisol to 5β tetrahydrocortisol (THF), and allo-THF (5α).

Methods: 24-hour urine samples were obtained from obese adolescents with T2D (n=10) and without T2D (n=31) and from normal weight healthy controls (n=18). Body fat (BF) % was estimated by electrical impedance. Tetrahydrocortisone (THE), THF (5β), Allo-THF (5α), α-cortolone, β-cortolone + β-cortol, α-cortol were assayed using gas chromatography with mass spectrometry. 11-βHSD1 activity was calculated as the ratio of [THE + allo-THF + 0.5x (β-cortolone + β-cortol) + α-cortol] / [THE + α-cortolone + 0.5x (β-cortolone + β-cortol)]. 5α reductase activity was calculated as the ratio of allo-THF/β THF. Differences of means among groups were assessed using multiple linear regression analysis adjusting for age, sex, pubertal status, BF%, diagnosis and sex*diagnosis interaction effect. Statistical significance was set at p≤0.05. Pearson correlation coefficients were calculated to analyze bivariate relationships between 11β-HSD1 activity and BF%.

Results: Age, sex, pubertal status and 11β-HSD1 activity were comparable among groups. The major determinant of 11β-HSD1 activity was BF%, which correlated inversely with enzyme activity (r= -0.50, p<0.0001). The correlation was stronger among males (r= -0.53, p= 0.0051) than females (r=-0.28, p=0.12). Some obese subjects with T2D had high 11β-HSD1 relative to BF%.

Conclusion: In contrast to studies in diabetic adults, we demonstrated that whole body 11β-HSD1 activity correlates inversely with body fat among obese teenagers with and without T2D and normal weight controls. This finding suggests down-regulation of 11β-HSD1 and tissue cortisol production as an adaptive response to weight gain. Loss of this adaptive response might be associated with and may promote the development of T2D in some obese adolescents.

Acknowledgement: Derfner Foundation, 5K12HD043494-14, Department of Pediatrics

#76 Title & Authors: Nonsense Variants in the Gene IRF2BPL are Associated with a Neurodegenerative Course

Rebecca C. Spillmann, Nicholas Stong, Jill A. Rosenfeld, Mary Kay Koenig, Julián A. Martínez-Agosto, Matthew Herzog, Damara Ortiz, Elena Infante, Paul C. Marcogliese, Shinya Yamamoto, Michael F. Wangler, UDN Members, Hugo J. Bellen, Brendan Lee, Stanley F. Nelson, David B. Goldstein, Vandanà Shashi, Loren D.M. Pena

Background & Hypothesis: Whole Exome Sequencing (WES) and web-based identification tools such as GeneMatcher facilitate candidate gene investigation via team science. One such candidate gene, IRF2BPL (Interferon Regulatory Factor 2 Binding Protein-Like), was identified in four individuals with neurodevelopmental abnormalities that underwent WES. Objective: To compare clinical features and molecular data of the patients with variants in the IRF2BPL gene.

Results: The patients are between 6-20 years of age, including one patient who died at 15 years. Two are male and all four are Caucasian and one additionally identified as Hispanic. Early childhood development was normal in 2/4 of the patients, with the other two reporting speech delay. All patients who died at 15 years. Two are male and all four are Caucasian and one additionally identified as Hispanic. Early childhood development was normal in 2/4 of the patients, with the other two reporting speech delay. All patients began to experience neurological regression at 3-14 years of age. Deterioration of gait and loss of intentional movements was the initial presentation in all of the patients. Subsequent symptoms include development of dysphagia requiring G-tube placement (3/4), dysmetria and spasticity (2/4), incontinence (2/4), axial hypotonia (2/4), and a progressive movement disorder with ataxia and dystonia (3/4). A brain MRI was normal between 5-8 years of age in two of the patients, with a third patient having a small cerebellum and corpus callosum anomalies of unclear significance. On a repeat MRI at 14years of age, the oldest patient was found to have developed diffuse cerebral atrophy, with severe volume loss by 20 years of age. Three of the individuals had clinical symptoms or suspicion of seizures, and the majority had abnormal EEGs regardless of clinical symptoms. Molecular data: Three nonsense variants were detected in the four cases (p.E172X, p.Q188X, and p.Q127X) and were de novo in two of the cases in which parents were available for testing. IRF2BPL is a very intolerant gene with an RVIS score of 9.26% and a pLI score of 0.97. All variants are absent from the gnomAD and ExAC control databases, and two of the variants are outside a repetitive region and are well covered in the majority of controls. The CADD score of the variants, > 28.4, places them among the most deleterious. Conclusions: We describe four individuals with an undiagnosed neurological phenotype and nonsense variants in IRF2BPL. Despite variable age of symptoms, there is overlap in the neurodegenerative nature of the disease course, including loss of gross/fine motor and self-help skills, abnormal EEG, clinical seizures, and abnormal movements. The bioinformatics signature of the gene and variants supports pathogenicity: IRF2BPL is intolerant to functional variation, the nonsense variants are absent from the gnomAD and ExAC reference databases, and are among the most deleterious. Additional work in a fly model is ongoing to clarify gene function and potentially inform the clinical phenotype.
#77 Novel phenotype of KCNMA1 de novo gene mutation controlled with stimulants

Rebecca Gibson, Marie McDonald, William Gallentine and Mohamad Mikati

**Background and hypothesis:** There are less than 20 patients reported with KCNMA1 mutations that have presented with developmental delay involuntary dystonic or choreiform movements of the mouth, tongue and extremities, and epilepsy with no information about any effective therapies. Here we describe a patient with a de novo KCNMA1 mutation with the unique presentation of predominantly atonic spells with a remarkable response to dextroamphetamine therapy, both previously not reported.

**Objective:** Report a 23 year old female with a novel KCNMA1 mutation consistent with novel phenotype and response dextroamphetamine.

**Methods:** Case report

**Results:** Patient delayed development during her first 2 years of life saying her first word and walking at the age of two years. At that age she started to experience episodes of falling with loss of tone of the body at times also with some concurrent dystonic posturing. These consisted of suddenly becoming limp and collapsing to the floor for 15-20 seconds x100 or more times/day that may involve laughter, tightened grasp, and in about half of them stiffening of the upper extremities and neck turning. These often were also preceded by prolonged staring. These episodes were not controlled during sequential trials of ethosuximide, valproate, phenobarbital, zonisamide, levetiracetam, temazepam, clonazepam, ketogenic diet trial, gabapentin, carbamazepine, lamotrigine, memantine and modafinil. MRI was negative video EEG showed no changes during multiple recorded spells. Extensive metabolic workup was unrevealing. Whole exome sequence analysis demonstrated a heterozygous for de novo N536H nonsense mutation in the KCNMA1 gene. At the age of 8 years old was placed on dextroamphetamine for hyperactivity after which she exhibited an unexpected remarkable of the spells from about 100 or more per day, to none except 5-10 that would occur the first hour in the morning before taking the medication or when the patient skips. Of note, fasting and caffeine drinks have also helped decreased spell frequency. At the age of 23 she continues to have this remarkable response to dextroamphetamine XR except when she misses doses. On exam, patient has moderate to severe cognitive delay, diffuse mildly decreased tone and scoliosis, and a shuffling crouched gait.

**Conclusions:** Our patient demonstrates a novel KCNMA1 mutation and novel phenotype of predominantly dystonia spells that were very well controlled with dextroamphetamine.

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#78 Title: Close the Loop: Emergency medicine and pediatric physician perceptions of feedback on the diagnostic process.

**Authors:** Rebecca Ruf, MD, Sarah Cohen, MD, Ashley Naughton, MD, Amanda Wessel, MD, James Fox, MD

**Background:** Diagnostic errors account for a significant number of adverse events, patient deaths, and malpractice claims according to a recent report by the Institute of Medicine. Despite accurate diagnosis playing a central role in patient care, there is no systematic process for providing physicians with feedback regarding the diagnostic process. In fact, little is even known about how physicians perceive and seek to improve their own diagnostic accuracy.

**Objective:** As part of a needs assessment, this project sought to improve our understanding of how physicians self-assess their diagnostic accuracy and their perceptions of a proposed system to provide diagnostic feedback.

**Methods:** Emergency medicine, pediatric, and internal medicine-pediatric residents and faculty were asked to complete an online anonymous Qualtrics survey. Participation was voluntary. Descriptive statistics were used to analyze responses. This is an IRB-exempt study.

**Results:** 54% of residents and 72% of faculty responded to the survey. Of those who responded, 91% of residents and 96% of faculty questioned their diagnostic accuracy at least monthly. All residents and faculty reported reviewing patient records after providing patient care. They were surprised about the ultimate diagnosis 33% (range 5-68%), and 26% (range 9-100%) of the time respectively. Aside from their own efforts, physicians received very little feedback on their diagnoses with 88% of respondents reporting never receiving feedback or receiving it only several times a year. 90% of residents and 95% of faculty indicated they would benefit from a systematic method for diagnostic feedback. They identified that incorporating a new process into their workflow and discomfort giving feedback would be barriers to implementation of such a system.

**Conclusions:** Physicians who care for acutely ill children frequently question their diagnostic accuracy yet infrequently receive the important feedback needed to improve their diagnostic impressions. Based on these results, our team is currently developing a system to address this educational gap while minding perceived barriers.
Title & Authors: Pediatric massive transfusion: a systematic review and summary of best-evidence practice strategies.


Background & Hypothesis: Pediatric patients often require massive transfusion protocol (MTP) initiation in a variety of settings ranging from trauma to refractory bleeding in transplant surgery. MTPs in adult populations have been studied extensively, supporting balanced ratio transfusion as a means to improve outcomes. However, due to physiologic differences in hemostasis and variations in mechanism of injury, adult MTPs cannot be extrapolated to pediatric populations. Limited data exists to support an optimal MTP in pediatric patients.

Objective: To review details of care from current pediatric MTPs and summarize best practice strategies.

Methods: PubMed, EMBASE, and Web of Science were searched using MeSH index and free text terms. This yielded articles from years 1946-2017. Articles were independently reviewed by two reviewers. Studies were reviewed for their definition of MT, factors predicting MT, complications of MT, blood product ratios, hemostatic adjuncts, protocol logistics, and clinical outcomes.

Results: From the search and review, 29 articles were identified including 13 retrospective analyses, 4 case series, 4 case reports, 4 review articles, 2 prospective cohort studies, 1 quality improvement study, and 1 survey of transfusion protocols. Seven unique definitions of pediatric MT were reported; the most common was >1 total blood volume within 24 hours, by 35% (10/29) of articles. A collective total of 18,369 patients were included, with 1163 receiving MT (6.3%). Individual reported mortality rates for studies including >1 patient receiving MT ranged 6 to 51% with overall mortality for patients receiving MT in studies reporting mortality at 25.5% (254/996). Of the articles reporting predictors of MT, Greater ISS was most commonly reported by 75% (6/8) of articles. Of the articles reporting complications of MT, the most common was coagulopathy by 60% (6/10) of articles. 45% (13/29) of articles reported a formal MTP or adopted one during the study. 80% (8/10) of the studies reporting their MTP utilized weight-based ratios for PRBC, FFP, and platelet release, with only two sites reporting non-weight based ratios similar to adults. However, all sites that did report blood product release demonstrated large discrepancies in ratios of blood products administered. Although not formally included in the majority of MTPs, rFVIIa was the most commonly reported hemostatic adjunct by 54% (7/13) of studies mentioning adjuncts.

Conclusions: Current practices of pediatric MTP demonstrate a wide variety of site-specific interventions with great variation in outcomes. Many sites differ from adult MTPs in regards to ratios of blood products released. Current literature lacks randomized clinical trials regarding pediatric MTP practices, and more prospective studies of safety, protocol adherence, and clinical outcomes are necessitated.

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Title & Authors: Estimating Radiation Exposure During Pediatric Cardiac Catheterization; A Potential for Radiation Reduction with Air Gap Technique

Chamberlain RC, Shindhelm AC, Fleming GA and Hill KD

Background & Hypothesis: The air gap technique (AGT) is a novel approach to radiation dose optimization during cardiac catheterization where an “air gap” is used in place of an anti-scatter grid to reduce dosing and is untested in children. We hypothesized that the AGT would have differential effects on absorbed radiation dose depending on patient size, with greatest benefit in larger patients and with lateral imaging where scatter irradiation is greatest.

Objective: Compare the simulated effective radiation dosing between standard imaging and air gap imaging techniques during pediatric cardiac catheterization

Methods: Fluoroscopy and cineangiography were performed using a Phillips Allura Fluoroscope on tissue simulation anthropomorphic phantoms ages 0, 5 and 15 years. Testing was first performed using a standard imaging approach (anti-scatter grid removed in the neonate and 5 year; kept in place for the teenager). Images were then repeated using the air gap technique, constructed so as to appear identical to the baseline images. Air Kerma per 1000 frames was measured and input to Monte Carlo simulation software (PCXMC, Amsterdam, Netherlands) to estimate effective dose in millisieverts.

Results: Effective radiation doses for the neonate and 5 year phantom were similar or increased when using the AGT compared to standard imaging for both PA and lateral imaging. In the teenage phantom the AGT reduced effective doses by ~ 1/3rd for fluoroscopy and cineangiography. AGT increased geometric magnification but image blur was not significant for any of the phantom imaging.

Conclusions: The air gap approach is an effective technique for dose reduction in larger patients where scatter irradiation is significantly increased, particularly when higher magnification is needed.
#81 Title & Authors: Evaluating Safety and Ventricular Systolic Function After Autologous Cord Blood Administration in Neonates with Hypoplastic Left Heart Syndrome

Chamberlain RC, Cotten CM, and Campbell MJ

**Background & Hypothesis:** Hypoplastic left heart syndrome (HLHS) is a congenital heart disease with high morbidity and mortality. Safety and efficacy evaluations of nucleated cord blood cell administration in infants with HLHS are limited. We hypothesize that autologous cord blood cells can be safely administered to newborns with HLHS.

**Objective:** Determine safety of autologous cord blood infusion in neonates with HLHS.

**Methods:** Prospective, single institution study of neonates with HLHS to receive autologous cord blood in the peri-operative period, July 2011 to 2015. Randomization was to single or multiple cord blood (1.5 x 10^7 cells/kg fresh, volume and red cell reduced) infusions. Right ventricular systolic function was evaluated by echocardiography, measured by qualitative assessment, ejection fraction and percent area change, retrospectively.

**Results:** Seven patients received pre-operative cord blood infusions within 48 hours of birth; three received a second post-operative infusion. Average gestation was 39 (37-40) weeks with 42.9% male and 85.7% Caucasian. Most (85.7%) patients underwent first stage palliation with Sano modification and all survived to initial discharge. One adverse event, necrotizing enterocolitis, was reported 2 months from infusion and post-discharge. All patients had preserved right ventricular systolic function after cord blood infusion with an average ejection fraction of 62% at hospital discharge.

**Conclusions:** In our cohort, autologous cord blood administration in neonates with HLHS was safe with no deleterious effect on right ventricular systolic function at discharge.

#82 Title & Authors: Inpatient Penicillin Allergy Evaluation Program Enriches Anti-Microbial Stewardship

Renee Kleris MD, Monica Tang, MD, Christina Sarubbi PharmD, Rebekah Wrenn PharmD, Deverick Anderson MD, MPH, Patricia Lugar MD, MS

**Background & Hypothesis:** A national urgency has prompted federal funding for antibacterial resistance leadership groups and antimicrobial stewardship programs. Penicillin skin testing is an important addition to these programs as skin testing can safely identify patients who are no longer allergic and may receive penicillin. Inpatient penicillin allergy evaluations will reduce broad spectrum antimicrobial use and patients who are cleared of their prior allergy will be able to be started on first line antimicrobial agents.

**Objective:** The stewardship allergy assessment team (STAAT) consists of representation from pharmacy, infectious disease, and allergy who offer penicillin skin testing (PST) and evaluation for inpatients with penicillin allergy. One goal of the program is to identify patients who no longer have penicillin allergy and allow for targeted antibiotic selection. This team strives to improve patient care by expanding accessibility to this evaluation.

**Methods:** Healthcare providers place consults to STAAT for inpatients with penicillin allergy. For eligible patients, the evaluation consists of PST (prick and intradermal) with Pre-Pen and Penicillin G and if negative a supervised oral challenge to amoxicillin. Data related to allergy history, indication for consult, test results, changes in antibiotic selection, length of stay, cost and satisfaction with the evaluation are recorded.

**Results:** STAAT has completed a total of 70 penicillin drug allergy evaluations. Indications for testing included: pneumonia, fever of unknown origin, neurosyphilis, bacteremia, intravascular, bone/joint, urinary tract, intraabdominal, and skin/soft tissue infections. 66 (94%) were cleared of previous penicillin allergy after negative skin testing and supervised oral challenge to amoxicillin. Two patients had positive PST and 2 had an equivocal PST. A total of 33 (47%) patients were started on a penicillin or penicillin derivative after clearance of their allergy. Of the 66 patients who were cleared, all (100%) electronic medical records were updated to reflect this updated status.

**Conclusions:** A multidisciplinary approach allows for increased accessibility to penicillin allergy evaluations for inpatients. This collaboration has additionally lead to greater patient and healthcare provider satisfaction.
#83 Title & Authors: Achieving rapid viral control in HIV-infected infants by adjunctive therapy with a novel Hsp90 inhibitor.

Ria Goswami, Holly Heimsath, Riley J. Mangan, Joshua Eudailey, Guido Ferrari, Timothy Haystead and Sallie R. Permar.

**Background & Hypothesis:** HIV-1 transmission via breastfeeding, accounting for ~50% of the 150,000 annual pediatric infections, often goes unrecognized for many months delaying initiation of ART. Delayed ART fails to rapidly suppress viral infection, resulting in the establishment of viral reservoir. HIV-mediated bystander immune activation further plays a significant role in generating new viral target cells, thereby mediating cell-to-cell HIV spread, and increasing reservoir size. Cellular chaperone protein Hsp90 has been shown to be involved in major stages of HIV replication and immune activation. Therefore, we hypothesize that, including an Hsp90 inhibitor in the current ARV regime will achieve rapid virologic control, thereby further limiting establishment of HIV reservoir. A reduced reservoir in postnatally-infected infants will result in their functional cure by lengthening the time to viral rebound upon discontinuation of therapy.

**Objective:** To investigate whether including an inhibitor of Hsp90 in the current ARV regime will achieve rapid viral suppression in a physiologically relevant *in vitro* model of HIV infection of pediatric tonsils.

**Methods:** Mononuclear cells isolated from pediatric tonsillar tissues (n=10-15), were infected with either a CXCR4-tropic (HIVNLGI) or a CCR5-tropic (HIVJRFL) GFP-expressing HIV-1. The infections were treated with a novel inhibitor of Hsp90 (Hs10), along with ARVs. The frequency of productively infected cells was measured by flow cytometry. The 50% cellular infectious dose (CID50) of the HIV infected mononuclear cells was evaluated using a Tzm-bl based reporter assay.

**Results:** Incorporation of Hs10 in the ARV regime resulted in a greater inhibition of HIV replication (HIVNLGI mean inhibition: 92.64% and HIVJRFL mean inhibition: 86.50%), compared to ARV alone (HIVNLGI: mean inhibition 65.48% and HIVJRFL mean inhibition: 76.55%). Hs10 treatment prevented cell-to-cell viral spread by reducing the proportion of bystander activated (CD4+CD25+) T cells by 67.34%, and downregulating CD4+ T cell expression by 99%. Furthermore, Hs10 treatment increased the CID50 of the infected tonsillar cells by 161%, and thereby reduced infectious viral transfer *in vitro*.

**Conclusions:** Our data demonstrate that incorporation of an Hsp90 inhibitor in the ARV regime can result in rapid HIV suppression by reducing bystander T cell activation and altering target cell phenotype. Our next step is to evaluate the effects of this adjunctive therapy on viral dynamics in SHIV-infected neonatal rhesus macaques.

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#84 Dexmedetomidine extraction by the extracorporeal membrane oxygenation circuit: results from an ex vivo study

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**BACKGROUND:** Dexmedetomidine is a sedative administered to minimize distress and decrease the risk of life threatening complications in children supported with extracorporeal membrane oxygenation (ECMO). The ECMO circuit can extract drug and decrease drug exposure, placing the patient at risk of therapeutic failure.

**OBJECTIVE:** This ex vivo study determined the extraction of dexmedetomidine by the ECMO circuit.

**METHODS:** Dexmedetomidine was studied in three closed-loop circuit configurations to isolate the impact of the oxygenator, hemofilter, and tubing on circuit extraction. Each circuit was primed with human blood according to standard practice for Duke Children’s Hospital, and flow was set to 1L/min. Drug was dosed to achieve therapeutic concentrations. Dexmedetomidine was added to a separate tube of blood to serve as a control and evaluate for natural drug degradation. Serial blood samples were collected over 24 hours and concentrations were quantified with a validated assay. Drug recovery was calculated at each time point: Ct/Ci*100, with Ct and Ci the concentrations at time = t and 1 minute, respectively.

**RESULTS:** Dexmedetomidine was highly extracted by the oxygenator evidenced by a mean recovery of 62-67% at 4 hours and 23-34% at 24 hours in circuits with an oxygenator in-line. In contrast, mean recovery with the oxygenator removed was 96% at 4 hours and 93% at 24 hours. Dexmedetomidine was stable over time with a mean recovery in the control samples of 102% at 24 hours (Figure 1).

**CONCLUSIONS:** These results suggest dexmedetomidine is extracted by the oxygenator in the ECMO circuit which may result in decreased drug exposure in vivo. Figure 1. Dexmedetomidine extraction by ECMO circuit configuration. Values are mean and error bars indicate 95% confidence interval.
#85 Title & Authors: Trends in incidence and selected characteristics of pediatric patients with non-accidental trauma in the United States

Sehyr Imran M.D., Chad Cross Ph.D., PStat®, Samrat U Das M.D.

Background & Hypothesis: Intentional injuries play a major role in child mortality and morbidity worldwide. There is a dearth of literature examining trends in the prevalence and outcomes of intentional injuries amongst children, based on nationwide data from the United States (US), especially after 2007.

Objective: Our aim was to study the current epidemiology and socioeconomic factors associated with incidence and outcomes of intentional injuries amongst children in the US.

Methods: We used 2013 - 2014 data from the Nationwide Inpatient Sample, a discharge database representative of all short-term, nonfederal hospitals in the United States. Pediatric patients were identified using the age cutoff of 18 years. International Classification of Diseases (ICD 9) codes for non-accidental trauma (NAT) were used to identify patients discharged with a primary diagnosis of NAT. Trends in the incidence and outcomes of pediatric NAT were compared for different age groups, gender, race and socioeconomic status (SES) based on quartiles (Qx) of median household income.

Results: In 2013 to 2014 there were a total of about 2-3 million pediatric discharges per quartile. Out of these a total of 8985 had a primary diagnosis of NAT. Approximately 40% of the cases of NAT were of patients from families with the lowest SES (Q1). There was a demonstrable trend of increased rate of NAT cases as a function of SES (Q1: 109.1; Q2: 89.7; Q3: 71.9; and Q4: 33.1), and this was generally true across the strata of age groups, gender, ethnicity, and payer type. In-hospital mortality rates demonstrated the same trend (Q1: 2.4; Q2: 1.5; Q3: 1.1; and Q4: 0.4)

Conclusions: Children from low SES households have a higher incidence of NAT and have worse outcomes, including significantly higher in-hospital mortality. This trend is consistent across all age groups and ethnicities. To our knowledge, this is the first report studying the socioeconomic status of children with non-accidental trauma in the United States. While past studies, all before 2008, have shown no difference between abuse and non-abuse groups based on SES, our study of more current epidemiology is strongly suggestive of a link between SES and incidence of NAT. Research to study epidemiology of NAT in more recent years is required to see if this a shift in trend. This is necessary in order to develop appropriate healthcare policies.

#86 Title & Authors: Insight into the phenotype of infants with Pompe disease identified by newborn screening with the common c.-32-13T>G “late-onset” GAA variant

Stephanie Austin, MS, MA; Mugdha V. Rairikar, MBBS, DCH; Laura E. Case, DPT, PCS; Lauren A. Bailey, MS, CGC; Zoheb B. Kazi, MBBS; Ankit K. Desai, MBBS; Kathryn L. Berrier, MS, CGC; Julie Coats, MPT, C/NDT; Rachel Gandy, DPT, PCS; Rebecca Quinones, DPT, PCS; Priya S. Kishnani, MD

Objective: Newborn screening (NBS) has led to early diagnosis and early initiation of treatment for infantile onset Pompe Disease (IOPD). However, guidelines for management of late onset Pompe disease (LOPD) via NBS, especially with the IVS c.-32-13T>G are not clear. This IVS variant is noted in 68-90% cases with LOPD and has been presumed to result in “adult” disease in heterozygosity, with a few cases with earlier onset and a mild to no phenotype in homozygosity. Our study evaluates newborns with LOPD having IVS variant with a diligent multidisciplinary approach to determine if they have an early presentation.

Methods: Seven children with LOPD identified by NBS with IVS variant (3 compound heterozygous, and 4 homozygous) were evaluated with clinical, biochemical (CK, AST, ALT, and urinary Glc4), cardiac evaluation, physical therapy (PT), occupational, and speech/language therapy.

Results: All seven patients demonstrated motor involvement by age 6 months; the three compound heterozygotes had symptoms as neonates. Heterozygous patients had more involvement with persistent hyperCKemia, elevated AST and ALT, swallowing difficulties, limb-girdle weakness, delayed motor milestones, and were initiated on ERT. The homozygous patients had normal laboratory parameters, and presented with very subtle yet LOPD specific signs, identified only by meticulous assessments.

Conclusion: This patient cohort represents the first carefully phenotyped cohort of infants with LOPD with the c.-32-13T>G “adult” variant detected by NBS in the USA. It emphasizes not only the opportunity for early detection of skeletal and other muscle involvement in infants with c.-32-13T>G variant but also a high probability of overlooking or underestimating the significance of clinically present and detectable features. It can thus serve as a valuable contribution in the development of evaluation and treatment algorithms for infants with LOPD.
#87 Title & Authors: Pharmacokinetics of Hydroxychloroquine in Pregnancies with Rheumatic Diseases

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**Background & Hypothesis:** Pregnancies in women with rheumatic disease often result in poor outcomes. Hydroxychloroquine (HCQ) reduces disease activity and flares; however, pregnancy causes significant physiologic changes that may alter levels of HCQ, thereby leading to therapeutic failure. We hypothesized HCQ levels will decline with progression of pregnancy.

**Objective:** The aim of this study was to characterize the population pharmacokinetics (PK) of HCQ in pregnancies complicated by rheumatic diseases.

**Methods:** We performed a population PK analysis using samples collected through the Duke Autoimmunity in Pregnancy (DAP) registry from 2013-2016. We measured HCQ concentration using HPLC/MS and analyzed data using nonlinear mixed effect modeling in Phoenix NLME. We calculated differences between pregnancy and postpartum Empirical Bayesian Estimates (EBEs) using paired t-tests. We computed expected steady state drug concentration profiles for HCQ during pregnancy and postpartum using the individual clinical data and EBEs developed from the final PK model.

**Results:** 145 serum samples were obtained from 50 study patients; 25 of whom had paired pregnancy and postpartum specimens. 5/50 subjects had average concentrations (pregnancy and postpartum) <100 ng/mL consistent with medication non-adherence and were excluded from further analysis. The population estimated apparent volume of distribution (V/F) was 1850 L/70kg and the estimated apparent clearance (CL/F) was 51 L/hr. Compared to postpartum values, the median V increased significantly during pregnancy (p<0.001), whereas CL and AUC24 did not change.

**Conclusions:** A one-compartment population PK model was developed for HCQ in pregnant women with rheumatic disease. Estimates for serum clearance were within the expected range of values for plasma in non-pregnant adults. Because clearance and AUC did not change during pregnancy compared to postpartum, our modeling in this small patient cohort does not support a need to adjust HCQ dose during pregnancy.

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#88 A prediction model to identify patients at high-risk of developing significant anti-drug antibodies: Experience with infantile Pompe disease receiving alglucosidase alfa utilizing acid α-glucosidase variants and HLA-type

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**Background & Hypothesis:** Pompe disease faces the challenges of anti-drug antibodies (ADA) against alglucosidase alfa. Immune tolerance induction (ITI) is a standard of care to prevent these ADA in cross-reactive immunological material (CRIM)-negative infantile Pompe disease (IPD). Approximately 32% of CRIM-positive IPD and a subset of adult patients develop significant ADA but there is no way to predict these patients based on their genotype. Our hypothesis is the immune response is driven not only by amino acid sequences in alglucosidase alfa (T-cell epitopes) that are seen as foreign based on GAA variants but also by major histocompatibility complex (MHC) genotype (HLA-class) which interacts with these T-cell epitopes and presents it to the helper T-cells.

**Objective:** To develop a tool to identify Pompe patients who are at high-risk of mounting an immune response against alglucosidase alfa that leads to development of high and sustained antibody titers.

**Methods:** We identified 24 carefully phenotyped IPD patients who had their GAA variant analysis, CRIM-status, HLA type, and ADA assessed. We classified them into CRIM-positive high-ADA (n=10), CRIM-positive low-ADA (n=9), and CRIM-negative high-ADA (n=5) groups. High-ADA had titers ≥51,200 at ≥6-months on alglucosidase alfa. Therapeutic and endogenous GAA sequences were analyzed using EpiMatrix (in-silico platform for epitope identification and prediction) and neo-epitope content was quantified on a patient HLA-specific basis using a “differential individualized T-cell epitope measure (iTEM)” score. This score reflects all predicted T-cell epitopes within alglucosidase alfa sequence that is foreign to the patient, given their native GAA sequence across both alleles. Association of iTEM scores and ADA titers was assessed. We classified them into CRIM-positive high-ADA (n=10), CRIM-positive low-ADA (n=9), and CRIM-negative high-ADA (n=5) groups. High-ADA had titers ≥51,200 at ≥6-months on alglucosidase alfa. Therapeutic and endogenous GAA sequences were analyzed using EpiMatrix (in-silico platform for epitope identification and prediction) and neo-epitope content was quantified on a patient HLA-specific basis using a “differential individualized T-cell epitope measure (iTEM)” score. This score reflects all predicted T-cell epitopes within alglucosidase alfa sequence that is foreign to the patient, given their native GAA sequence across both alleles. Association of iTEM scores and ADA titers was assessed. We classified them into CRIM-positive high-ADA (n=10), CRIM-positive low-ADA (n=9), and CRIM-negative high-ADA (n=5) groups. High-ADA had titers ≥51,200 at ≥6-months on alglucosidase alfa. Therapeutic and endogenous GAA sequences were analyzed using EpiMatrix (in-silico platform for epitope identification and prediction) and neo-epitope content was quantified on a patient HLA-specific basis using a “differential individualized T-cell epitope measure (iTEM)” score. This score reflects all predicted T-cell epitopes within alglucosidase alfa sequence that is foreign to the patient, given their native GAA sequence across both alleles. Association of iTEM scores and ADA titers was assessed.

**Results:** Patients with iTEM score >10 were classified as “High iTEM”. Patients with high iTEM scores had 52 times higher odds of developing high ADA than patients with low scores (overall agreement 88%, p=0.0005).

**Conclusions:** This model provides a significant improvement over utilizing CRIM-status alone (overall agreement 63%, p=0.053) at identifying patients at high-risk of developing ADA. Given the risk of ADA in other lysosomal storage disorders this model has the potential to identify high-risk patients and guide the optimal implementation of ITI for mitigating these challenges. The quick turnaround time needed for HLA typing also makes it viable to incorporate this tool as part of the management for Pompe patients picked up via newborn screening.
#89 Title & Authors: Cardiac Arrests in the Duke Pediatric Intensive Care Unit-Defining the Problem and Developing Solutions

Candice Ray, MD; Matthew Pizzuto, MD; Heather Harrison, RN; Alicia Espinosa, RN; David A. Turner, MD; Sameer Kamath, MBBS; Kimberly Jackson, MD

**Background & Hypothesis:** Approximately 6,000 pediatric patients receive cardiopulmonary resuscitation (CPR) each year. Only about 46% of these patients who have an in-hospital cardiac arrest survive to discharge, and these patients represent a significant proportion of the annual mortality. At the time of initiation of this project, there was no system in place to track the incidence and outcomes of cardiac arrest occurring in the Duke PICU.

**Objective:** To determine the incidence and outcomes of CPR performed on patients admitted to the PICU at Duke, identify opportunities for improvement in the care of these patients during and after cardiac arrest, and reduce the number of patients requiring CPR.

**Methods:** From January to December 2017, all cardiac arrests in the PICU were tracked and reviewed in detail at a multidisciplinary monthly pediatric cardiac arrest conference. Data points collected included: cause of arrest, time to CPR, quality of CPR including rate and depth, medications administered, rate of return of spontaneous circulation (ROSC), need for Extracorporeal Cardiopulmonary Resuscitation (ECPR), and outcome. A committee was created to provide education on multiple CPR related topics to the all disciplines of the team including nurses, respiratory therapists, residents, PICU fellows, and PICU attendings.

**Results:** There were 22 cardiac arrests in 17 patients (1.7% PICU admissions). The vast majority (88%) of cardiac arrests occurred in medical patients, with the remainder (12%) occurring in trauma/post-surgical patients. Respiratory failure was the most common cause of cardiac arrest (64%). ROSC was achieved in 77% of cardiac arrests and there was only one ECPR case. 8 of 17 patients (47%) survived to hospital discharge. Of those patients who did not survive, the average CPR duration was CPR 24 minutes. There were 5.9 cardiac arrests per 1000 patient days and 15.4 days between cardiac arrest events. 59% of patients had their arrest on the first day of PICU admission, with majority of those patients being admitted from regional referral hospitals.

**Conclusions:** The incidence of cardiac arrest in the PICU at Duke, along with rates of ROSC, causes of arrest, and survival to discharge are consistent the most recently reported national benchmarks in tertiary care centers across the United States. In March of 2018, we will be implementing monthly multidisciplinary educational sessions on CPR related topics such as improving the quality of CPR and increasing awareness of pre-arrest warning signs. Our PICU cardiac arrest committee will continue to track and thoroughly review all cardiac arrests to identify areas for quality improvement initiatives with the ultimate goal of reducing the rate and improving outcomes of patients who experience cardiac arrest in the PICU.

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#90 Title & Authors: Multidisciplinary Handover Standardization to Enhance Transitions from the Emergency Department to Intensive Care Unit

Authors: Stephanie P Schwartz, MD, Emily C Sterrett, MD, MS, W Clayton Bordley, MD, Kyle J Rehder, MD, and Sameer S Kamath, MD, MBBS.

**Background & Hypothesis:** The Joint Commission and World Health Organization recommend using standardized handover processes to reduce error and harm related to care team transitions. Recent literature describes a diverse set of handover types, but there are no published studies or models of handovers between the pediatric emergency department (PED) and pediatric intensive care unit (PICU).

**Objective:** Use Model for Improvement methodology to standardize PED to PICU handovers in our academic institution and improve patient outcomes during the PED to PICU care transition.

**Methods:** Multidisciplinary focus groups and computer-based surveys identified existing and potential failure modes. We ensured high reliability in process changes by structuring small tests of change and using existing workflows. Statistical process control charts of monthly survey data on communication metrics, handover content, patient outcomes, and provider satisfaction for handover reports informed iterative PDSA cycles.

**Results:** 302 patients were admitted from the PED to PICU from October 2016 to November 2017. 202 handovers evaluations were completed (33.4%). Initial test of change revealed a statistically significant improvement in physician presence for bedside handovers. Positive special cause variation was observed in both reports of provider satisfaction and understanding of patients’ conditions and issues after implementation of PED physician presence at PICU bedside during handover. A negative trend in critical content missing from the handover report was canceled after implementing a PICU ‘sterile cockpit’ and scripting the bedside handover.

**Conclusions:** Implementing a standardized bedside handover process for PED to PICU care transitions is feasible. Our results suggest a multidisciplinary and multispecialty bedside team, ‘sterile cockpit’, and checklist handover scripts improve provider satisfaction and handover content.
#91 Title & Authors: Reducing excessive variation in infant sepsis evaluation

Emily C. Sterrett, MD, MS & Ernestina Belt, MD

**Background:** The evolving epidemiology of sepsis among infants over the last few decades has created significant ambiguity for bedside providers leading to incomplete risk stratification, inappropriate test utilization and unnecessary antibiotic exposure and hospitalization of well-appearing infants.

**Objective:** To improve and standardize the care of well-appearing febrile infants age 7-60 days who present to the Duke University Hospital Emergency Department and Inpatient Pediatrics. Specific metrics include: 1) reduce unnecessary antibiotic exposure, 2) reduce unnecessary chest radiographs, 3) increase use of urinalysis for risk stratification, and 4) reduce hospital length of stay for culture-negative infants to less than 30 hours and less than 42 hours for low-risk and high-risk infants, respectively.

**Methods:** We participated in a national improvement collaborative through the American Academy of Pediatrics Value in Inpatient Pediatrics Network. Using Model for Improvement methodology and multi-specialty stakeholder buy-in, we created a standardized approach to risk stratification and hospitalization of well-appearing febrile infants. Work-aids included readily accessible care algorithms, a smart phone application and specific order sets. Project metrics were tracked monthly using p-charts and special cause variation was determined using traditional rules for statistical process control. Performance failures were addressed using provider feedback.

**Results:** One year of baseline data was collected and compared monthly to implementation data starting in December 2016. Special cause variation was noted for 3 of our metrics. Low risk infants aged 31-60 days admitted to the hospital, decreased from 30% to 0%. Low risk infants not receiving antibiotics decreased from 37% to 0%. The proportion of infants for whom a urinalysis was obtained improved from a baseline of 90% to 100%. There was no special cause variation in CXR utilization or hospital length of stay.

**Conclusions:** Multi-specialty consensus on care algorithms empowered providers to use less antibiotics and reduce unnecessary hospitalization. Future work will include implementation of procalcitonin testing for enhanced risk stratification, consistently limiting unnecessary CXRs, decreasing hospital length of stay and spreading our algorithm-directed care to Duke’s regional emergency departments and referring hospitals.

#92 Title & Authors: Shifting the Paradigm: From Physician Burnout to the Workplace

Heather S. McLean MD, Carol L. Stanley MS CPHQ, Jessica Sperling PhD, Megan Gray MSW, Meredith Parker BSW, Tracy Spears MS, Jennifer M. Lawson MD

**BACKGROUND:** Reports of physician burnout have reached alarming rates, and most interventions focus on the individual. Emphasis on burnout distracts from consideration of workplace environment and related drivers as contributors to the potential to thrive and engage. Shifting the focus from physician burnout to the workplace environment is a practical approach towards meaningful improvement work.

**METHODS:** A mixed method study of pediatric physician faculty was used to identify key drivers of workplace satisfaction. Participants completed an anonymous web-based survey that combined the Maslach Burnout Inventory© (MBI) and Areas of Worklife Survey© (AWS) that informed semi-structured focus groups. Two-sided t-tests, descriptive statistics, and data visualization were performed in addition to a qualitative analysis.

**RESULTS:** 155 (91%) of 171 clinical physician faculty completed the survey. Physicians had significantly better average scores than the general population in the depersonalization (1.1 vs. 1.7; p<0.0001) and personal accomplishment domains (4.9 vs. 4.3; p<0.001); there was no difference in the emotional exhaustion domain of the MBI (2.5 vs. 2.3; p=0.0274). The average workload score of the AWS was significantly worse (2.2 vs. 3.0; p<0.0001) than the general population while the community (3.9 vs 3.4; p<0.0001) and value (3.5 vs 3.2; p<0.0001) domains were significantly better. Faculty identified the following drivers of workplace dissatisfaction in focus groups: 1) Excessive and mismatched workload demands; 2) Lack of autonomy and control; 3) Punitive and discordant performance measures; 4) Lack of communication and decision-making input; and 5) Tension between clinical productivity and academic achievement. Rewarding relationships and teamwork were described as positive workplace drivers.

**CONCLUSIONS:** Pediatric faculty experienced multiple workplace concerns in addition to rewarding relationships and teamwork. This study identified opportunities for improvement work at multiple levels. Examples include targeting clinical care redesign, physician compensation plan, and promotion criteria.
#93 Title: Systematic Analysis of Epic BestPractice Advisory Leads to a Dramatic Reduction in Firing with No Clinical Harm

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**Background & Hypothesis:** BestPractice Advisories (BPAs) are an automated feature within Epic designed to notify providers of recommendations for safe care. They are often suspected of firing excessively, leading to alert fatigue. The pediatric “dosing weight” BPA was identified as a likely example, with an average of 617 alerts per day and a recommended action rate of only 6.7%.

**Objective:** The intent of this project was to analyze and reduce firing of the dosing weight BPA while preventing any weight-related drug dosing errors.

**Methods:** This was a collaborative project among the Pediatric Residency Safety Council, DHTS, and Children's Core Safety. Adjustments were made to the BPA parameters according to plan-do-study-act (PDSA) cycles, and outcomes were followed monthly. The primary outcome was number of BPA firings. The secondary outcome was the recommended action rate of the BPA (the rate at which a dosing weight was entered in response to the alert). Balancing measures included SRS reports of weight-related medication errors.

**Results:** Modification of the BPA parameters resulted in a 92.0% reduction in firings and a three-fold improvement in recommended action rate during the first PDSA cycle. Post-intervention, there were three dosing errors for medications selected from order sets, all of which were caught by pharmacists and did not result in patient harm. Thus, the BPA was adjusted in a second PDSA cycle to fire during order sets as well. This resulted in a slight increase in firings but a further improvement in recommended action rate. There were no subsequent weight-related drug errors. Overall, in two PDSA cycles, there was an 88.9% reduction in BPA firings.

**Conclusions:** Clinician feedback and involvement in modifications to electronic medical records can lead to substantial reductions in alert fatigue while still ensuring optimal clinical care.

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#94 Title & Authors: Standardizing Weaning of High Flow Nasal Cannula in the Pediatric Intensive Care Unit – A Quality Improvement Project

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**Background & Hypothesis:** High flow nasal cannula (HFNC) therapy is a specialized apparatus that allows delivery of supplemental oxygen at rates higher than a patient’s spontaneous inspiratory demand. Retrospective studies have suggested that the use of HFNC in infants with bronchiolitis reduces the rates of intubation, and several prospective studies regarding its use in pediatrics are ongoing across the globe. Relatively little evidence exists for standardization of implementation or weaning of HFNC therapy in patients with bronchiolitis. Currently, Duke Children's Hospital and Health Center relies on clinician-led weaning of therapy, rather than a standardized protocol. Given that standardization of inpatient asthma care using the Modified Pulmonary Index Score led to decreased hospital length of stay at Duke, we proposed that a similar system could reduce ICU and hospital length of stay for patients with bronchiolitis.

**Objective:** To develop and implement a standardized protocol for weaning HFNC therapy for children with bronchiolitis with a goal of reducing time on therapy and PICU length of stay.

**Methods:** A standardized weaning guideline for patients with bronchiolitis was developed modeled after that used at Duke based on the Modified Pulmonary Index Score. Data were collected in REDCap and analyzed with Excel and SPSS. **Inclusion criteria:** Children ages 0-24 months of age with a primary diagnosis of bronchiolitis started on HFNC therapy. **Exclusion criteria:** underlying cardiac, neuromuscular, anatomic, or immunologic conditions.

**Primary outcome:** PICU length of stay. **Secondary outcomes:** duration of HFNC therapy, hospital length of stay and duration of mechanical ventilation. **Balancing measures (safety outcomes):** need for escalation of respiratory support in patients weaned by protocol and ICU readmission.

**Results:** Thus far, 7 eligible patients have been enrolled in the project. The mean (±SD) duration of HFNC therapy is 57.4 (±12.1) hours. The mean (±SD) ICU length of stay is 3.4 (±1.1) days and hospital length of stay is 5.0 (±1.0) days. No patients have had escalation of respiratory support, and there have been no ICU readmissions.

**Conclusions:** Based on an initial small sample size, our high flow weaning algorithm has not resulted in any adverse events, escalations of respiratory support, or PICU readmissions. Additional patients are needed to determine if this protocol reduces ICU and hospital length of stay.
### #95 Title & Authors: Work-life balance behaviors cluster in work-settings and relate to burnout and safety culture: A cross-sectional survey analysis

Stephanie P. Schwartz, MD, Kathryn C. Adair, PhD, Jonathan Bae, MD, Kyle J. Rehder, MD, Tait D. Shanafelt, MD, Jochen Profit, MD, and J. Bryan Sexton, PhD

**Background & Hypothesis:** Healthcare is approaching a tipping point as burnout and dissatisfaction with work-life integration (WLI) in healthcare workers continues to escalate. A scale evaluating common behaviors as actionable examples of poor work-life balance was introduced to measure WLI. When aggregated at the work-setting level, individual work-life behaviors constitute a climate where specific behaviors are commonly shared by members of the same work-setting. The work-life climate scale was used as part of a routine safety culture assessment and we hypothesized that healthcare workers reporting better WLI would have better safety culture norms including better teamwork and safety scores and lower burnout scores.

**Objective:** 1. Explore differences in WLI behaviors by role, specialty, and other respondent demographics in a large healthcare system. 2. Evaluate the psychometric properties of the work-life climate scale, and the extent to which it acts like a climate, or group-level norm when used at the work-setting level. 3. Explore associations between work-life climate and other healthcare climates including teamwork, safety, and burnout.

**Methods:** A cross-sectional survey study of US healthcare workers within a large healthcare system.

**Results:** 10,627 of 13,040 eligible healthcare workers across 440 work-setting within 7 entities of a large healthcare system (81% response rate) completed the SCORE safety culture survey in 2016. The work-life climate scale internal consisten
cy was α=.83. Univariate ANOVA demonstrated differences that varied significantly in work-life behaviors between healthcare worker role, length of time in specialty, and work-setting. Respondents working less than 6 months in specialty had the best WLI, and relative to other roles, physicians of all stages had the worst WLI. When aggregated at the work-setting level, RE-ANOVAs revealed both between and within-work setting variance for the work-life climate scale and ICCs reflected clustering – meaning that WLI functions like a group norm, and not just an individual difference. T-tests of top vs bottom quartile work-settings revealed that positive work-life climate was associated with better teamwork climate, safety climate, local leadership, and improvement readiness, as well as lower personal burnout and burnout climate (p <0.001).

**Conclusions:** Problems with WLI are common in healthcare workers. Frequency of imbalance differs significantly based on position and time in specialty. The consistent association with other safety culture domains, opens the door for initiatives to improve teamwork, safety, and burnout by targeting specific WLI behaviors.

### #96 Title & Authors: Pediatric Ambulatory Hypertension Recognition and Management: A Resident Quality Improvement Project

Rachel Randell, MD, Jonathan Pelletier, MD, William Burgon, Michael Smith, MD, Anna Williams, MD, Adam Bensimhon, MD and Carolyn Avery, MD, MHS

**Background & Hypothesis:** Hypertension (HTN) is an increasingly common pediatric health problem. Timely diagnosis and intervention are key to preventing adverse health effects associated with HTN, and yearly screening is recommended by the American Academy of Pediatrics. Despite this recommendation, measured blood pressure values qualifying as hypertension often go unrecognized in the ambulatory care setting.

**Objective:** To determine the diagnostic rate of HTN by providers at an academic ambulatory care center and to determine if rates can be increased with multiple small-scale, multidisciplinary interventions targeting education and systems.

**Methods:** First, diagnostic rates were determined by calculating the proportion of patients with diagnostic codes for HTN or elevated blood pressure out of all patients with recorded blood pressures qualifying as HTN during routine well child examinations at one ambulatory care site. Data were updated nightly through a third-party server. The first Plan-Do-Study-Act (PDSA) cycle targeted resident provider education through a didactic session, disseminated diagnostic algorithm and multiple e-mail correspondences.

**Results:** Out of all clinic visits with a documented blood pressure qualifying as HTN, the proportion with diagnostic coding indicating abnormal blood pressure was 34% prior to intervention. Surprisingly, this proportion decreased to 7% one month after the first PDSA cycle. As a result, a change to the provider note template was made to incorporate a “hard stop” to review blood pressure. Analysis for this cycle is currently underway.

**Conclusions:** Diagnostic rates for HTN were higher than reported in similar settings; however, recognition and diagnosis of HTN remains an area for much improvement. Findings of decreased diagnostic rates after provider education intervention may suggest the importance of other factors, such as systems, to improve timely recognition and management of HTN. A second PDSA cycle implementing a template change is currently underway.
#97 Title: “Who is this patient’s PCP?” - A Quality Improvement Initiative to Improve the Accuracy of the EMR PCP Field

Authors: Rebecca Ruf, MD, Scott Sexton, MD, Carolyn Avery, MD, and Jane Trinh, MD

Background: Many patients seen in the pediatrics and medicine-pediatrics (med-peds) resident clinics do not have an accurate primary care provider (PCP) listed in the electronic medical record (EMR). This is a barrier to continuity of care, hinders the identification of resident patient panels that could be used for quality improvement, and prevents clinical notifications from reaching the appropriate provider.

Objective: To improve the accuracy of the PCP field for patients in the pediatrics and med-peds resident clinics by 50% in 3 years.

Methods: An accurate PCP was defined as a current clinic resident or attending who completed the new patient visit and/or who had seen the patient most frequently for wellness or return visits. Baseline accuracy of the PCP field was assessed using chart review of a random selection of patients seen in the resident clinics in the prior 3 months. Three PDSA cycles were then completed with provider-focused interventions. Chart review was completed after each cycle to assess the efficacy of the intervention.

Results: At baseline, the listed PCP was accurate in 56% of the pediatric patients seen in the resident clinics. Interventions included an email introduction of the QI project to all clinic residents and preceptors, a reminder email to providers, and a subsequent email with individualized data from the previous cycle to each PCP. Chart review at three month intervals between interventions demonstrated improvement in accuracy of the PCP field to 80% initially, and sustained at 75% and 78% in subsequent cycles.

Conclusions: The accuracy of the PCP field for pediatric patients in the resident clinics significantly increased with several provider-focused interventions, and this improvement has been maintained for one year. A more accurate PCP field will enable residents to focus on quality metrics based on their own patient panels. The PDC has established “patients with documented PCP” as a 2019 quality metric. Though the department of pediatrics has met this target, it has acknowledged that accuracy of this field remains an issue. Focused efforts similar to what has been done in the resident clinics could improve accuracy across the health system.

#98 Title & Authors: Title: SALMoN; improving use of a rounding checklist in the Pediatric Intensive Care Unit (PICU).

Sameer Kamath M.D (Pediatrics), Heather Harrison RN (Nursing), Alicia Espinosa RN (Nursing), Travis Heath (Pharmacy) and Kristy Paley (Nutrition Services)

Background & Hypothesis: With the increasing complexity and volume of patients within the PICU, the potential exists to overlook items such as frequency of laboratory tests, medication dose adjustments, need for ancillary services and attention to social issues. Checklists that remind providers of such issues during rounds improve care.

Objective: Improve use of a standardized checklist on every patient every day during rounds from 0% to 90% in six months with the ultimate goal of improving patient care in the PICU.

Methods: We surveyed the PICU team for need of a checklist and identified critical elements that could complement the rounding process in the survey. After deliberation with nursing leadership, we picked a tool focused upon Social Issues, Need for Ancillary Services, Laboratory Tests, Medication Safety, Order review and Nutrition (SALMoN). Key driver diagram was created to strategize implementation of this quality improvement initiative. Residents and nurses were educated about the checklist. Flyers were placed at bedside as reminders. Checklist was included within the resident note template to improve reliability of process. Compliance with checklist was audited through chart review till sustenance was established. Data on compliance was shared with the PICU team in the form of run charts and control charts.

Results: Checklist compliance varied from 52% to 100% for the first 11 weeks after the go live date in February 2017. Several PDSA cycles were used to improve compliance culminating in insertion of the checklist within the resident note template as a high reliability intervention. Compliance improved significantly following this intervention and has sustained at >90%.

Next Steps: Through this simple intervention we hope to show an improvement in laboratory costs, pharmacy costs, timely involvement of ancillary services and nutritional status of PICU patients.
#99 Title & Authors: “Sterile Cockpit”: An initiative for minimizing interruptions during handoff and rounds in the PICU

Sameer Kamath, MD; Kyle Rehder, MD; Jonathan Gehlbach, MD; Alicia Espinosa, RN and Heather Harrison, RN

**Background & Hypothesis:** Handoff represents transition of care from one provider to another and rounds represent a focused review of events/data followed by formulation of a plan of care. Interruptions during handoff or rounds can result in errors and patient harm.

**Objective:** The goal of this initiative was to limit interruptions during handoff and rounds.

**Methods:** Interruptions during handoff and rounds was identified as a priority to improve patient safety by PICU leadership. An educational flyer was created and placed in strategic locations throughout the unit (Figure 1). Signage was placed outside MD workroom and at PICU entrance to remind staff about handoff times. Providers were educated to not interrupt RN handoffs between 7-7:30 AM / PM each day. One provider was identified as the designated contact person (CP) during rounds or handoff. Name and number of CP provided to unit clerk to enable routing of calls and queries. RN’s were encouraged to filter questions through the charge nurse before approaching the CP. Rounds and Handoff were designated as “Sterile Cockpit” to discourage interruptions for non-emergent issues. Audit tool was placed in strategic locations to enable reporting of violations.

**Results:** Non PICU staff were the most frequent interrupters of handoff followed by nurses and then residents. Over time the number of interruptions during handoff and rounds decreased. Staff reported interruptions during handoff more often than those occurring during rounds.

**Conclusions:** Through this quality improvement initiative we reduced interruptions during handoff across all disciplines. In the next phase we will continue maintaining a sterile cockpit during handoff and strive to replicate our success during rounds.

#100 Title & Authors: Variation in analgesia/sedation practice by time of the day in a Pediatric Intensive Care Unit (PICU)

Sameer Kamath, Kyle Rehder, Tammy Uhl, Travis Heath, Caitlin Daley and Ira Cheifetz

**Background & Hypothesis:** Exposure to high dose sedatives and opioids is associated with ICU delirium, drug dependence and other morbidities. Excessive use of sedatives at night disrupts sleep wake cycle, can delay extubation, and may facilitate development of delirium. Two adult studies and one pediatric study done in a cardiac ICU demonstrated increased night time administration of sedatives and opioids in critically ill patients. Diurnal variation in sedation practices in a mixed medical and surgical PICU has not been previously reported. We hypothesized that despite use of a standardized analgesia/sedation protocol, there was diurnal variation in sedation practices in our PICU.

**Objective:** Study the diurnal variation in sedation practices in our PICU.

**Methods:** Single center, retrospective review of prospectively collected data from all PICU patients requiring mechanical ventilation and receiving analgesics and/or sedatives between July 1st 2016 and June 30th 2017. An analgesia/sedation protocol was developed by a multidisciplinary team with the goal of standardizing sedation practices in the PICU. Per protocol, Fentanyl (infusion and PRN bolus doses) was the drug of choice followed by addition of a benzodiazepine (midazolam infusion or lorazepam bolus dosing)) for persistent agitation. Both change in the rate of infusions (fentanyl and midazolam), as well as number of boluses (fentanyl, midazolam and lorazepam) administered, at various time points throughout a 24 hour period were analyzed. Day shift was defined as 7 am to 7 pm and night shift was defined as 7pm to 7 am. Data was provided and analyzed by Duke Performance services.

**Results:** 2057 boluses of sedatives/analgesics were administered with 56% of them given during the day and 44% at night. 4043 changes were made in infusion rates of sedatives/analgesics with 52% occurring during the day and 48% occurring at night. Morphine (46%) was the most frequently administered bolus medication followed by fentanyl (34%) while dexmedetomidine (34%) was the most frequently titrated infusion followed by fentanyl (33%).

**Conclusions:** There was no diurnal variation in administered bolus doses of analgesics or sedatives when using a validated sedation scale coupled with an evidence based, standardized analgesia and sedation order set in our PICU.
#101 Title & Authors: Pressure Injury (PI) reduction at Duke Children’s Hospital (DCH)

Sameer Kamath, Michelle Rice, Tammy Uhl, Aaron Rose, Heather McLean

**Background & Hypothesis:** In 2015, Duke Children’s had 28 hospital acquired pressure injuries (HAPI) with 10 of these injuries, classified as severe harm. The majority of pressure injuries (PI) are preventable, thus providing an opportunity for improvement, and are a key indicator of nursing quality and patient care.

**Objective:** The group was tasked to implement best practice PI prevention bundles across DCH, reduce serious harm PI events, audit unit bundle compliance and increase staff engagement on our journey to zero.

**Methods:** Duke Children’s examined its rate of HAPI, identified units with the highest incidence and assessed gaps in practice. Key components of the project included increasing awareness, optimizing practice and improving data collection. Weekly skin rounds with on the spot education were initiated in high-risk units in 2015 to raise awareness. Nursing practice and care policies were aligned with Solutions for Patient Safety Best PI Prevention Practice, and Skin Care Champions (SCCs) were educated on bundle implementation and process audits. In addition, all PI are reported in RL6 for data collection and tracking, EHR changes were facilitated to improve documentation of bundle elements, and a process audit chart was created to track unit compliance with bundle elements. Finally, a PowerPoint was used to educate SCCs; Skin Boards were created for staff education material (see attachment) and a PI K -card was created with SPS skin care bundle elements to enable audit and ongoing education.

**Results:** Duke Children’s has experienced a statistical shift in both practice and outcome metrics since the start of the project. Pressure injuries overall are identified earlier and severe harm pressure injuries have decreased across Duke Children’s Hospital since 2015. In addition, to a significant reduction in serious harm events, PI bundle compliance is excellent and staff have embraced skin care wholeheartedly.

**Conclusions and Next Steps:** PI reduction is possible through increased vigilance and adherence to best practices. The group intends to spread PI prevention best practices across all patient care units of DCH and help identify best practices to reduce respiratory device related PI in children.

#102 Title & Authors: Improving Pain Management in Pediatric Patients Admitted for Uncomplicated Vaso-Occlusive Episodes.

Amy Lee MD, Julie Childers MD, PhD, Amanda Johnson MD, Ashley Naughton MD, David Noyd MD, Heather Hills PharmD, BCOP, Zachary Long RN, CNIV, CPN, Megan Jordan MD, Heather McLean MD, Jennifer Rothman MD, Kristen Meade MD.

**Background & Hypothesis:** Vaso-occlusive episodes (VOEs) are common and painful complications of sickle cell disease (SCD) that account for the majority of emergency department (ED) visits and hospitalizations. Despite evidenced-based recommendations for management of VOEs, pain control is often delayed and suboptimal, in part due to concerns about potential side effects, provider discomfort with the doses and frequencies of opioids needed to control pain in this population, and insufficient re-assessments and adjustments.

**Objective:** The global aim of our quality improvement project was to increase the efficacy of pain management during the first 36 hours of pediatric inpatient admission for uncomplicated VOE. Our SMART aim was to increase the absolute percentage of patients experiencing a 2-point drop in pain scores at 36 hours by 20% in 6 months.

**Methods:** We created a comprehensive, multi-modal pain management protocol with input from nursing, pharmacy, and physician staff and based on available evidence-based recommendations, and distributed it to all of the pediatric residents. An education session on pain management tools and the pain management protocol was provided at the 1-month mark during noon conference. We performed pre-education and post-education surveys. In addition, we collected data for outcome measures (drop in pain scores from presentation to 36 hours), process measures (time to initiation of orders meeting the protocol), and balancing measures (length of stay, adverse effects, and 7-day and 30-day readmission).

**Results:** There was improvement in resident comfort with managing SCD patients admitted for VOE and improved comfort with using opioids for pain management. There was a decrease in adverse effects of opioids after initiation of protocol. While we did not meet our SMART aim goal, most patients experienced at least a 10% reduction in pain by 36 hours.

**Conclusions:** We suspect that the lack of demonstrable response reflects the complexity of measuring pain management as opposed to lack of efficacy of the protocol. We hope that with continued improvement and use of the VOE-specific standardized pain management protocol, we will be able to enhance care for SCD patients and foster collaborative engagement in promoting patient safety.