Duke Department of Pediatrics
2017 Research Retreat
Tuesday, April 18, 2017
Trent Semans Center

KEYNOTE ADDRESS

Looking Back and Looking Forward -- The FDA and Research in Children

Robert M. Califf, MD
Former Commissioner of U.S. Food and Drug Administration
Donald F. Fortin, MD, Professor of Cardiology, in the School of Medicine
Professor of Medicine, Division of Cardiology

pediatrics.duke.edu
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<th>Time</th>
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<tr>
<td>9-11am</td>
<td>Poster hanging</td>
<td>All submitters, 6th floor</td>
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<td>*Note: Room access is restricted 11-2pm</td>
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<tr>
<td>12-1:30pm</td>
<td>Donor luncheon</td>
<td>Invitation only, 6th floor</td>
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<tr>
<td>2pm-3pm</td>
<td>Platform presentations accepted to upcoming PAS meeting</td>
<td>Classroom 4 (4th floor)</td>
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<td>*Moderated by Dr. Brian Smith</td>
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<td>2:00pm</td>
<td>Dr. Sarah Armstrong - Is the switch to low-fat milk making children more healthy? Associations between milk-fat type consumed and child obesity from the National Health and Nutrition Examination Survey (NHANES), 1999-2014</td>
<td>6th floor</td>
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<tr>
<td>2:15pm</td>
<td>Dr. Mihai Puia Dumitrescu - Safety of Off-label use of Caffeine Citrate in Premature Infants</td>
<td>6th floor</td>
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<td>2:30pm</td>
<td>Dr. Sarah Armstrong - Race, ethnicity, and poverty influences on physical activity in youth from a nationally representative sample, 2007-2014</td>
<td>6th floor</td>
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<td>2:45pm</td>
<td>Dr. Mihai Puia Dumitrescu - Evaluation of Gentamicin-Induced Ototoxicity in Hospitalized Infants</td>
<td>6th floor</td>
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<td>3-5:15pm</td>
<td>Poster viewing - light food and drinks</td>
<td>Authors present at posters, 6th floor</td>
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<td>3:00-5:00</td>
<td>Authors present at posters</td>
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<td>5:15pm</td>
<td>Poster takedown &amp; make way to Learning Hall</td>
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<td>5:25-7:30</td>
<td>Top Abstracts and keynote address</td>
<td>Learning Hall (2nd floor)</td>
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<td>5:25pm</td>
<td>Opening remarks</td>
<td>Dr. Ann Reed</td>
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<td>William Cleland Professor of Pediatrics &amp; Chair, Department of Pediatrics</td>
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<tr>
<td>5:30pm-6:15pm</td>
<td>Keynote Robert M. Califf, MD The Donald F. Fortin Professor of Cardiology</td>
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<td>“Looking back and Looking Forward—the FDA and Research in Children”</td>
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<td></td>
<td>Introduction by Jennifer Li, MD</td>
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<td>6:15pm</td>
<td>Recognition of Top 5 Basic Science abstracts - Rasheed Gbabdegesin, MD, MBBS</td>
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<td>6:20pm</td>
<td>Top Basic Research Abstract Dr. Michael Deel</td>
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<td>The transcriptional co-activator TAZ is a potent mediator of alveolar rhabdomyosarcoma tumorigenesis.</td>
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<td>6:32pm</td>
<td>Recognition of Top 3 QI abstracts - Heather McLean, MD</td>
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<td>6:37pm</td>
<td>Top QI Abstract Dr. Lindsay Terrell</td>
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<td>Improving the Medical Care of Children in Foster Care: An Academic-Community QI Collaborative</td>
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<td>6:49pm</td>
<td>Recognition of Top 5 Clinical Research abstracts - Sallie Permar, MD, PhD</td>
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<td>Top Clinical Research Abstracts</td>
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<td>6:54pm</td>
<td>Dr. Ria Goswami – HIV replication in infant tonsils is inhibited in presence of Hsp90 Inhibitor, Hs10</td>
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<td>7:06pm</td>
<td>Dr. Sherika Hill – ADHD and High-Risk sexual behaviors and consequences in adolescence and adulthood</td>
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<td>7:18pm</td>
<td>Closing Remarks Dr. Coleen Cunningham</td>
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<td>1</td>
<td>Elie Abdelnour, Monisha Sachdev, Marlee Szabo, Arsen Hunanyan, Mohamad Mikati.</td>
<td>No Loss in GAD67-positive and Neuropeptide Y-positive GABAergic Cells in the Hippocampus of Knock-in Alternating Hemiplegia of Childhood Mouse Model</td>
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<td>2</td>
<td>Agboola O, Markert ML, Chen BJ.</td>
<td>Thymus Transplantation for Chronic Graft Versus Host Disease</td>
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<td>3</td>
<td>Yayan Alwaraawrah, Keiko Danzaki, William Eisner, Amanda Nichols, and Nancie Maclver</td>
<td>Diet Induced Obesity Promotes Changes in T cell Metabolism Consistent with Pro-inflammatory Phenotype</td>
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<td>4</td>
<td>Mehrmean Arshad, Raul Salinas, Patrick C. SeedYe</td>
<td>Investigating the role of MprA and its in vivo targeting in Urinary Tract Infection pathogenesis</td>
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<td>5</td>
<td>Agnes S. Chao1, Laura G. Dubois2, Kelly Pegram1, J. Will Thompson2,3,</td>
<td>Molecular Analysis of Oligodendrocytic Oxyysterols in Human Maternal Breast Milk.</td>
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<td>Eric J. Benner1.</td>
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<td>6</td>
<td>HE Cole, D Easterhoff, TC Bradley, TC Gurley, J Pollara, G Ferrari, GD</td>
<td>Development of new methods to genotype Fc-receptors in humans and rhesus macaques</td>
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<td>Tomaras, MA Moody</td>
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<td>7</td>
<td>Keiko Danzaki1, Amanda Nichols, William Eisner, Donte Saucillo, Valerie</td>
<td>Insulin and Insulin-like growth factor 1 as regulators of T cell metabolism and function</td>
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<td>Gerriets, and Nancie Maclver</td>
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<td>8</td>
<td>Michael D Deel, MD; Katherine K Slemmons; Ashley R Hinson, MD; Lisa ES</td>
<td>The transcriptional co-activator TAZ is a potent mediator of alveolar rhabdomyosarcoma tumorigenesis.</td>
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<td>Crose, PhD; Nina Kuprasertkul; Kristianne Oristian; Rex C Bentley,</td>
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<td>MD; Corinne M Linardic, MD PhD</td>
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<td>9</td>
<td>Jenny J. Li, Candy Chen, Margaret E. DeMonia, Katrina K. Slemmons,</td>
<td>Expression of oncogenic RAS in alveolar rhabdomyosarcoma cells leads to oncogene-induced senescence.</td>
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<td>Lisa Crose, Corinne M. Linardic</td>
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<td>10</td>
<td>Maria Dennis1, Joshua Eudailey1, Morgan Parker1, Bonnie Phillips3,</td>
<td>Impact of Maternal IM/IN HIV-Env immunization during pregnancy on postnatal HIV transmission.</td>
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<td>Genevieve Fouda1, Koen Van Rompay2, Kristina De Paris3, and Sallie P</td>
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<td>L.B.M. Dosier, H. Zhu, H. Zhang, G. Palmer, T.J. McMahon</td>
<td>The Role of L-Amino Acid Transporter (LAT-1) in the Hypoxia-Induced Export of Anti-Adhesive S-Nitrosothiols (SNOs)</td>
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<td>13</td>
<td>Sarah Hart-Unger1, Yukitomo Arao2, Katherine J. Hamilton2, Sydney L. Lierz2, David E. Malarkey2, Sylvia C. Hewitt2, Michael Freemark1 and Kenneth S. Korach2</td>
<td>Sex steroids and the pathogenesis of fatty liver disease: analysis of estrogen receptor α mutant mice.</td>
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<td>14</td>
<td>Jay Gupta1, Amy Cheng1, Daniel Y. Joh1, Trenton Dailey-Chwalibog2,</td>
<td>Inkjet Printed Immunoassay and Volumetric Absorptive Microsampling Devices Enable Point-of-Care Protein Hormone and Cytokine Analysis</td>
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<td>Angus M. Hucknall1, Qingshan Wei3, Aydogan Ozcan4, Benjamin Guesdon2,</td>
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<td>Michael Freemark5, Ashutosh Chilkoti1</td>
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<td>Gentzon Hall, Brandon Lane, Megan Chryst-Ladd, Guanghong Wu, Liming Wang, Gina Kovalik, Eugene Kovalik, Robert Spurney, Rasheed Gbadegesin</td>
<td>A Novel Heterozygous Truncating Variant in ATP-binding Cassette A–13 (ABCA13) is Associated with Familial Focal Segmental Glomerulosclerosis</td>
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<td>Harold Leraas MA, Jina Kim MD, Uttara Nag MD, Ehsan Benrashid MD, Jeffrey Lawson MD, PhD, James Otto PhD, Elisabeth Tracy MD</td>
<td>Development of a neonatal swine model for pediatric femoral arterial thrombosis after cardiac catheterization</td>
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<td>18</td>
<td>Hannah Itell, Erin McGuire, Mubeen Mosaheb, Meng Chen, Sallie Permar, and Genevieve Fouda</td>
<td>Development and Application of a Multiplex Assay for the Quantification of Antibody Responses to Common Pediatric Vaccines</td>
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<td>19</td>
<td>CL Jones, LC Armand, TC Gurley, KO Saunders, AM Trama, J Pollara, G Ferrari, GD Tomaras, MA MOODY</td>
<td>Development of a method to phenotype Fc-receptor-mediated antibody binding to immune cells</td>
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<td>20</td>
<td>Amit Kumar1, Claire Powers1, Josh Eudailey1, Elena Giorgi2, David Martinez1, Ayooluwa Douglas1, Lisa Stamper1, Genevieve Fouda1, Erin McGuire1, Feng Gao1 and Sallie Permar1</td>
<td>HIV-1 Transmitted/founder Viruses from Peripartum Transmission are Resistant to Neutralization by Maternal Plasma</td>
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<td>21</td>
<td>Brandon Lane, Gentzon Hall, Megan Chryst-Ladd, Guanghong Wu, Jen-Jar Lin, XueJun Qin, Elizabeth R. Hauser, Rasheed Gbadegesin</td>
<td>Dysregulation of WT1(-KTS) is associated with the kidney-specific effects of the LMX1B R246Q mutation.</td>
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<td>Han-Hyuk Lim, Haiping Yi, Takashi K. Kishimoto, Fengqin Gao, Baodong Sun, and Priya S Kishnani</td>
<td>A pilot study on using rapamycin-carrying synthetic vaccine particles (SVP) in conjunction with enzyme replacement therapy to induce immune tolerance in Pompe disease</td>
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Variable IgG transplacental transfer in the setting of maternal HIV infection

**Uttara P. Nag**, MD1; James C. Otto, PhD1; Christopher C. McCoy1, MD; Harold J. Leraas, BS2; Jina Kim, MD1; J. Chris Brady, BS1; Joseph W. Turek1, MD; Jeffrey H. Lawson, MD PhD1; Elisabeth T. Tracy, MD1

Proteomic Analysis of Changes Induced by Infant Cardiopulmonary Bypass

**Amanda Nichols**, Sivan Cohen, and Nancie Maclver

Effects of Malnutrition on T follicular helper (Tfh) cell and Germinatal Center B cell Development and Function.

**Kristianne M. Oristian**, Lisa E.S. Cross, Rex Bentley, Nina Kuprasertkul, David G. Kirsch, and Corinne M. Linardic

Loss of MST/Hippo signaling promotes tumorigenesis in a genetically engineered mouse model of fusion-positive alveolar rhabdomyosarcoma.

**Jordan Richardson**, Arsen Hunyanan, Ph.D., Adriana Azar, Monisha Sachdev, April Ratliff, Mohamad Mikati M.D.

Impairment in Social Interaction, Memory, and Depression due to Long Term Higher End Doses of Dexamethorphan

**Monisha Sachdev**, Arsen Hunyanan, Elie Abdelnour and Mohammad A Mikati.

Efficacy of Dexamethorphan in Preventing Sudden Unexpected Death in Epilepsy and Spreading Depolarization: A study in the Mashl+-/- knock-in mouse model.

**Tulika Singh**, Holly Heimsath, Josh Eudailey, Dawn Dudley, Matthew Aliota, Christina Newman, Mariel Mohns, Meghan Breitbart, David O'Connor, and Sallie Permar

Zika virus envelope-specific monoclonal antibodies isolated from Zika virus infected rhesus monkeys following primary and secondary virus exposure

**Melody Su**, Cody S. Nelson, Robert Pass, Ravit Boger, Sallie R. Permar

Cytomegalovirus diversity in glycoprotein B subunit vaccinees and placebo recipients

**Tao Sun**, Haiqing Yi, Chunyu Yang, Priya S Kishnani, and Baodong Sun

Sbtd1 plays a dominant role in glycogen transport to lysosomes in liver

**Marlee Szabo**, Elie Abdelnoura, Monisha Sachdeva, Arsen Hunyanana, Mohammad Mikatia

Quantification of Purkinje cells in mouse model of Alternating Hemiplegia of Childhood

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

Lactoferrin and protection against postnatal cytomegalovirus infection in premature infants

**Muhammad H. Alkazemi**, MS, Ruiyang Jiang, MD, Steven Wolf, Gina Marie-Pomann, PhD, J. Todd Purves, MD, PhD, John S. Wiener, MD, and Jonathan C. Routh, MD, MPH

Radical and Partial Nephrectomy in Children and Young Adults: Equivalent Readmissions and Postoperative Complications

**Sarah Armstrong**, MD; Eliana Perrin, MD, MPH; Ashley Skinner, PhD

Race, ethnicity, and poverty influences on physical activity in youth from a nationally representative sample, 2007-2014

**Sarah Armstrong**, MD; Eliana Perrin, MD, MPH; Ashley Skinner, PhD

Is the switch to low-fat milk making children more healthy? Associations between milk-fat type consumed and child obesity from the National Health and Nutrition Examination Survey (NHANES), 1999-2014

**Stephanie L. Austin**, MS1, Rachel D. Torok, MD2, Chaniya Phornphutkul, MD3 Kathleen M. Rotondo, MD4, Anne F. Buckley, MD, PhD5, Gregory H. Tatum, MD2, Stephanie B. Wechsler, MD1,2, Priya S. Kishnani, MD1

PRKAG2 as a Mimicker of Pompe Disease
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<tr>
<td>Deeksha Bali, Haoyue Zhang, Jian Dai, Patricia McCaw, James Beasley, Denise Peterson, Ashlee Stiles, Dwight Koeverl, Marie McDonald, Catherine Rehder, Sarah Young</td>
<td>Diagnostic testing and novel approach to Fabry Disease screening in the US population (&quot;the Program&quot;)</td>
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<td>David Bearl, MD, Robert D.B. Jaquiss, MD, Travis P. Vesel, MD</td>
<td>Indications and Outcomes of Temporary Mechanical Circulatory Support in Pediatric Patients with Cardiac Failure</td>
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<td>Megan W. Berube, Rachel G. Greenberg, Monica E. Lemmon, Carolyn E. Pizoli, Margarita Bidegain, Reese H. Clark, C. Michael Cotton</td>
<td>Multicenter cohort study of opioid use for neonates treated with therapeutic hypothermia for neonatal hypoxic-ischemic encephalopathy</td>
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<td>Herrera TI4, Edwards L1, Malcolm WF1, Smith PB1,3, Fisher K1, Pizoli C2, Gustafson KE1, Goldstein RF1, Cotten CM1, Goldberg RN1</td>
<td>Outcomes of Preterm Infants treated with Hypothermia for Hypoxic-Ischemic Encephalopathy</td>
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<td>Reid C. Chamberlain, M.D., Jonathan H. Pelletier, M.D., Sarah Blanchard, M.D., Christoph P. Hornik, M.D., M.P.H., Kevin D. Hill, M.D., M.S., and Michael J. Campbell, M.D.</td>
<td>Evaluating Appropriate Use of Pediatric Echocardiograms for Chest Pain in Outpatient Clinics</td>
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<td>Jake J. Deines, MD; Jianhong Chang, PhD; Karin Reuter-Reser, PhD, CPNP-AC</td>
<td>Altered Cerebral Blood Flow in Mild Pediatric Traumatic Brain Injury</td>
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<td>Desai AK1, Kazi ZB1, Martin RF2, Terry FE2, Martin WD2, De Groot AS2, Kishnani PS1</td>
<td>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</td>
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<td>Amy C. Gauldtey, Maggie Bromberg, Mark Connelly, Tracy Spears, and Laura E. Schanbar</td>
<td>Parent and Child Report of Pain and Fatigue in JIA: Does Disagreement between Parent and Child Predict Functional Outcomes?</td>
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<td>Jonathan A Gehlbach, MD, Andrew G Miller, RRT-ACCS-NPS, Christoph P Hornik, MD, Ira M Cheifetz, MD</td>
<td>Deadspace to Tidal Volume Ratio as a Predictor of Extubation Success</td>
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<td>Ria Goswami, Riley Mangan, Holly Heimath, Joshua Eudailey, Philip Hughes, Guido Ferrarti, Timothy Haystead, Barton Haynes and Sallie Permar.</td>
<td>HIV replication in infant tonsils is inhibited in presence of Hsp90 Inhibitor, Hs10.</td>
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<td>KG Gray, CM Cotten, RR Clark, RG Greenberg</td>
<td>Safety of Diazoxide for Infants in the Neonatal Intensive Care Unit (NICU)</td>
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<td>Pinar Gusmus Balikcioglu, MD, James Bain, PhD, Michael Muehlbauer, PhD, Thomas L O’Connell, PhD, Stu art Alan Chalew, MD and Michael Freemark, MD</td>
<td>Pathogenesis of Type 2 Diabetes in Obese Adolescents: Metabolites of Serotonin and Mitochondrial Function in 24Hour Urine Samples</td>
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<td>Christine I Ha, Ankit K Desai, Justin Waterfield, Zoheb B Kazi, Stephanie L Austin, Edward H Bossen, Priya S Kishnani, Anne F Buckley</td>
<td>Outside the fiber: interstitial pathology of skeletal muscle in infantile Pompe disease</td>
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<td>Harold J. Leraas, M.A., Jina Kim, M.D., Zhifei Sun, M.D., Uttara P. Nag, M.D., Reed W. Kamyszek, B.S., Henry E. Rice, M.D., Obinna O. Adibe, M.D., Alexandra J Borst, MD, Jennifer A Rothman, MD, Jeffrey H. Lawson, M.D., Elisabeth T. Tracy, M.D.</td>
<td>Postoperative Venous Thromboembolism in Children is Increased in Setting of Cancer or Infection</td>
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<td>Mrudu Herbert, M.D, Priya S. Kishnani, M.D.</td>
<td>c.-32-13T&gt;G mutation in late-onset Pompe disease and associated cardiac manifestations: implications for Newborn Screening</td>
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<td>Sherika Hill, PhD MHA1, Bernard Fuemmeler, PhD MPH2, Christoph Hornik, MD MPH3, Gary Maslow, MD MPH1,3, Gordon Worley, MD3</td>
<td>ADHD and High-Risk Sexual Behaviors and Consequences in Adolescence and Adulthood</td>
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<td>Zoheb B. Kazi1, Ankit K Desai1, Angelika Erwin2, Chris Makris3, Bradley Troxler3, David Kronn4, Seymour Packman5, Marta Sabbadini5, Jean-Marc Nuoffer6, James Weisfeld-Adams7, William Rizzo8, Clarisa Maxit9, Marianne Rohrbach10, Diana Ballhausen11, Katalyn Scherer12, Omar Abdul-Rahman13, Alexandra M. Joseph14, Alison McVie-Wylie14, Susan Richards14, Priya Kishnani1</td>
<td>Prophylactic immune modulation in infantile Pompe disease using low-dose methotrexate induction: A safe, inexpensive, widely accessible, and efficacious strategy</td>
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<td>Julie J. Kim-Chang, MD1, Patricia L. Lugar, MD, MS1, Anjeni Keswani, MD2</td>
<td>The Significance of Anti-FcɛRI Antibodies in Chronic Idiopathic Urticaria: The Role of Autoimmunity in the Differential Response to Treatment.</td>
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<td>Renee Klieris M.D. and Patricia L. Lugar M.D., M.S.</td>
<td>Peri-operative Hypersensitivity Reactions and Anaphylaxis: Outpatient Evaluation and Management</td>
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<td>Elizabeth Kotzen, Gentzon Hall, Megan Chryst-Ladd, Guanghong Wu, Brandon Lane, Shashi Nagaraj, John Foreman, Michael Randles, Rachel Lennon, David Howell, Rasheed Gbadegesin.</td>
<td>Basement membrane nephropathy like phenotype in a family with an ARHGAP24 mutation known to cause familial FSGS</td>
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<td>Effects of breast milk-derived non-broadly neutralizing antibodies</td>
<td>Riley J. Mangan, Jonathon E. Himes, Ria Gosswami, Thomas L. Jeffries, Joshua Eudailey,</td>
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<td>on oral SHIV acquisition and viral reservoir establishment in infant rhesus macaques</td>
<td>Holly Heimssath, Amit Kumar, Quang Nguyen, Carolyn Weinbaum, Margaret Gilbert, Faith Shiro,</td>
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<td>Type 2 Diabetes is Associated with Reduced Metabolic Inactivation of</td>
<td>Mark Miller, MD, Pinar Gumus Balkiccioegl, MD, James Bain, PhD, Michael Muehlbauer, PhD,</td>
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<td>Cortisol due to Increased Whole body 11β Hydroxy Steroid Dehydrogenase Activity in Obese African-American Children</td>
<td>Thomas L O'Connell, PhD, Stuart Alan Chalew, MD and Michael Fremark, MD</td>
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<td>B-cell sensitivity to incretins and glucose in healthy men and</td>
<td>Mark Miller, Cris Slenz, David D’ Alessio</td>
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<td>Proteomic Analysis of Changes Induced by Infant Cardiopulmonary Bypass</td>
<td>Uttara N Nag, MD; Harold J Leraas, BS; Jina Kim, MD; Brian Ezekian, MD; Christopher Reed, MD;</td>
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<td>Does initial response to corticosteroid therapy in MCD/FSGS predict disease recurrence or response to plasmapheresis post kidney transplant? A study by the Midwest Pediatric Nephrology Consortium.</td>
<td>Jeffrey H Lawson, MD; Elisabeth T Tracy, MD</td>
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<td>The Use of Echocardiography for 'Rarely Appropriate' Indications in Pediatric Patients Presenting with Syncope is associated with Increased Cost with Minimal Diagnostic Yield: A Retrospective Cohort Study.</td>
<td>Jonathan H. Pelletier, Sarah Blanchard, Reid C. Chamberlain, Christoph P. Hornik, Kevin D. Hill, Michael J. Campbell</td>
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<td>Infantile systemic hyalinosis, an ultra-rare condition with a well delineated phenotype but no pathophysiologic understanding or treatment</td>
<td>Loren DM Pena, Kelly Schoch, Rebecca Spillmann, Yong Hui Jiang, Allyn McConkie-Rosell, Nicole Walley, Jennifer Sullivan, Camilla Sanders, Members of UDN</td>
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<td>Evaluation of Gentamicin-Induced Ototoxicity in Hospitalized Infants</td>
<td>M Puia Dumitrescu, DK Benjamin, PB Smith, R Clark, D Gonzalez, CP Hornik</td>
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<td>Clinical approach towards the management of infants with late onset Pompe disease identified by newborn screening</td>
<td>Mugdha Rairikar MBBS, DCH; Lauren A. Bailey MS, CGC; Kathryn L. Berrier MS, CGC; Ankit Desai MBBS; Zoheb B. Kazi MBBS; Laura E. Case DPT, PCS; Priya S. Kishnani, MD</td>
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<td>Modality of Primary HIV Disclosure and Association with Mental Health, Stigma, and Antiretroviral Therapy Acceptance in HIV-Infected Tanzanian Youth</td>
<td>Julia Ramos BS, Leonia Laurean BA, Severa Luhanga MPH, Blandina Mmbaga MD PhD, Dorothy Dow MD, MSc-GH</td>
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<td>Electrical Status Epilepticus of Sleep, Continuous Myoclonus and Superrefractory Status Epilepticus in Patients with KCNA2 Mutations.</td>
<td>Monisha Sachdev, Marina Gainza-Lein, Dmitry Tchapijnikov, Tobias Loddenkeper and Mohammad A. Mikati.</td>
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**BOLD** = Presenting Author  
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### Title & Authors: No Loss in GAD67-positive and Neuropeptide Y-positive GABAergic Cells in the Hippocampus of Knock-in Alternating Hemiplegia of Childhood Mouse Model.
Elie Abdelnour, Monisha Sachdev, Marlee Szabo, Arsen Hunanyan, Mohamad Mikati.

**Background & Hypothesis:** Alternating Hemiplegia of Childhood (AHC) is caused by a mutation in the ATP1A3 gene coding for the alpha-3 subunit of the Na/K ATPase, expressed most prominently in GABAergic interneurons. We hypothesize that hippocampal GABAergic cell loss might be the underlying mechanism of increased excitability.

**Objective:** To investigate whether seizure predisposition in a knock-in mouse model (E815K) carrying the most severe mutation causing AHC in humans, could be explained by GABAergic interneuron cell loss in the hippocampus.

**Methods:** Mice brains were collected, sectioned, and stained for GABAergic interneurons with GAD67 and Neuropeptide Y (NPY) markers. Stereological analysis was then done to determine GAD67-positive and NPY-positive cell number in the CA1 region of the dorsal hippocampus. Mean cell counts of the two groups were then compared for significance (Student t test).

**Results:** E815K mice manifested frequent spontaneous seizures and reproduced the hemiplegia and dystonia manifestations of AHC. CA1 cell counts of GAD67-positive cells did not show significant difference between the heterozygous E815K mice (HET, N=4) and wild type WT littermate controls (N=5), (6286±832 cells v/s 6999±1139, p=0.3414). Similarly, CA1 count of NPY-positive cells did not show significant difference between HET and WT (2531±855 cells v/s 2417±728 cells, p=0.8350).

**Conclusions:** The absence of differences in the above investigated GABAergic neuron cell numbers suggests that other mechanisms, neurophysiological or differences in numbers of other cell types or structure, need to be investigated as potential causes of seizure predisposition in this mouse model of AHC.

### Title & Authors: Thymus Transplantation for Chronic Graft Versus Host Disease
Agboola O, Markert ML, Chen BJ.

**Background & Hypothesis:** Graft-versus-host-disease (GVHD) is a common complication of allogeneic hematopoietic cell transplant (HCT). GVHD-related thymic damage results in a loss of thymic negative selection and further anti-host reactivity. GVHD has been classically divided into acute and chronic variants based upon the time of onset using a cutoff of 100 days. The overall incidence of GVHD remains between 30% and 60% and carries approximately a 50% mortality rate. We sought to assess whether thymus transplantation is able to restore thymic function and prevent the production of autoreactive T-cells.

**Methods:** We performed bone marrow transplantation (BMT) on BALB/c mice with bone marrow cells from C57BL/6 (B6) mice using an established chronic GVHD model. Bone marrow cells from B6 mice given to the BALB/c mice were either T-cell depleted (control) or not (induced GVHD group/intervention group). Thymus from newborn, non-BMT BALB/c mice was inserted under the kidney capsule of the intervention group at 4 weeks post-BMT. We then clinically assessed whether thymus transplantation was able to prevent the development of chronic GVHD and increase survival.

**Results:** 100 days BALB/c mice who received thymus transplant at 4 weeks had earlier reversal of clinical GVHD scores and earlier recovery of their body weight (n=14, p=0.008 and p=0.03 respectively). In addition, they showed an increased rate of survival vs GVHD induced control (75% vs 40% respectively, p=0.0043).

**Conclusions:** This preliminary study suggests a novel approach using transplantation of recipient-type thymus at 4 weeks post-BMT may prevent the development of chronic GVHD and increase survival. Further studies are needed to replicate data and explore underlying mechanisms.
#3 Title & Authors: Diet Induced Obesity Promotes Changes in T cell Metabolism Consistent with Pro-inflammatory Phenotype
Yazan Alwarawrah, Keiko Danzaki, William Eisner, Amanda Nichols, and Nancie MacIver

**Background & Hypothesis:** Low-grade inflammation is responsible for many obesity-associated pathologies such as insulin resistance and nonalcoholic fatty liver disease. T cells play an important role in mounting immune response and the modulation of inflammation, and have been identified as a key player in obesity-associated inflammation leading to insulin resistance. We and others have found that altered T cell metabolism can influence T cell differentiation and function; however, it is not yet known if or how obesity influences the T cell metabolic state. We hypothesize that diet induced obesity promotes changes in T cell metabolism leading to increased proinflammatory T cells functions.

**Objective:** To assess the effect of diet induced obesity (DIO) on T cell subpopulations and metabolism in the visceral adipose tissue (VAT) and peripheral lymphoid tissue (spleen).

**Methods:** 3 week old C57BL/6 mice were fed high fat (DIO mice) or regular chow diet (control mice) until they reached 15 weeks or 26 weeks of age. From each mouse, VAT and spleen were collected and T cells were isolated and analyzed by flow cytometry for: T cells subpopulation markers, key metabolic enzyme expression, glucose uptake and cytokine production. In addition, the expression of around 240 enzymes involved in several metabolic pathways were measured in CD4+ T cells by RT-qPCR.

**Results:** Proportions of Treg and Th17 cells were decreased in the VAT of DIO mice. VAT resident CD4+ T cells from DIO mice were found to have increased glucose uptake. Additionally, Treg cells were found to have an increased expression of the glucose transporter GLUT1. The metabolic enzyme qPCR panel showed slight differences in expression between control vs. DIO mice with a tendency toward a decrease in the expression of some enzymes involved in oxidative phosphorylation and fatty acid transport along with an increase in enzymes that positively influence glycolysis in DIO mice.

**Conclusions:** Increased expression of Glut1 and glycolytic metabolism promotes effector T cell (Th1 and Th17) function, but inhibits Treg suppressive capacity. In our studies, we found that obesity seems to steer T cell metabolism towards a glycolytic phenotype that induces inflammation with a decrease in the anti-inflammatory T cells.

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#4 Investigating the role of MprA and its in vivo targeting in Urinary Tract Infection pathogenesis
Mehreen Arshad, Raul Salinas, Patrick C. Seed

**Background:** Gram negative infections are a major cause of morbidity and mortality worldwide. Uropathogenic *Escherichia coli* (UPEC) is one of the most common Gram negative organisms and the major cause of urinary tract infection (UTI). Over the past decade, UPEC antibiotic resistance against all of the commonly used outpatient antibiotics has rapidly increased. This underscores the critical need to investigate novel antimicrobial strategies. Motivated by studies in our laboratory and others demonstrating the importance of polysaccharide encapsulation for UPEC virulence, we recently identified a highly active small molecule inhibitor of UPEC capsule biogenesis, referred to as DU011. Preliminary studies suggest that DU011 binds to MprA, a widely conserved xenobiotic receptor and transcriptional repressor of an MDR efflux pump, MprA is required for UPEC capsule expression and a knockout of *mpfA* in UPEC renders those bacteria avirulent in a murine sepsis model. We hypothesize that MprA also plays a role in UTI pathogenesis through its regulation of the *E. coli* polysaccharide capsule

**Methods:** Groups of six week old female C3H/HeN were infected with isogenic strains of GFP-labelled UTI89, Δneu (capsule knockout strain), ΔmprA and complemented ΔmprA. Animals were inoculated with ~2 × 10⁷ bacteria and sacrificed at 48 hrs. Bladder and kidney samples were homogenized and plated.

**Results:** Animals infected with Δneu or ΔmprA have a significantly lower bacterial counts in the bladder compared to wild type UTI89 (p-value 0.007 and 0.03 respectively). There was no significant difference in bladder counts between wild type UTI89 and ΔmprA complemented strain. Interestingly, bacteria were isolated from the kidneys of all animals and there was no significant difference between the isolates. However, this may be a limitation of our animal model.

**Conclusions:** These studies have provided proof-of-concept that in vivo targeting of mprA results in reduced bacterial virulence and increased host immune system mediated bacterial clearance from the bladder. Future studies will include investigating the effect of treatment with DU011, a small molecule inhibitor of *E. coli* capsule known to target MprA, on transurethral infection by UTI89.
#5 Molecular Analysis of Oligodendrogenic Oxysterols in Human Maternal Breast Milk.

Agnes S. Chao1, Laura G. Dubois2, Kelly Pegram1, J. Will Thompson2,3, Eric J. Benner1. 1Division of Neonatology, Department of Pediatrics; 2Duke Proteomics and Metabolomics Shared Resource; 3Department of Pharmacology & Cancer Biology, Duke University Medical Center, Durham, NC 27710 USA.

Background: Perinatal white matter injury is the leading cause of neurodevelopmental deficits in survivors of premature birth. There are no treatment options available. A significant challenge to the development of novel therapeutic strategies in neonates is the appropriate concern for safety. We discovered that the naturally occurring oxysterol, 20-aHydroxycholesterol (20HC), can promote oligodendrocyte production from postnatal neural stem cells in vitro and rescue perinatal white matter injury in mice. Here, we investigated the oxysterol content in human maternal breast milk. Our analysis did not detect 20-ahydroxycholesterol (20HC) in breast milk but did identify four breast milk-associated oxysterols. Breast milk-associated oxysterols were then tested in vitro for their ability to promote oligodendrogenesis.

Objective & Hypothesis: We hypothesize that breast milk contains specific oxysterols that promote oligodendrogenesis similarly to 20HC.

Methods: We obtained human maternal breast milk from 12 healthy donors at Duke University Medical Center (DUMC). These samples were produced by mothers who recently delivered healthy full term infants. In addition to freshly pumped milk from donors, we also analyzed human milk from the donor breast milk bank. All samples were analyzed by liquid chromatography tandem mass spectrometry (LC-MS/MS) to determine the concentration of cholesterol, 20HC, 22HC, 24HC, 25HC, and 27HC. Primary neural stem cells were then exposed to breast milk-associated oxysterols and allowed to differentiate for 18 days. Following differentiation, oligodendrocyte production was analyzed by immunohistochemistry and western blotting for CNPase or myelin basic protein.

Results: We identified four oxysterols (22HC, 24HC, 25HC, and 27HC) in human breast milk from full term mothers and donor bank samples. Breast milk-associated 22HC, 24HC, and 25HC induced oligodendrocyte production from neural stem cells in vitro similarly to 20HC. 27HC failed to induce oligodendrocyte production in vitro.

Conclusions: Oxysterols are present in human maternal breast milk suggesting that neonatal exposure may be safe. Systemic administration of breast milk-associated oxysterols following perinatal white matter injury may be further developed into a novel and safe therapeutic strategy to mitigate perinatal brain injury.

#6 Title & Authors: Development of new methods to genotype Fc-receptors in humans and rhesus macaques

HE Cole, D Easterhoff, TC Bradley, TC Gurley, J Pollara, G Ferrari, GD Tomaras, MA Moody

Background & Hypothesis: Antibodies have antigen binding regions (Fab domains) and constant regions (Fc domains)—both regions can be important for antibody function. The interaction of antibodies with cellular effectors is usually mediated by binding of antibody Fc with cell surface receptors (FcRs) that vary by their intrinsic affinity for different isotypes and subclasses. Polymorphisms of FcRs can affect antibody binding, leading to different functional activities by effector cells. While there are described polymorphisms in both humans and macaques, it is unclear if the total diversity of polymorphisms in either population has been defined. Objective: To develop new methods for genotyping FcRs in humans and macaques. Methods: Single-gene PCR and next generation sequencing (NGS) was performed on human and rhesus macaque samples. Results: Single-gene PCR on human samples was able to detect polymorphisms of CD16 and CD32 that are known to affect Fc-FcR interactions. This method is being adapted to an NGS format to allow for higher throughput and to allow discovery of new alleles in humans. Single-cell PCR on rhesus samples is more variable, suggesting that the diversity of genes in macaques is higher than in humans. Conclusions: Genotyping of human FcRs to date has focused on a small number of alleles known to have biological effects, potentially skewing our understanding of the diversity of these genes in the population. NGS-based methods will allow for detection of known and unknown polymorphisms, with the potential to discover new alleles. Similarly, rhesus macaque genotyping is limited by known polymorphisms and genetic sequences. To bypass this problem, we will perform NGS-based whole-genome sequencing of macaques that have detectable alleles using annotated primer sets and those that have unknown FcR alleles, to determine if variation in primer binding regions is affecting the results. This method will be applied to Indian-origin, Chinese-origin, and hybrid rhesus macaques, to determine the degree of diversity present in animal populations commonly used for HIV-1 and other pathogen studies.
Background & Hypothesis: TAZ is required for transit through the G2/M phase of the cell cycle, opposes aRMS myogenic differentiation, and supports aRMS cancer cell stemness. Preclinical studies revealed decreased cell and tumor growth with porphyrin compounds alone and synergistically in combination with vincristine. Conclusions: Our work demonstrates that TAZ is important in aRMS tumor biology. Genetic and pharmacologic inhibition of TAZ abrogates aRMS tumor growth. While PF is currently not therapeutically tractable, we hypothesize that targeting TAZ may attenuate PF activity and could be a promising novel target for treating this aggressive sarcoma.

#8 Title & Authors: The transcriptional co-activator TAZ is a potent mediator of alveolar rhabdomyosarcoma tumorigenesis. Michael D Deel, MD; Katherine K Slommons; Ashley R Hinson, MD; Lisa ES Crose, PhD; Nina Kuprasertkul; Kristianne Oristian; Rex C Bentley, MD; Corinne M Linardic, MD PhD

Background & Hypothesis: Despite increasing evidence that the transcriptional co-activator TAZ conveys stem-like characteristics to adult epithelial cancers, the role of TAZ in sarcomas is poorly understood. Alveolar rhabdomyosarcoma (aRMS) is a pediatric soft tissue sarcoma driven by the signature PAX3/7-FOXO1 (PF) chimeric transcription factor. Survival for aRMS is <30% at 5 years, and there are no therapies that target the transcriptional programming of PF. Given that TAZ is a co-activator of wildtype PAX3-mediated transcription, and that TEADs (the main binding partner for TAZ) are among the top enriched transcription factor motifs in PF binding sites, we hypothesized that TAZ serves as a PF co-activator, and interrogated the role of TAZ in aRMS tumorigenesis. Objective: To define the requirement for TAZ in aRMS tumorigenesis utilizing in vitro and in vivo genetic and pharmacologic approaches. Methods: After determining in NCI Oncogenomics data sets that TAZ is upregulated in human aRMS transcriptomes, we evaluated whether TAZ is also upregulated in a microarray from our previously published model of PF transcriptional changes. To assess TAZ abundance in human aRMS tumors, we performed immunohistochemical staining of 64 human aRMS samples from tissue microarrays (TMAs) obtained from the COG. Next, we examined TAZ loss-of-function using two independent, lentivirally-delivered, stable or conditionally expressed TAZ shRNAs to interrogate the role of TAZ in supporting aRMS tumorigenesis. Finally, we performed pharmacological studies using porphyrin compounds, which have been shown to interfere with TAZ/TEAD transcriptional activity.

Results: Genetic and pharmacologic approaches revealed high nuclear TAZ expression in aRMS tumors. TAZ suppression decreases aRMS cell growth and proliferation, and decreases tumor growth and prolongs survival in murine xenografts. Median survival was 17 days in control groups and 31 days in the two shRNA groups (p=0.006, p=0.041). TAZ is required for transit through the G2/M phase of the cell cycle, opposes aRMS myogenic differentiation, and supports aRMS cancer cell stemness. Preclinical studies revealed decreased cell and tumor growth with porphyrin compounds alone and synergistically in combination with vincristine. Conclusions: Our work demonstrates that TAZ is important in aRMS tumor biology. Genetic and pharmacologic inhibition of TAZ abrogates aRMS tumor growth. While PF is currently not therapeutically tractable, we hypothesize that targeting TAZ may attenuate PF activity and could be a promising novel target for treating this aggressive sarcoma.
#9 Title & Authors: Expression of oncogenic RAS in alveolar rhabdomyosarcoma cells leads to oncogene-induced senescence

Jenny J. Li, Candy Chen, Margaret E. DeMonia, Katrina K. Slemmons, Lisa Crose, Corinne M. Linardic

Background & Hypothesis: Rhabdomyosarcoma (RMS) is the most common pediatric soft tissue sarcoma. The two predominant histologic variants of RMS, embryonal and alveolar rhabdomyosarcoma (eRMS and aRMS, respectively), have very different clinical behavior and molecular makeup. eRMS is associated with an intermediate prognosis and has a five year survival rate of >80% while aRMS is more aggressive with a 5-year survival rate below 40%. eRMS frequently has multiple genetic alterations, including mutations in RAS isoforms and TP53, whereas aRMS has a more simple genetic karyotype and is driven by chromosomal translocations resulting in PAX3-FOXO1 or PAX7-FOXO1 fusion oncoproteins. RAS is a well-known oncogene commonly mutated in human cancers including eRMS, where RAS mutations are associated with higher tumor grade. However, mutations in RAS genes are rarely found in aRMS. Therefore, we hypothesize that oncogenic RAS is either unnecessary or detrimental to the formation and progression of aRMS.

Objective: To explore the role of RAS in aRMS.

Methods: Oncogenic HRAS, wild type HRAS, dominant negative HRAS, and four different RAS effector activating mutants were stably expressed in human RMS cell lines using retroviral constructs. Phenotypic changes in these generated cell lines were examined using manual cell counting, immunoblotting, and BrdU and differentiation assays. Drug studies using pharmacological activation of AKT and ERK, two of the downstream effector pathways of RAS signaling, are in progress.

Results: We found that while ectopic oncogenic HRAS expression was tolerated in the human eRMS cell line RD, it was detrimental to cell growth in the human aRMS cell lines Rh28, Rh3, and Rh30. Growth inhibition was mediated by oncogene-induced senescence and associated with increased RB pathway activity and expression of the cyclin-dependent kinase inhibitors p16 and p21. Activation of AKT and ERK, downstream effectors of RAS signaling, both contributed to this growth inhibition.

Conclusions: This work suggests that oncogenic RAS has fundamentally divergent roles in eRMS and aRMS, and may provide insight into the differential origins and therapeutic requirements for these RMS subtypes.

#10 Title & Authors: Impact of Maternal IM/IN HIV-Env immunization during pregnancy on postnatal HIV transmission. Maria Dennis1, Joshua Eudailey1, Morgan Parker1, Bonnie Phillips3, Genevieve Fouda1, Koen Van Rompay2, Kristina De Paris3, and Sallie Permar1. 1DHVI, DUMC, Durham, NC, USA; 2California National Primate Research Center, University of California, Davis, CA, USA; 3Dept of Microbiology and Immunology, UNC, Chapel Hill, NC, USA

Background & Hypothesis: Despite the proven effectiveness of antiretrovirals (ARVs) preventing mother-to-child-transmission of HIV-1, 150,000 pediatric HIV-1 infections occur annually, largely in the postnatal period when maternal ARV adherence is at its lowest. We previously defined a combined intramuscular (IM)/intranasal (IN) maternal vaccine regimen that elicited strong, functional systemic IgG responses and robust milk IgA responses in hormonally-induced lactating monkeys. Our hypothesis is that these responses may be sufficient for protection against infant virus acquisition. Objective: In the current study we test whether the maternal antibody (Ab) responses elicited by IM/IN HIV-Envelope (Env) immunization during pregnancy and lactation will provide passive protection to infants in utero and via breastfeeding against postnatal virus challenge. Methods: We employed an IM MVA prime/combined IM/IN Env protein boost strategy in uninfected pregnant monkeys starting in the second trimester of pregnancy using MVA expressing the clade C transmitted/founder Env 1086.C gp120 or RSV-F protein (control group). All infants were orally challenged starting at 6 weeks of age in a weekly low-dose oral SHIV challenge model with the clade C tier 2 SHIV1157ipd3N4, isolated from an HIV-infected Zambian infant. The concentration of IgG/IgA Env-specific Abs, epitope specificity and breadth, tier 1/2 neutralization, and ADCC activity was measured longitudinally in both maternal and infant plasma as well as breast milk. Results: Similar to the previous study, this immunization strategy elicited robust IgG/IgA Env-specific Abs, which bound to known important immunodominant epitopes (V2, V3) and exhibited cross-clade breadth, mediated tier 1 virus neutralization and ADCC activity against the SHIV challenge virus, and were adequately transferred across the placenta (median: 149%; 17-384%). Despite the transfer of these maternal IgG responses, there was no significant difference between vaccine groups in the number of infants infected, challenges to infection, or peak viral load. Interestingly, a small number of animals in both groups were resistant to infection, and further assessment of innate factors is being evaluated to determine if there is any predictor of oral SHIV susceptibility. Conclusions: Maternal immunization and Ab transfer alone did not mediate protection, suggesting that active HIV vaccination or passive immunization with broadly neutralizing Abs of the infants will likely be required to protect infants against HIV-1 acquisition.
#11 Title & Authors: The Role of L-Amino Acid Transporter (LAT-1) in the Hypoxia-Induced Export of Anti-Adhesive S-Nitrosothiols (SNos)

**Background & Hypothesis:** Red blood cell hemoglobin forms S-nitrosothiols (SNOS) from precursor and nitric oxide (NO) or nitrite and the SNO can be exported, regulating vascular tone. However, the conduit by which SNOS leave the RBC has not been identified. RBC adhesivity to vascular endothelial is increased in a SNO deficient state. We hypothesize that the membrane L neutral amino acid transporter (LAT1) is a key participant in the export of SNO from the red blood cell and by inhibiting export of SNO from the red blood cell will increase red blood cell adhesion to human umbilical vein endothelial cells. Additionally, addition of an extracellular small molecule NO donor can rescue this increased adhesion.

**Objective:** We investigate the role of the system L neutral amino acid transporter (LAT) in SNO export.

**Methods:** We examined the role of LAT1 in the hypoxia-induced export of SNOS from RBCs, and the influence of LAT1 on RBC adhesivity to endothelial cells in vitro and in vivo. We used a graduated-height flow chamber to determine the percentage of RBCs that were adherent to human umbilical vein endothelial cells (HUVECs) under variable shear stress conditions. For in vivo, testing we investigated effects of transfusion of RBC treated with LAT1 inhibitor in a mouse imaging model.

**Results:** The LAT1 inhibitor JPH-203 markedly attenuated the export of SNOS from RBCs in hypoxia. RBCs treated with JPH 203 were excessively adherent to human umbilical vein endothelial cells (HUVECs) in a flow-chamber model, and this adhesion could be overcome with low micromolar (10 μM) S-nitrosocysteine (CSNO). In nude mice anesthetized with isoflurane and transfused with labeled human RBCs exposed to the LAT1 inhibitor, there was increased sequestration of the human RBCs within microvessels in an intravital microscopy model, as compared to that after transfusion of control, vehicle-treated RBCs.

**Conclusions:** In summary, these results point to a role for LAT1 in the export of SNOS from RBCs. The basal export of RBC SNOS appears to limit RBC adhesion to the endothelium in vitro and in vivo. These findings have implications for conditions and diseases in which RBC function is known to be dysregulated, including pulmonary arterial hypertension, sickle cell disease, sepsis, and the storage of RBCs for transfusion.

#12 Title & Authors: Variable neutralization sensitivity of infant transmitted/founder and maternal HIV-1 viruses to paired maternal plasma
Ayooluwa O. Douglas, David R. Martinez, Joshua A. Eudailey, Amit Kumar, Feng Gao, and Sallie R. Permar

**Background & Hypothesis:** Each year, more than 150,000 infant HIV-1 infections occur via mother-to-child transmission (MTCT). While antiretroviral treatment (ART) strategies have been highly effective at reducing MTCT risk, it is likely that additional immune based strategies will be required to achieve the goal of an HIV-1 free generation. In the absence of ART prophylaxis, only 30-40% of infants born to HIV-1 infected mothers become infected. Furthermore, only one to a few viral variants in mothers are transmitted to each infant, suggesting that there is a selective bottleneck in the setting of MTCT as observed in sexual transmissions. This suggests a possible role of maternal factors, such as maternal humoral immune responses, in preventing the transmission of HIV-1 to infants. **Objective:** To define the phylogenetic relationships between maternal viral population and infant viruses that established infection, transmitted/founder (T/F) viruses, and to define the role of maternal autologous virus neutralizing antibodies in potentially selecting for neutralization resistant infant T/F viruses. **Methods:** Using single genome amplification (SGA), eight infants were screened for homogeneous HIV-1 full length env gene populations. From eight infants screened, four infants with homogeneous virus populations were used to define T/F viruses. Full length HIV-1 env genes were amplified from these 4 mother-infant pairs from the historic Woman and Infant Transmission Study (WITS), an observational cohort of HIV-infected women followed prior to the availability of ART therapy. The env gene products were transfected into 293T cells with an SG3 backbone. Mother-infant viruses were tested in a TZM-bl neutralization assay. **Results** Of the four maternal-infant pairs, three of the infants were infected with 1 T/F virus and one infant was infected with 2 T/F viruses. The mean inhibitory dilution (ID50) by maternal plasma against infant T/F viruses was 47.2 (range 20 - 126). 2 of 5 infant T/F viruses had similar sensitivity to maternal plasma compared to maternal non-transmitted viruses (ID50 = 20-21.1). 3 of 5 infant T/F viruses were more neutralization sensitive to maternal plasma compared to maternal non-transmitted viruses (ID50 = 26, 43, and 126 for TFs; mean of 20, 40.6, and 92 for non-transmitted variants). We have also isolated 19 monoclonal antibodies (mAbs) from a transmitting mother whose infant TF virus was relatively resistant to maternal plasma neutralization (ID50 = 21). **Conclusions:** Our preliminary data suggest that T/F viruses have variable neutralization sensitivity to maternal plasma. Future studies will investigate the neutralization sensitivity of infant T/F viruses to maternal mAbs that neutralize HIV-1.
Sex steroids and the pathogenesis of fatty liver disease: analysis of estrogen receptor α mutant mice.

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**Background & Hypothesis:** Fatty liver disease (NAFLD) is a common complication of insulin resistance and the metabolic syndrome and is now the most frequent cause of chronic hepatic failure in obese adolescents. The propensity to liver fat deposition is controlled by gonadal steroid action: NAFLD is less common in teenage girls than boys and more common in estrogen-deficient states like Turner syndrome. Conversely, excess androgens promote hepatic lipogenesis in females and, together with visceral adiposity and insulin resistance, may explain the higher rates of fatty liver disease in teenagers with polycystic ovary syndrome. **Objective:** The mechanisms by which estrogen protects against liver fat deposition and the site(s) of estrogen action are currently unclear. We used novel mouse models to explore the roles of estrogen and testosterone signaling in the pathogenesis of hepatic steatosis. **Methods:** We analyzed liver fat deposition and gene expression in global estrogen receptor (ER)α knockout mice (αERKO) and liver-specific ERα knockout mice (LERKO) fed high fat diets (HFD). Hepatic steatosis was assessed by scoring macrovesicular and microvesicular steatosis as by measuring serum ALT levels. We analyzed serum testosterone to assess its correlation with hepatic steatosis and insulin sensitivity. **Results:** Steatosis was greater and ALT levels higher in wild-type (WT) males than females. Body weight, liver fat accumulation, and serum ALT levels were far greater in HFD-fed global αERKO females than in HFD-fed WT controls. In contrast liver-specific LERKO females did not accumulate excess liver fat and had normal ALT levels. Insulin resistance and glucose intolerance developed in αERKO females but not LERKO females. Microvesicular steatosis and ALT levels in αERKO females were associated with a rise in serum testosterone, and microarray analysis showed up-regulation of dihydrotestosterone action in livers of female αERKO mice. **Conclusions:** ERα-mediated transcription in non-hepatic tissues is essential for estrogen-mediated protection against hepatic steatosis in HFD-fed females. The balance between non-hepatic estrogen signaling and hepatic or non-hepatic testosterone action may control the development of hepatic steatosis and NAFLD.

Title & Authors: Inkjet Printed Immunoassay and Volumetric Absorptive Microsampling Devices Enable Point-of-Care Protein Hormone and Cytokine Analysis

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**Background & Hypothesis:** Childhood malnutrition is a major global health concern. Conventional methods for evaluating severe acute malnutrition (SAM) rely on anthropometric measurements, which are unreliable and subjective. Recent studies in Uganda showed that the strongest biochemical predictors of mortality and growth failure in malnourished children were serum levels of two critical metabolic hormones: leptin and IL-6. Unfortunately, standard approaches for protein hormone analysis (namely, ELISA) are not practical for use by healthcare workers (HCWs) in low- and middle-income countries (LMICs), which often lack vital laboratory resources and infrastructure. Furthermore, sample collection in remote areas is challenging due to necessity of cold storage and rapid transportation to the laboratory. Thus, we hypothesize that a point-of-care (POC) system for sample collection and analysis will allow us to tailor treatment rationally and cost-effectively, to the physiological and metabolic needs of malnourished children, in order to improve patient outcomes. **Objective:** To develop a scalable and sustainable route towards protein hormone biomarker testing by healthcare workers in LMICs in a manner that addresses the limitations presented above. **Methods:** We will combine the use of two enabling technologies: First, for specimen collection, we will use lowcost volumetric absorptive microsampling devices (the “Neoteryx Mitra”) that are user-friendly and ensure analyte stability without cold storage. Second, for on-site biomarker analysis, we have developed an innovative, selfcontained POC assay platform that automatically runs to completion in one step, after addition of sample from Mitra device, and can be read by a smartphone detector. **Results:** Serum and whole blood specimens stored in Neoteryx Mitra devices are environmentally stable after a minimum of 4 weeks of storage at room temperature. The D4 assay displays analytical figures-of-merit that are comparable to standard laboratory-based ELISAs for leptin and IL-6. **Conclusions:** Our strategy offers a new algorithm for assessment of malnourished children in LMICs. Promising results would justify larger clinical trials and epidemiological studies, with the ultimate goal of broad implementation in public health systems in the developing world.
Background: Focal segmental glomerulosclerosis (FSGS) is a major cause of chronic kidney disease (CKD) and end-stage kidney disease (ESKD) worldwide. Over 50% of patients with FSGS develop ESKD within a decade. FSGS is characterized by steroid resistant nephrotic syndrome, rapid progression to ESKD, focal scarring of the glomerular capillary tuft and effacement of visceral epithelial cell (i.e. podocyte) foot processes. Over the past three decades, the study of hereditary forms of FSGS has highlighted podocyte injury as central to disease pathogenesis. Characteristic features of FSGS-associated podocytopathy include 1) dysregulation of actin cytoskeletal dynamics and hypermotility, 2) inappropriate cell cycle re-entry and hyperproliferation, and 3) apoptosis. Using whole exome sequencing (WES), we identified a novel heterozygous truncating variant (p.2330fsX2354) in ABCA13 that segregated with disease in a kindred with familial FSGS. ABCA13 is a ~576 kDa membrane transport protein with no known functions in podocytes. ABCA13 is the largest member of a family of 13 transport proteins involved in cellular drug and lipid efflux. Through a series of preliminary in vitro analyses, we sought to characterize the pathogenic effects of ABCA13 knockdown (KD) on podocyte motility. We focused on the PI-3K/AKT/Rac1/JNK/Paxillin signaling pathway as an established regulator of podocyte motility. **Hypothesis:** Impaired ABCA13 expression and activity cause podocyte dysmotility via dysregulation of PI-3K/AKT/Rac1/JNK/Paxillin signaling. **Objective:** To examine the functional effects of siRNA-mediated ABCA13 KD on podocyte motility. **Methods:** WES, lentivirus-mediated siRNA gene KD, scratch wound healing assays, and immunoblotting Results: Wound healing in stably-expressing ABCA13 siRNA podocytes is significantly increased (p <0.01) relative to controls. Additionally, AKT activation and AKT-mediated inhibitory phosphorylation of Rac1 (p-Ser71) are significantly reduced (p<0.05) in ABCA13 KD podocytes relative to controls. Finally, activation of JNK and phosphorylation of its downstream effector paxillin (p-Ser178) are significantly (p<0.05) upregulated in ABCA13 KD podocytes relative to controls. **Conclusions:** ABCA13 KD induces podocyte hypermotility via perturbation of the PI-3K/AKT/Rac1/JNK/paxillin signaling axis in vitro. These findings may provide insight into the pathobiology of the p.2330fsX2354 variant in our FSGS kindred. Further studies are planned to evaluate the effects of ABCA13 KD on podocyte apoptosis and proliferation in vitro and to explore the effects of ABCA13 deletion on podocyte function in vivo.

**Authors:** Gentzon Hall, Brandon Lane, Megan Chryst-Ladd, Guanghong Wu, Liming Wang, Gina Kovalik, Eugene Kovalik, Robert Spurney, Rasheed Gbadegesin

**Conclusions:** Neonatal pigs can be used to replicate prothrombotic states in infants undergoing catheterization through femoral arterial access. Further research into the quantitative and qualitative changes in inflammatory markers, clotting factors, and other biomarkers will help to elucidate differences between pediatric and adult states of hypercoagulability and may provide insight into pediatric risk factors for thrombosis due to vascular injury.

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**Background:** Age related changes in the pediatric coagulome may predispose infants undergoing cardiac catheterization to developing femoral artery thrombosis after cardiac catheterization. Infants (ages 0-12 months) have the highest rate of femoral arterial thrombosis following cardiac catheterization of any pediatric age group and a significantly higher rate than adults. However, clinical studies of pediatric thrombosis are limited by small volume of blood samples and lack of ability to assess arterial endothelium histologically. The goal of this study was to develop a piglet model of femoral arterial catheterization that would allow for investigation of risk factors for thrombosis in pediatric patients due to vascular injury. **Objective:** To create a neonatal swine model of femoral arterial catheterization that would allow for investigation of femoral artery thrombosis in young animals and characterization of changes in inflammatory markers, clotting factors, and endothelial response to injury.

**Methods:** Newborn pigs (age 1-7 days) were randomly assigned to one of four groups: Sham procedure, Sham procedure with heparin anticoagulation, Femoral arterial catheterization, and Femoral catheterization with heparin treatment. Blood was collected from animals before any intervention (sham or catheterization). Animals then underwent femoral artery catheterization with a 3 French arterial sheath or sham procedure of anesthesia without additional intervention. Blood was collected 1 hour, 4 hours, and 24 hours post-procedure. Blood was processed to isolate plasma, red blood cells, and buffy coat. At 24 hours post-procedure animals were sacrificed and femoral arteries were collected for histologic examination. Harvested vessels were sectioned and stained using H&E.

**Results:** Femoral arterial thrombosis was demonstrated at the site of access in both catheter and catheter with heparin groups by Doppler ultrasound of the femoral artery. In swine undergoing catheterization, thrombosis was also noted on histologic examination with H&E stain. **Conclusions:** Neonatal pigs can be used to replicate prothrombotic states in infants undergoing catheterization through femoral arterial access. Further research into the quantitative and qualitative changes in inflammatory markers, clotting factors, and other biomarkers will help to elucidate differences between pediatric and adult states of hypercoagulability and may provide insight into pediatric risk factors for thrombosis due to vascular injury.
Background: Establishment of the GI tract microbiota begins at birth and co-evolves with the immune system, dynamically influencing one another for the first 2 years of an infant’s life. HIV-1 envelope epitopes have been reported to mimic microbiota antigens commonly found in the gut. Thus, part of developing a successful vaccine to HIV-1 may be early immunization of children before the microbiome and microbiome-specific immunity have fully developed, allowing the immune system to differentiate between helpful commensal bacteria and foreign viral invasion. Objective: This study aims to define the relationship between the developing microbiome of the infant rhesus monkeys and the immunologic response from distinct vaccination regimens. Methods: Four groups, 1) Conventional, 2) Co-Administration, 3) Protein Only, and 4) Extended Interval, each consisting of 5 infant rhesus monkeys were immunized in varying HIV Env prime/boost modalities and intervals, and by systemic and mucosal routes of immunization. Envelope-specific antibody responses were measured by ELISA, and neutralization assays in TZM-bl cells. Phylogenetic profiling of infant microbiomes was conducted by extracting 16S ribosomal RNA from stool samples. The variable region 4 (V4) of 16S rRNA was amplified and amplicons sequenced using the Illumina MiSeq platform. 16S rRNA reads were quality filtered, demultiplexed, and clustered into operational taxonomic units (OTUs) using vsearch. A subsequent diversity analysis was performed with QIIME. Results: The Protein Only regimen yielded the earliest HIV-1 Env-specific IgG response to gp120, but the maximal response did not differ between groups. The Protein Only regimen induced the strongest and most persistent Tier 1 neutralizing antibodies. The Extended Interval regimen had the highest mucosal 1086C gp140 IgG and IgA in stool samples. Bacterial communities in stool of the infant vaccinees demonstrated variable abundance but similarly taxonomic profiles by genera. Interestingly, we identified positive correlations between the vaccine-elicited IgG responses and the bacterial genera Bifdobacteria, Succinivibrio, and Megasphaera spanning multiple vaccine groups. Conclusions and Future Directions: Abundance of certain bacteria in the infant gastrointestinal tract may be associated with the magnitude of the IgG response to infant HIV Env vaccination. Introducing these resident bacteria as a probiotic early in life may achieve increased IgG response to HIV-1 Env vaccination.

#18 Development and Application of a Multiplex Assay for the Quantification of Antibody Responses to Common Pediatric Vaccines

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Background: The World Health Organization (WHO) recommends that all infants receive the following panel of 9 vaccines during the first 18 months of life: BCG, hepatitis B, DTP, polio, Haemophilus influenzae type B (HiB), pneumococcal conjugate, rotavirus, measles, and rubella. With current research efforts to develop a pediatric HIV vaccine, it is important to evaluate how an additional vaccine would fit into the recommended program schedule, as previous research has shown that vaccine co-administration can interfere with elicited immune responses. Objective: Develop a binding antibody multiplex assay (BAMA) to measure antibody responses elicited by multiple pediatric vaccines concurrently and use this assay to compare pediatric vaccine-elicited antibody responses in HIV vaccinated infants and placebo recipients from the completed PACTG 230/326 trials. Methods: Binding to pertussis toxin, diphtheria toxin, hepatitis B surface antigen, respiratory syncytial virus F protein, HbO-HA, tetanus toxoid, and rubella virus capsid protein was measured in a panel of 50 plasma samples by both ELISA and BAMA. Antibody concentrations were calculated using WHO reference reagents and were compared between the two assays. To characterize the specificity of the multiplex assay, a sample with high binding to each antigen was pre-absorbed with homologous and heterologous inhibitors and binding signal was compared to the same sample, non-treated. The pediatric vaccine BAMA assay was then used to determine antibody concentrations in vaccine and placebo recipients from the PACTG 230/326 trials at 24 weeks of age. Results: Concentrations of antibodies against the pediatric vaccines determined by ELISA and BAMA strongly correlated (r > 0.85). Using the ELISA concentrations to determine if a sample had antibody levels above established protective levels, we found that most antigens in the BAMA assay had false positive and negative rates <20%. Pre-absorption of plasma with homologous protein led to a substantial decrease (≥84%) in binding signal, whereas low levels of heterologous inhibition (≥12%) were observed. Antibody concentrations against all of the antigens were comparable between HIV vaccine and placebo recipients from the PACTG 230/326 cohorts. Conclusions: The pediatric vaccine multiplex assay agrees with the standard ELISA, and can be used to quantify responses to the routine vaccines represented in our antigen panel. Preliminary data on applying this assay to the PACTG 230/326 trials suggest that infant HIV vaccination does not interfere with antibody responses to vaccines commonly administered in infancy. This assay can be similarly applied to other large cohorts to assess multiple pediatric vaccine antibody responses simultaneously and to study vaccine interference.
#19 Title & Authors: Development of a method to phenotype Fc-receptor-mediated antibody binding to immune cells.

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**Background & Hypothesis:** Antibodies have antigen binding regions (Fab domains) and constant regions (Fc domains)—both regions can be important for antibody function. The interaction of antibodies with cellular effectors is usually mediated by binding of antibody Fc with cell surface receptors (FcRs). Different isotypes, subclasses and polymorphisms of FcRs can affect antibody binding, leading to different functional activities by effector cells. **Objective:** To develop a flow cytometry-based assay for antibody binding to cell surface FcRs. **Methods:** Monoclonal antibodies (mAbs) were synthesized as wild type human IgG1 or with mutations known to enhance binding to human CD16 and to diminish binding to human CD32. Two mAbs were prepared: 7B2 against the HIV-1 Envelope gp41 immunodominant domain and CH65 against the influenza hemagglutinin receptor binding site. We tested the ability of these mAbs to bind surface FcRs on human and rhesus macaque immune cells under different conditions. **Results:** We compared wild-type IgG1 molecules to modified IgG1. As expected, modified IgG1 bound better to CD16 (Fc-gamma-R III) on human NK cells, while the wild-type IgG1 bound more strongly to cells expressing CD32 (Fc-gamma-R II) or CD64 (Fc-gamma-R I). When we tested the panel against a variety of rhesus macaque cells, we found a range of patterns—for some macaques the two types of IgG1 were equivalent in binding to CD16, for some the modified form bound better, and for others neither antibody type bound well. Binding to CD32/CD64 was also varied, and taken together, there were different among macaques. **Conclusions:** Variations of Fc that modify human Fc-FcR interactions can be detected in a flow cytometry-based assay. Using the same assay in rhesus macaques demonstrated a more diverse range of binding patterns. These data indicate that passive infusion of antibodies in rhesus macaques may have different abilities to engage immune cells that could lead to differences in functional outcomes.

#20 HIV-1 Transmitted/founder Viruses from Peripartum Transmission are Resistant to Neutralization by Maternal Plasma.

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**Background:** Despite extensive genetic diversity of HIV-1 variants in chronic infection, maternal to child transmission of HIV-1 involves selective transmission of one or few viral variants. Infant transmitted-founder (T/F) viruses are of particular interest, since they represent the target of a potential maternal or infant vaccine. The interaction between the maternal immune system and selection of infant T/F viruses is not well understood. **Hypothesis:** Identifying key features of Infant T/F viruses, which help their escape from maternal serum, will inform the development of a vaccine to prevent mother to child transmission (MTCT). **Methods:** Plasma samples from HIV infected mother/infant pairs enrolled in the Women and Infants Transmission Study (WITS) were selected based on availability and documented (HIV-<7 days, HIV->7 days) peripartum HIV-1 transmission status. Single genome amplification and sequencing of the HIV-1 env gene variants in plasma of 16 mother-infant pairs was performed. Infant T/F viruses were cloned into an expression vector and used to prepare pseudoviruses. Overlap PCR method was used to prepare pseudoviruses from 5-10 non-transmitted maternal variants from each mother. Neutralization sensitivity of pseudoviruses from each mother-infant pair was tested against maternal plasma (1:20 dilution) and a panel of broadly neutralizing antibodies (bnAbs) in a TZM-bl cells neutralization assay. ID50 were compared using 1-sided permutation test. **Results:** 63% (10/16) of infants were infected with 1 T/F virus while 37% (6/16) were infected with 2 or more T/F viruses (22 total T/F viruses). T/F viruses from 12 infants were fully resistant (ID50<40) to paired maternal serum. However, 4 infants' T/F viruses were sensitive to paired maternal plasma (18%). In contrast, 44% (59/134) of nontransmitted maternal variants were sensitive to autologous plasma neutralization. Infant T/F viruses were significantly more resistant to paired maternal plasma than non-transmitted maternal variants (p= 0.01898). VRC01 neutralized all the infant T/F viruses. **Conclusion:** Resistance of 82% of infant T/F virus compared with 56% non-transmitted maternal viruses’ resistance to neutralization by maternal plasma demonstrates that infant T/F variants may be selected by their ability to escape neutralization. Identifying these unique infant T/F properties will help to define maternal antibody response that could be boosted during pregnancy to further reduce MTCT. Our results are relevant to ongoing infant passive vaccination trials with the bnAb VRC01, as it potently neutralized all infant T/F viruses.
#21 Title & Authors: Dysregulation of WT1(-KTS) is associated with the kidney-specific effects of the LMX1B R246Q mutation.

Brandon Lane, Gentzon Hall, Megan Chryst-Ladd, Guanghong Wu, Jen-Jar Lin, XueJun Qin, Elizabeth R. Hauser, Rasheed Gbadegesin

**Background & Hypothesis:** Mutations in the LIM homeobox transcription factor 1-beta (LMX1 B) are a cause of nail patellar syndrome, a condition characterized by skeletal changes, glaucoma and focal segmental glomerulosclerosis. Recently, a missense mutation (R246Q) in LMX1 B was reported as a cause of glomerular pathologies without extra-renal manifestations, otherwise known as nail patella-like renal disease (NPLRD). The mechanisms by which LMX1 B R246Q causes a renal-specific phenotype is unknown. We propose that two new families with kidney disease spanning multiple generations have NPLRD caused by a disruption of key podocyte related genes due to the LMX1 B R246Q mutation.

**Objective:** To identify the genetic cause of kidney disease in the two families using a combination of whole exome sequencing and linkage analysis. To determine how this mutation may be causing a kidney disease phenotype using cultured human podocyte cell lines.

**Methods:** Whole exome sequencing and linkage analysis to identify the causal mutation. Real-time PCR analysis, western blotting, and immunofluorescence analysis of key podocyte genes in stable lentiviral infected immortalized human podocyte cell lines overexpressing either wildtype or R246Q mutant LMX1 B.

**Results:** We have identified two additional NPLRD families with the R246Q mutation and observed dominant negative and haploinsufficiency effects of the mutation on the expression of podocyte genes such as NPHS 1, GLEPP1, and WT1. Specifically, we observed a novel LMX1 B R246Q-mediated downregulation of WT1(-KTS) isoforms in podocytes.

**Conclusions:** We have shown that the renal-specific phenotype associated with the LMX1 B R246Q mutation may be due to a dominant negative effect on WT1(-KTS) isoforms that may cause a disruption of the WT1 (KTS):(+KTS) isofrom ratio and a decrease in the expression of podocyte genes. Full delineation of the LMX1 B gene regulon is needed to define its role in maintenance of glomerular filtration barrier integrity.

#22 A pilot study on using rapamycin-carrying synthetic vaccine particles (SVP) in conjunction with enzyme replacement therapy to induce immune tolerance in Pompe disease

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**Background & Hypothesis:** A major obstacle to enzyme replacement therapy (ERT) with recombinant human acid α-glucosidase (rhGAA) for Pompe disease is the development of high and sustained titers of anti-rhGAA antibodies in a subset of patients, which often leads to a loss of treatment efficacy. Current clinical immunomodulatory protocols are not antigen-specific and can cause side effects due to systemic immune suppression. A recent study demonstrated that novel synthetic vaccine particles containing rapamycin (SVP-Rapa) could induce durable and antigen-specific immune tolerance to co-administered protein in mice and non-human primates. **Objective:** To test the ability of SVP-Rapa to suppress anti-rhGAA antibody responses to ERT in Pompe disease (GAA-KO) mice.

**Methods:** During the 12-week course of weekly ERT, rhGAA was co-administrated intravenously with SVP-Rapa (treatment group) or empty nanoparticles (empty NP, mock treatment) for the first 3 weeks. A third group of mice was injected intraperitoneally with methotrexate (MTX; positive controls) at 0, 24, and 48 h following the first three rhGAA injections. Blood was collected every 2 weeks for testing anti-rhGAA antibody titer. Monthly Rota-rod performance was tested for monitoring motor function improvement. GAA enzyme activity and glycogen content were analyzed in liver, heart, and skeletal muscles.

**Results:** Anti-rhGAA antibody titers started rising from 2 weeks of ERT for the empty NP-treated mice and from 6 weeks for the MTX-treated mice; in comparison, no anti-rhGAA antibodies were developed in the SVP-Rapa-treated mice until 12 weeks. In addition, SVP-Rapa treatment significantly improved glycogen clearance in skeletal muscles and motor function by ERT compared with mice treated with empty NP or MTX.

**Conclusions:** Our data suggest that co-administration of tolerogenic SVP-Rapa may be an innovative and safe approach in ERT for Pompe disease to induce durable immune tolerance against rhGAA, and it will further improve the clinical outcomes in patients treated with ERT.
#23 Variable IgG transplacental transfer in the setting of maternal HIV infection
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**Background & Hypothesis:** Due to the high success of maternal antiretroviral treatment during pregnancy, the number of HIV-exposed uninfected (HEU) infants is increasing each year. HEU infants have up to 4-fold higher morbidity and mortality rates associated with respiratory and diarrhea-related illnesses compared to HIV-unexposed (HU) infants. Interestingly, maternal HIV infection is associated with poor IgG transplacental transfer, potentially contributing to the high susceptibility of HEUs to common infections.

**Objective:** To define determinants of IgG transplacental transfer efficiency in HIV-infected mothers.

**Methods:** We measured HIV and non-HIV-specific IgG serum concentration in 167 HIV-infected mothers and their infants in US and Malawian cohorts by a binding antibody multiplex assay (BAMA). The measured antigen-specific IgG antibodies were: HIV gp120, HIV V1V2, HIV V3, HIV gp41, tetanus toxoid, rubella capsid antigen, hepatitis B surface antigen, influenza hemagglutinin, and diphtheria toxin. IgG transplacental transfer efficiency was calculated as the ratio of infant IgG cord blood concentration over maternal IgG serum concentration X 100. IgG subclass responses of variably transferred HIV and non-HIV-specific antibodies were measured by BAMA. Finally, the Fc region glycan profile of variably transferred HIV and non-HIV-specific antibodies was measured by capillary electrophoresis.

**Results:** From 167 HIV-infected mother infant pairs, 3 patterns of maternal IgG transplacental transfer were observed: good, variable, and poor IgG transfer. 22 pairs had efficient IgG transfer (>100%) against most tested antigens, while 93 pairs had poor IgG transfer (< 80%) across most tested antigens. Finally, 52 pairs the IgG transfer was variable from one antigen to another. Maternal gp120 and V3-specific IgG serum magnitude responses negatively correlated with IgG transplacental transfer of gp120 and V3-specific IgG antibodies, but this trend was not observed for other HIV and non-HIV-specific IgGs. Neither the Fc region glycosylation profiles nor IgG subclass was predictive of transplacental transfer efficiency in women with variable transfer of HIV and non-HIV-specific IgGs. Interestingly, 5% of US and 17% of Malawian HEUs were born with plasma levels of tetanus toxoid-specific IgG below the protective serum titer threshold. Furthermore, 18% of US and 77% of Malawian HEUs birth levels of plasma diphtheria-specific IgG were below the protective serum titer threshold.

**Conclusions:** Our results indicate that in both African and US HIV-infected pregnant women, the transplacental transfer of IgG is variable and neither IgG subclass, nor IgG Fc region glycan profiles is predictive of IgG transplacental transfer efficiency. Moreover, HEUs receive suboptimal levels of maternal protective IgG against common neonatal pathogens, likely contributing to their increased susceptibility to infectious diseases.

#24 Title & Authors: Proteomic Analysis of Changes Induced by Infant Cardiopulmonary Bypass
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**Background & Hypothesis:** Bleeding and thrombotic events cause significant morbidity and mortality in children on extracorporeal membrane oxygenation (ECMO) or cardiopulmonary bypass (CPB). Existing laboratory studies fail to reliably predict an d guide management of these events.

**Objective:** To better characterize the complex and overlapping interactions that make the pediatric coagulome and its response to vascular injury, we performed an unbiased proteomic analysis of infants undergoing CPB.

**Methods:** Blood samples were collected from infants (0-12 months) at initiation of CPB, and at 1, 4, and 24 hours after initiation. 2D-difference gel electrophoresis (2-DIGE) was utilized to identify changes in plasma protein concentrations across time points. Inflammatory cytokines and vascular injury markers were assessed by ELISA.

**Results:** Ten infants with congenital cardiac anomalies were enrolled with mean age 125±121 (range 3-330) days. No immediate complications were observed. Using 2D-DIGE, >1400 individual protein spots were observed, and 89 proteins demonstrated significant concentration change (>30%, p <0.02). 70/89 proteins were upregulated at one or more time points after initiation of CPB, while 19/89 were downregulated. 29/89 protein spots were identified by mass spectrometry. These included multiple proteins not previously described as hemostatic proteins, with at least 7 identified as novel proteins with unknown functions. Of the cytokines analyzed with ELISA, IL-2, IL-8 and IL-10 were elevated at 4h after initiation of bypass and IL-6 was elevated at both 4 and 24 hours after initiation of bypass.

**Conclusions:** In response to CPB in infants, changes are observed in the concentrations of numerous plasma proteins. Associations these plasma protein changes with clinically significant bleeding and thrombotic events that occur during the course of support may provide predictive biomarkers for better management of bleeding and thrombosis in these infants.
#25 Title & Authors: Effects of Malnutrition on T follicular helper (Tfh) cell and Germininal Center B cell Development and Function. Amanda Nichols, Sivan Cohen, and Nancie MacIver

Background & Hypothesis: In previous studies, we have found that malnutrition (from fasting or calorie restriction) leads to decreased CD4+ T helper cell number, function, and metabolism. These T cell changes in malnutrition are associated with both an increased susceptibility to infection as well as protection against autoimmune diseases. Multiple subsets of CD4+ T helper cells exist, and each has distinct functional and metabolic phenotypes. T follicular helper (Tfh) cells are a subset of CD4+ T helper cells and are known to promote the differentiation and proliferation of B cells within germinal centers (GC) in order to produce long lived, high affinity antibodies. Tfh cells, therefore, have an important role in driving protective immunity, autoimmunity, and vaccine development. However, it remains unknown if and how these cells are affected in states of undernutrition when protective immunity is generally impaired.

Objective: Our objective was to determine the effects of malnutrition (fasting) on the development, function, and metabolism of Tfh cells and GC B cells.

Methods: We fasted C57BL/6 mice for 48 hours and isolated lymphocytes from Peyer’s patches to determine Tfh cell and GC B cell number and function using flow cytometry. To study Tfh cell metabolism, we isolated CD4+ T cells from spleen and in vitro differentiated them into Tfh cells, followed by study of induced Tfh cell metabolism.

Results: Fasting led to decreased numbers of Tfh cells and GC B cells from Peyer’s patches. Moreover, Tfh cells and GC B cells from fasted animals made less IL-21 and IgA, respectively, demonstrating impaired function. Induced Tfh cells from fasted animals also had impaired glucose metabolism, which was characterized by decreased expression of the T cell glucose transporter Glu1, decreased uptake of glucose, and decreased expression of HIF-1alpha, which is known to promote a glycolytic phenotype in T cells. Additionally, when fasted mice were treated with twice daily leptin injections, which have been shown previously to rescue CD4+ Th1 and Th17 cells during malnutrition, Tfh cell function and GC B cells numbers were rescued. Likewise, rescue of glucose metabolism with overexpression of the Glut1 transgene also reversed Tfh cell defects seen in fasting. We also observed interesting changes in Tfh cell numbers in high-fat diet-induced obese mice, including decreased Tfh and GC B cell numbers, accompanied by a striking increase in the non-GC Tfh cell population.

Conclusions: Malnutrition (fasting) reduced Tfh cell and GC B cell development and impaired the function of these cells. Moreover, fasting impaired Tfh cell glucose uptake and metabolism. These Tfh cell defects were reversed by either treatment with the nutritionally regulated hormone leptin or by rescue of glucose metabolism.

#26 Title & Authors: Loss of MST/Hippo signaling promotes tumorigenesis in a genetically engineered mouse model of fusion-positive alveolar rhabdomyosarcoma. Kristianne M. Oristian, Lisa E.S. Crose, Rex Bentley, Nina Kuprasertkul, David G. Kirsch, and Corinne M. Linardic

While improvement in survival for pediatric cancer patients over the last 40 years has been encouraging, certain cancer types evade cure. One such example is fusion-positive alveolar rhabdomyosarcoma (aRMS), a pediatric soft tissue sarcoma of mesenchymal origin with skeletal muscle features and a 5-year survival rate of <50%. A hallmark of this aggressive malignancy is the t(2;13) translocation fusion gene PAX3-FOXO1 (PF). Primary cell-based modeling experiments in our laboratory and others have shown that PF is necessary but not sufficient for aRMS tumorigenesis, indicating additional molecular alterations are required to initiate and sustain tumor growth. Previously we showed that PF-positive aRMS tumorigenesis relies on alterations in Hippo pathway signaling, including upregulation of the YAP transcriptional co-activator, and downregulation of the MST/Hippo kinase, which occurs when the scaffold protein RASSF4 (itself a PF transcriptional target) binds and inhibits MST1/Hippo. We hypothesized that ablating MST/Hippo signaling in an existing genetically engineered mouse model (GEMM) of aRMS would accelerate tumorigenesis and provide insight into the role of this pathway in aRMS. To assess the role of MST/Hippo signaling in aRMS, MST1/2-floxed (Stk3F/F; Stk4F/F) mice were crossed with an established MSTWT aRMS GEMM driven by conditional expression of Pax3-Foxo1 from the endogenous Pax3 locus and conditional loss of Cdkn2a in Myf6-expressing cells. Statistical analysis revealed that compared to MSTWT aRMS control animals, MSTNull have significantly accelerated tumorigenesis (median survival 112 vs. 224 days, p < 0.0001) and increased tumor penetrance (76% vs. 27%). MSTNull animals developed tumors disproportionately in the head and neck as compared to control, and incurred multiple tumors per animal. Tumors were analyzed via immunohistochemistry for MyoD and myogenin, markers of RMS, as well as Myf5, an early marker of myogenesis. Tumors in both cohorts were positive for aRMS markers. Tumor-derived cell lines were used for in vitro cell-based assays and molecular interrogation. We have identified the MST/Hippo signaling axis as an important tumor suppressor mechanism in aRMS. The rapid onset and increased penetrance of tumorigenesis in this GEMM provides a powerful tool for interrogating aRMS biology and screening novel therapeutics.
**#27 Impairment in Social Interaction, Memory, and Depression due to Long Term Higher End Doses of Dextromethorphan**

Jordan Richardson, Arsen Hunanyan, Ph.D., Adriana Azar, Monisha Sachdev, April Ratliff, Mohamad Mikati M.D

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**Background & Hypothesis:** DXM, a commonly used antitussive and a drug that is now also being used in many neurological diseases in children, has acute and long term toxicities that have been demonstrated in animal models in adult rodents. However its effects on the developing brain have not been studied. Because DXM blocks NMDA receptors, the developing brain may be particularly vulnerable to its potential toxic effects.

**Objective:** The objective of this study was to understand the short and long term toxicity of Dextromethorphan (DXM) on the developing brain.

**Methods:** We determined the behavioral effects of DXM after acute (20 min post dose) and after chronic administration (daily dosingx10 days P30-40 with behavioral testing done on P41-45) in C57BL/6 mice. We studied three groups: DXM 40 mg/kg/day, 20, and vehicle. Such doses result in serum levels that are comparable to both levels achieved in overdose by abusers and during treatment of neonatal hyperglycinemia in infants.

**Results:** Acute toxicity: There were no differences among the 3 groups in any of the following tests: Open Field time in Peripheral, Rotarod, or Grip Strength. (ANOVA p>0.05). However, there was a difference in the Beam Walking Test in both time (p=0.041) and amount of slips (p=0.023) and Crosses in the Open Field Test (p=0.002). Long-term toxicity: There were no statistical differences among the 3 groups in any of the following tests: Forced Swimming, Novel Object, and Social Affiliation or Novelty. (p>.05 in all cases), however there were very strong trends that require further investigation.

**Conclusions:** In acute doses, DXM caused motor skill impairments and hyperactivity. Long-term administration of DXM at high doses in the developing brain may cause impairment in Social Interaction, Memory and Depression. Further tests are being done on these parameters. This is of potential clinical importance in treating pediatric patients with neurological impairments with DXM.

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**#28 Title & Authors:** “Efficacy of Dextromethorphan in Preventing Sudden Unexpected Death in Epilepsy and Spreading Depolarization: A study in the Mashl–/– knock-in mouse model.”

Monisha Sachdev, Arsen Hunanyan, Elie Abdelnour and Mohamad A Mikati.

**Background & Hypothesis:** Sudden unexpected death in epilepsy (SUDEP) is the most common cause of death in epilepsy. Its underlying mechanism has been shown to involve spreading depolarization (SD). Mutations in the alpha subunit of the Na+/K+ pump (ATP1A3) result in Alternating Hemiplegia of Childhood (AHC), a disease that manifests as episodes of hemiplegia, dystonia and epileptic seizures with high risk for SUDEP. Our lab has developed a knock in mouse model carrying the most common mutation that causes AHC in humans (Mashl+/– mouse). SD has been shown to be propagated by NMDA receptor activation and dextromethorphan (DXM), which has anti NMDA blocking properties, has been shown to attenuate SD. It is not known however, if the intake of DXM can attenuate SD or reduce the risk of SUDEP. The goals of this study are to address these questions.

**Objective:** Demonstrate occurrence of sudden unexpected death in epilepsy (SUDEP) in Mashl–/– knock-in mice (carrying Atp1a3 D801N mutation); determine at what age it is highest; and investigate whether DXM can ameliorate Mashl–/– predisposition to SUDEP as an illustration of the usefulness of this model to screen for potential anti-SUDEP agents.

**Methods:** The natural time course of SUDEP in the Mashl–/– mice, known to manifest spontaneous recurrent seizures and vestibular stimulation induced seizures, was examined Next, 4 groups were studied: 2 WT and 2 Mashl–/– mice, one of each receiving vehicle and one of each receiving DXM (20 mg/kg). Animals underwent vestibular stimulation once/day for 10 days 20 minutes after receiving vehicle/DXM. Then animals were subjected to flurothyl-induced seizures.

**Results:** Mortality of Mashl–/– to be significantly higher in the second quarter of life: n = 39, P < 0.05, x2 test (7/39 in the 1st quarter; 16/39 in the 2nd; 11/39 in the 3rd; and 5/39 in the 4th). Spontaneous recurrent seizures were observed in 3/22 (14%) Mashl–/– mice as compared to 0/40 (0%) WT mice. After undergoing vestibular stimulation, all Mashl–/– mice had seizures irrespective of whether they received DXM or vehicle. Preliminary results showed a trend that DXM treatment decreases seizure latency in both WT and Mashl–/– mice (WT, n = 2, P = 0.058; Mashl–/–, n = 2, P = 0.052). Mortality: WT-Vehicle 0/2, WT-DXM 0/2, Mashl–/–Vehicle 1/4, Mashl–/–DXM 2/4.

**Conclusions:** SUDEP occurred most commonly during the second quarter of life and by virtue of the occurrence of stimulus induced seizures this model can be used as model to screen for potential anti-SUDEP medications. Preliminary data gathered regarding DXM in this model do not appear favorable for a positive effect.
**#29 Title & Authors:** Zika virus envelope-specific monoclonal antibodies isolated from Zika virus infected rhesus monkeys following primary and secondary virus exposure  
*Tulika Singh, Holly Heimsath, Josh Eudailey, Dawn Dudley, Matthew Aliota, Christina Newman, Mariel Mohns, Meghan Breitbach, David O’Connor, and Sallie Perrin*

**Background & Hypothesis:** Zika virus (ZIKV) can be transmitted from mother to child and is causing congenital microcephaly and other birth defects. To design vaccines that prevent mother-to-child-transmission and congenital Zika syndrome, it is imperative to evaluate maternal immune responses that correlate with protection against placental virus transmission and fetal disease. We hypothesize that secondary exposure to ZIKV will elicit ZIKV envelope-specific neutralizing monoclonal antibodies (mAbs) that do not cross-react with Dengue virus (DENV).

**Objective:** 1) Isolate monoclonal antibodies following primary and secondary infection; 2) Evaluate binding of mAbs to ZIKV and DENV E-proteins, via ELISA; 3) Characterize genetic variation and clonal lineage of ZIKV E-protein-specific mAbs.  
**Methods:** Three rhesus monkeys were challenged subcutaneously with French Polynesian ZIKV, each at a different dose: 1 x 10^4, 10^5, and 10^6 PFUs on day 0 and re-challenged with 1 x 10^4 PFUs on day 70. We sorted single plasmablasts (CD14-/CD16-/CD20-/ CD3-/CD123-/CD11c-/CD80+/HLA-DR+) from PBMCs collected 7-10 days after primary infection, and 4 days after the re-challenge. Variable heavy (VH) and light chain (VL) genes were amplified, and mAbs were generated through transient transfection. MAbS were evaluated for binding via whole Zika-virion assay, and ELISA with ZIKV E-proteins and DENV serotypes 1-4 E-proteins.  
**Results:** 3 of 3 rhesus monkeys were protected against viremia upon secondary Zika virus exposure. MAbs were isolated from two ZIKV-infected rhesus monkeys following primary infection and secondary infection. Serum neutralization titers were boosted upon re-challenge in one animal. After plasmablast sorting and VH and VL amplification, 71 mAbs were tested for binding (32 from primary infection and 39 from secondary infection), with 15 binding to ZIKV envelope; of these, 3 mAbs demonstrated whole virion binding only, 5 mAbs confirmed binding only to the ZIKV E-protein, and 7 mAbs bound to both the virion and E-protein. High cross-reactivity between ZIKV and DENV E-proteins has been observed, with 11 Zika E-specific mAbs cross-reactive with DENV 1-4 E-proteins. 1 mAb was specific only to ZIKV E-protein. 11 of the 15 ZIKV-specific mAbs use VH gene family 4. The somatic hypermutation rate for ZIKV-specific mAbs from primary infection varied between 6-9%, and from secondary infection it varied between 3.5-16%.  
**Conclusions:** Generating ZIKV E-protein-specific mAbs will allow investigation of mAb evolution and epitope focusing, characterization of protective immune responses, and guide the development of mAb based therapeutics.

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**#30 Title & Authors:** Identification of antigen-specific B cells and isolation of monoclonal antibodies from syphilis-infected patients  
*AT Sponaugle, TC Gurley, LC Armand, S Stager, WC Binz, DJ Marshall, HX Liao, AC Seña, HP Zheng, JD Tucker, M Caimano, A Anand, C Karanian, J Salazar, MS Cohen, JD Radolf, MA Moody*

**Background & Hypothesis:** Because the pathogen cannot be easily cultivated, diagnosis of syphilis relies upon serological tests that are indirect (eg, RPR), technically challenging (eg, darkfield microscopy), or difficult to interpret in the face of repeat infection (eg, ELISA). In addition, some individuals retain elevated levels of anti-cardiolipin antibodies after treatment, the so-called “serofast state”, leading to clinical concerns for the need to repeat anti-syphilis therapy. To date, few studies have examined the immune response to syphilis infection at the level of the individual B cell to deconvolute this complex antibody response.  
**Objective:** To develop tools to study the antibody response to *Treponema pallidum* infection.  
**Methods:** We studied two cohorts of individuals recruited from populations at risk for syphilis infection. A study of acute HIV-1 infection performed from 2005-2012 in the US and Africa by the Center for HIV/AIDS Vaccine Immunology (CHAVI 001) enrolled a group of participants with syphilis infection; a second group of prospectively recruited participants with syphilis were recruited from the Durham County Health Department. For both groups, clinician-estimated staging of syphilis was recorded. Peripheral blood mononuclear cells (PBMCs) and plasma/serum were stored for each participant. Recombinant proteins derived from *T pallidum* (Tp15K, Tp17K, Tp47K) were biotinylated and formulated as tetramers using fluorescently-labeled streptavidin; these constructs were used to phenotype memory B cells from 22 participants (6 primary, 7 secondary, 4 latent, 5 serofast). Monoclonal antibodies were isolated from one participant.  
**Results:** Antigen-specific B cells that bound recombinant *T pallidum* proteins were detected in all participants. Tp15K showed the lowest frequency of antigen-specific B cells in all groups and were lowest in secondary syphilis. The frequency of Tp47K was highest in all groups although some syphilis patients did not have detectable antigen-specific B cells in PBMC. Sorting of cells using Tp17K resulted in the isolation of 2 antibodies reactive with the protein in ELISA; these antibodies both used variable heavy chain family 3 gene segments and were modestly mutated (2.0% and 3.0% heavy chain mutation frequency).  
**Conclusions:** We successfully used flow cytometry to isolate antigen-specific B cells from syphilis patients and produce human monoclonal antibodies. This technique has promise as a way to deconvolute the polyclonal antibody response to syphilis infection.
The HIV-1 Env binding and neutralizing domain of Tenascin C overlaps that of variable loop 3 glycan-dependent broadly neutralizing monoclonal antibodies. L. Stamper, F. Jaeger, T. Ohashi, I. Tablazon, H. Erickson, S.M. Alam, S. Permar. Background: Innate immune factors in the mucosa are the first line of defense against viral infections. Lactoferrin, secretory leukocyte protease inhibitor (SLPI), and mucins have been found in breast milk and other secretory fluids to have HIV-1 inhibitory activity. We previously isolated a novel, innate protein in breast milk, Tenascin-C (TNC), that mediates HIV envelope (Env) neutralization by interacting with the V3 loop domain. Identifying the fine specificity of the neutralizing domain of TNC could be important to develop strategies to help reduce breast milk and other forms of mucosal transmission of HIV-1. Methods: Truncated TNC proteins were assessed for their ability to bind to HIV-1 B.MN envelope (Env) gp120 through surface plasmon resonance and ELISA. TNC regions with high binding to Env gp120 were then tested for their ability to bind to the HIV-1 MN V3 peptide, the proposed HIV Env binding domain of TNC. Linear overlapping V3 peptides were assessed for binding to TNC. A truncated and mutated version of MN V3 peptide was used to narrow down the binding domain by ELISA. Truncated TNC proteins were used to measure HIV-1 neutralization activity in the TZMbl reporter cell assay. Mutants of MN.3 HIV-1 with the same amino acid mutations as linear peptides were used to assess neutralization of TNC in the context of the V3 loop conformation. Results: TNC fibrinogen terminal knob (fbg) bound to the HIV-1 Env with higher binding strength than full length TNC, and TNC fbg had higher binding affinity to a scaffolded and linear V3 than the other TNC regions. These results suggest that TNC fbg is the primary HIV Env binding site on TNC. Linear V3 peptide ELISA using various HIV strains, revealed that TNC bound most strongly to the V3 region of the HIV MN strain. When linear V3 amino acid sequences of different HIV-1 strains were aligned to that of the MN strain, a cluster of amino acids near the C-terminus of the V3 loop of MN.3 differed from the other V3 peptides. This region of V3 is also an epitope for glycan-dependent V3 broadly neutralizing antibodies. Moreover, deletion of this cluster of C terminus V3 residues abrogated TNC-V3 binding, whereas alanine replacement of each residue within this cluster reduced the affinity of TNC-V3 binding. Conclusion: Our data indicates that TNC fbg is the primary binding domain for the V3 loop of HIV-1 Env. TNC fbg domain appears to bind a unique amino acid cluster near the glycan V3 broadly neutralizing antibody epitope in the V3 loop. Future studies will focus on further characterizing HIV-1 neutralization mediated by V3 specific interaction of TNC. Mapping the binding domains of TNC to HIV-1 Env may help identify a vulnerable target for future strategies to prevent mucosal HIV-1 transmission.

Cytomegalovirus diversity in glycoprotein B subunit vaccinees and placebo recipients
Melody Su, Cody S. Nelson, Robert Pass, Ravit Boger, Sallie R. Permar

Background & Hypothesis: Congenital human cytomegalovirus (HCMV) affects 1 in 150 newborn infants, frequently resulting in brain damage, hearing loss, or neurodevelopmental delay. One possible strategy to prevent this disease is the development of a maternal vaccine. The most successful immunization strategy to date consisted of HCMV glycoprotein B (gB)/MF59 adjuvant, resulting in a 50% reduction in the rate of HCMV acquisition in multiple phase 2 clinical trials. gB facilitates viral entry in HCMV and is a known target of neutralizing antibodies. Polymorphisms in gB of vaccine recipients may indicate evasion of vaccine-elicited immune responses. We sequenced gB of CMV isolates from a sample cohort of 7 gB/MF59 adjuvant vaccinated and 11 placebo recipient postpartum women. Samples were isolated from urine, saliva, vaginal fluid, and blood. Objective: to investigate sequence diversity between HCMV infected gB vaccinees and placebo recipients for possible vaccine-exerted immune pressure and viral immune evasion
Methods: Nested PCR was performed to amplify (1) the full gB ORF and (2) a 540bp hypervariable region corresponding to gB neutralizing epitope domains I/II. The Nextera XT DNA Prep Kit was used to tagment the full gB gene, Nextera XT index primers assigned to both sample sets, and libraries sequenced on an Illumina Miseq platform. Consensus DNA sequences for full gB were extracted using Geneious. Domains I/II sequences were processed using the SeekDeep pipeline. Unique viral haplotypes were generated, and variants present <0.5% were excluded from analysis. All sequencing was done in duplicate to reduce background error. Results: Full gB ORF sequencing revealed a 94% pairwise identity for the gB nucleotide sequence and 93.1% for the amino acid sequence. 4 gB genotypes were detected in the subjects. gB1 was dominant across both treatment groups and all sample types except for vaginal fluid. The median number of viral haplotypes present (n) and median nucleotide diversity (π) for the dominant gB genotype were not different between vaccinees (n=3, π=0.00073) and placebo recipients (n=3, π=0.00060) (p=ns, Mann-Whitney U test). There was no difference in the median number of haplotypes present or nucleotide diversity of viruses replicating in urine (n=2, π=0.00060), saliva (n=3, π=0.00065), vaginal fluid (n=3, π=0.00047), and blood (n=5, π=0.00151) (p=ns, Kruskal-Wallis test). Conclusions: The variable pairwise identity and nucleotide alignments suggest that there is a substantial amount of diversity in the fusion protein of this DNA virus and that subsequent investigations may identify amino acid residues under selection pressure in vaccinees. The similar number of unique haplotypes and dominant gB nucleotide diversity scores between treatment groups and across diverse physiologic compartments indicates that vaccination and sample type may not influence the number of unique variants replicating or nucleotide diversity.
#33 Title & Authors: Stbd1 plays a dominant role in glycogen transport to lysosomes in liver.
Tao Sun, Haiqing Yi, Chunyu Yang, Priya S Kishnani, and Baodong Sun

Background & Hypothesis: Deficiency of acid alpha-glucosidase (GAA) causes Pompe disease (glycogen storage disease type II), resulting in a progressive lysosomal glycogen accumulation and dysfunction in cardiac and skeletal muscles and other tissues. In mammalian cells, a small portion of cellular glycogen is transported to and degraded in lysosomes by GAA, but the mechanism of how glycogen is transported to the lysosomes is still unclear. Recently starch binding domain-containing protein 1 (Stbd1) has been proposed to participate in glycogen trafficking to lysosomes, suggesting it might be a new therapeutic target for Pompe disease. Although our previous study showed that knockdown of Stbd1 in GAA-KO mice did not alter lysosomal glycogen storage in skeletal muscles, we could not exclude the possibility that the residual Stbd1 was sufficient to transport glycogen to the lysosomes in the muscle cells. Objective: To determine the impact of elimination of Stbd1 on glycogen transport to lysosomes. Methods: A GAA/Stbd1 double knockout (dKO) mouse model was generated by crossing Stbd1-KO mice with GAA-KO mice. Lysosomal glycogen accumulation was assessed by quantifying tissue glycogen content in fasted GAA-KO and dKO mice. Rescue experiments were also performed by injecting series of adeno-associated viral (AAV) vectors expressing human Stbd1 or its mutants into dKO mice. Results: There was no difference in glycogen accumulation in skeletal and cardiac muscles between dKO and GAA-KO mice, but glycogen content in liver of dKO mice was approximately 25% of that of the GAA-KO mice. This demonstrates that the transport of glycogen to lysosomes was suppressed in liver, but not in muscles, by the loss of Stbd1. Exogenous expression of human Stbd1 in dKO mice restored the liver glycogen content to the level of GAA-KO mice. A mutant that contains only the N-terminal 24 hydrophobic segment and the C-terminal CBM20 domain had the same capability as wild-type Stbd1 to restore liver glycogen content. Conclusions: This is the first report to demonstrate the function of Stbd1 in mouse. Our results demonstrate that Stbd1 plays a dominate role in glycogen transport to lysosomes in liver and the N-terminal transmembrane region and the C-terminal CBM20 domain are critical for this function. Stbd1 knockout does not affect glycogen transport to lysosomes in muscles.

#34 Title & Authors: Quantification of Purkinje cells in mouse model of Alternating Hemiplegia of Childhood
Marlee Szabo, Elie Abdelnour, Monisha Sachdeva, Arsen Hunanyan, Mohamad Mikati, Division of Pediatric Neurology, Duke University Medical Center, Durham, NC

Background & Hypothesis: Dystonia is closely tied to dysfunction in both the basal ganglia and cerebellum. Recent work has shown that perfusion of ouabain, a selective blocker of the alpha-3 isoform of the Na/K-ATPase, into the cerebellar cortex is sufficient to induce dystonia in mice. Cerebellar Purkinje cells (PCs) exclusively express the alpha-3 isoform of the Na/K-ATPase, making them particularly sensitive to mutations in the ATP1A3 gene. Multiple clinical studies of individuals with ATP1A3 disorders have observed reduction in Purkinje cell number in patients. Given that ATP1A3 is highly expressed in cerebellar PCs, and given that this region has been implicated in dystonia, we hypothesize that cerebellar Purkinje cell number will be reduced in one knock-in (E815K) model of Alternating Hemiplegia of Childhood (AHC). Objective: This study investigated whether dystonia in the E815K mouse model of AHC, which manifests prominent dystonia and is associated with ATP1A3 dysfunction, could result from cerebellar PC loss. Methods: Mouse brains were extracted, sectioned, and immunostained using GAD67 to identify GABAergic cells. PCs were identified by stain quality, morphology, and location. Cell numbers were quantified using stereological analysis in the cerebellar vermis and each of the cerebellar hemispheres. Mean cell counts were then compared using Student's t-test. Results: Vermis PC counts did not show a significant difference between heterozygous E815K mice (HET, N=4) and wild type littermate controls (WT, N=4), (123797 ± 38553 vs 112119 ± 18722, p = 0.60543). Similarly, the cerebellar hemispheres, when considered together, showed no significant difference in PC numbers between HET and WT (132886 ± 32817 vs 117716 ± 20214, p = 0.46116). Comparing the hemispheres separately as well as total PC number between HET and WT also yielded no significant results. Conclusions: These results indicate that the pathophysiology in dystonia in the model does not involve reduction in cells in the cerebellar cortex. This is reason to investigate PC physiology as well as other cell types as explanations for dystonia in the E815K model.
#35 Title & Authors: The role of nucleoporins in CALM-AF10 and CRM1-AF10 leukemogenesis.
Sei-Gyung K. Sze1, Sarah A. Port2, Ralph H. Kehlenbach2, Catherine P. Lavau1 & Daniel S. Wechsler1
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Background & Hypothesis: The t(10;11) CALM-AF10 translocation occurs in pediatric patients with T-ALL and AML and is associated with a poor prognosis. CALM-AF10 leukemias are characterized by upregulation of HOXA expression, which is a known driver of leukemogenesis. Interaction of the CALM-AF10 oncoprotein with the nuclear export factor CRM1/XPO1 is essential for upregulation of HOXA expression. We have previously shown that CRM1 interacts with HOXA chromatin, suggesting that CRM1 recruits CALM-AF10 to its target genes. CRM1 can also substitute for CALM, such that CRM1-AF10 also induces HOXA gene transcription and induces leukemias in mice. However, CRM1 does not contain any recognized DNA-binding domains, implying that other proteins must be involved. Nucleoporins (NUPs) such as NUP98 and NUP214 are components of the nuclear pore complex and interact with CRM1 during nucleocytoplasmic transport of macromolecules. Both NUP98 and NUP214 are also involved in leukemogenic translocations that result in HOXA transactivation.

Objective: To determine whether the NUP214 nucleoporin cooperates with CRM1-AF10 to transactivate HOXA genes and induce leukemias. Methods: A recent report identified several binding residues within CRM1 that are important in mediating interaction with NUP214 (Port, Cell Reports 2015). We created a mutated CRM1mutNUP-AF10 expression vector in which binding to NUP214 is impaired, but interaction with nuclear export signal-containing molecules is retained. Using Hoxa7- and Hoxa9-luciferase reporter assays, we measured Hoxa gene transcription in HEK293 cells transiently transfected with either CRM1-AF10 or CRM1mutNUP-AF10 constructs. Murine hematopoietic progenitor cells transduced with these fusion vectors were transplanted into sublethally irradiated mice that continue to be evaluated for leukemia development. Results: The ability of CRM1mutNUP-AF10 to activate Hoxa7- and Hoxa9-luciferase reporters was reproducibly decreased (by 10-20%) compared with CRM1-AF10. Mice were transplanted with CRM1-AF10- and CRM1mutNUP-AF10- transduced hematopoietic progenitors five months ago (September 2016) and continue to be monitored for development of leukemia. The average leukemia latency for CRM1-AF10 transduced cells is 100 days; thus far, none of the CRM1mutNUP-AF10-transplanted mice (n=5) has developed leukemia. Conclusions: Interfering with the ability of CRM1 to interact with NUP214 moderately diminishes Hoxa7 and Hoxa9 transcriptional activation. Ongoing in vivo experiments suggest that the interaction of CRM1 with NUP214 may contribute to CRM1-AF10 leukemogenesis. We are currently performing ChIP studies to directly assess the binding of CRM1mutNUP-AF10 to Hoxa genes. Targeting the CRM1-NUP interaction may be a novel therapeutic approach for CRM1-dependent leukemias.

#36 Title & Authors: HIV Env-specific vaccine-elicited immune responses in adult and infants immunized with a MF59-adjuvanted rgp120 vaccine.

Background & Hypothesis: We have previously reported that infants immunized with an MF59-adjuvanted rgp120 vaccine developed greater magnitude and longer duration potentially-protective anti-V1V2 IgG responses than adults from the moderately effective RV144 trial in which participants were immunized with an ALVAC-prime/Alum-adjuvanted AIDSVAX boost strategy. To determine if the differences between adults and infants are due to different vaccine regimens, we compared infant responses to that of adults immunized with the same rgp120 vaccine. We hypothesize that the distinct vaccine response between adults and infants is due to inherent differences in the adult and infant immune systems.

Objective: Compare Env-specific IgG responses in adults and infants immunized with an MF59-adjuvanted rgp120 vaccine and define the characteristics of infant vaccine-elicited monoclonal antibodies (mAbs).

Methods: The magnitude and duration of IgG against gp120 and the V1V2 region were measured in adult (AVEG 201 trial) and infant (PACTG 230 trial) vaccine recipients using a binding antibody multiplex assay (BAMA). In addition, binding and avidity against a clade B V2 peptide were assessed by ELISA. Antigen-specific B cells were sorted from four infant vaccinees and mAbs were produced. The immunogenetic characteristics (VH gene usage, CDR3 length, mutation rate), and epitope specificity of the mAbs were then evaluated.

Results: At peak immunogenicity, the magnitude of Env-specific IgG responses was higher in vaccinated infants than in adults (MNgg120 median MFI: infants 15,509, adults 2290; p<0.001). Moreover, potentially protective V1V2-specific IgG binding was greater than one log higher in vaccinated infants compared to adults (median infant MFI 23926, median adult MFI 1538; p<0.001) and remained higher 6-7 months post-peak immunogenicity (median MFI, infants: 11, infants: 2523; p=0.018). The avidity index (AI) of V2-specific IgG was comparable at peak immunogenicity between adults and infants (mean infant AI 0.6, mean adult AI 0.56, p=0.46). Out of 61 functional mAbs isolated from 4 infants, 30 were reactive against Bcon gp140 of which 21 were gp120 reactive (2 to V3, 12 to CD4-bs, 1 to C5, and 6 conformational). Infant vaccine-elicited Abs contained relatively long heavy chain CDR3 regions (median 19 aa) and displayed low levels of mutation in variable heavy genes (0.5 to 3.5%).

Conclusions: Our findings suggest that infant responses to HIV Env immunogens are equal or higher in magnitude and durability to those of adults and underline the need for more pediatric HIV vaccine trials.
**#37 Title & Authors:** Rhesus macaque-derived antibodies use V\textsubscript{H}3 to bind the influenza hemagglutinin stem  


**Background & Hypothesis:** Despite annual vaccinations, influenza evades host immune responses and causes worldwide seasonal epidemics. Changes in non-conserved regions of influenza proteins results in the need for annual vaccine updates. Eliciting antibodies against the conserved stem region of hemagglutinin might allow broader coverage against some influenza variants. **Objective:** To determine if rhesus macaques (RMs) develop stem antibodies similar to those found in humans. **Methods:** RMs were immunized with purified recombinant HA (H1 A/Solomon Islands/03/2006) from which we sorted single B cells and isolated monoclonal antibodies (mAbs). We tested plasma and mAbs for binding and blocking by ELISA, influenza microneutralization, hemagglutination inhibition (HAI), pseudovirus neutralization, and antibody-dependent cellular cytotoxicity (ADCC). We performed site-directed mutagenesis to test which mAb residues were critical for stem binding. **Results:** RMs developed binding antibodies against multiple influenza strains (eg, H1, H3, H5) and high titers of neutralizing antibodies against the immunizing strain. RM sera blocked the binding of a stem-directed mAb (CR6261) to the HA immunogen. We isolated 9 RM mAbs that bound H1 and H5 HA proteins; 7/9 (78%) blocked CR6261 binding by ELISA. All blocking mAbs used a similar variable region heavy chain gene segment (V\textsubscript{H}3-J); non-blocking mAbs V\textsubscript{H}4 gene segments. RM mAbs had minimal microneutralization or HAI activity, but the CR6261-blocking mAbs were active against multiple strains in a pseudovirus neutralization assay. Sequence analysis of the blocking mAbs showed that all had phenylalanine (Phe) residues in locations that could be important for binding: heavy chain complementarity determining region 1 (HCDR1) (position 29), framework 3 (FW3) (position 68), and in the center of HCDR3 (position 105). We selected 3 mAbs for further testing; these mAbs all mediated ADCC in vitro. Testing of site-directed mutants of the mAb Phe residues showed that the HCDR1 and HCDR3 residues were critical for binding, blocking, and ADCC activity, but changes to the FW3 residue had little impact. **Conclusions:** RMs immunized against influenza develop a response similar to that seen in some humans. Future studies are needed to show if RM-derived stem antibodies could provide protection against infectious influenza challenge.

**#38 Title & Authors:** “Lactoferrin and protection against postnatal cytomegalovirus infection in premature infants” Kristin Weimer, MD, PhD, Stevie Rowe, MD, Margarita Bidegain, MD and Sallie Permar, MD, PhD

**Background & Hypothesis:** Very low birth weight (VLBW) preterm infants are at high risk for life-threatening infections during hospitalization in the neonatal intensive care unit (NICU). Providing breast milk to preterm infants provides protection against infections and is highly recommended for all preterm infants. However, breast milk feeding carries the risk of pathogenic infection by cytomegalovirus (CMV) shed in their mother’s breast milk. While harmless in full term infants, postnatal CMV in VLBW infants can result in a severe sepsis-like illness. It has recently been shown that preterm infants with postnatal CMV infection have an increased risk of BPD at 36 weeks corrected gestational age. A potential solution is to harness the innate antimicrobial properties of human milk to naturally protect vulnerable infants against CMV acquisition. Lactoferrin is a natural breast milk and saliva protein known to have potent activity against CMV. A recent clinical trial showed that enteral administration of bovine lactoferrin to preterm infants was safe and protective against bacterial sepsis. Establishment of a role for lactoferrin in prevention of postnatal CMV transmission would implicate its use as a prophylactic agent to protect VLBW infants against the severe effects of postnatal CMV infection. We hypothesize that high CMV load in breast milk will be associated with an increased risk of postnatal transmission of CMV, while a high concentration of lactoferrin in breast milk and saliva will be associated with a decreased risk of postnatal transmission of CMV. **Objectives:** To determine the incidence of postnatal CMV acquisition in VLBW infants in the Duke NICU and to determine the relationship between breast milk and saliva lactoferrin concentration, breast milk CMV load, and acquisition of postnatal CMV. **Methods:** We will enroll 76 VLBW (< 1500 g) preterm, maternal breast milk fed infants hospitalized in the Duke NICU and their lactating mothers and follow them for 6 months, or until discharge. Maternal breast milk and infant saliva samples will be collected every 2 weeks. To determine the infant’s CMV exposure status, the mother’s milk will be tested for CMV IgG. Postnatal infant CMV acquisition and CMV load in maternal mother’s milk will be determined using a CMV quantitative PCR assay. Breast milk and saliva levels of lactoferrin will be determined using commercial ELISA kits. **Results & Conclusion:** To date, we have enrolled 71 VLBW infants exposed to maternal milk. Of the samples tested, at least 5/10 mothers from the mother-infant pairs were positive for CMV IgG in breast milk. So far, no infants are positive for CMV in saliva. Finding a natural protein, like lactoferrin, that can prevent postnatal CMV acquisition would identify a valuable prophylactic agent that can be given to protect this vulnerable population.
#AA  Title & Authors: Antibody-mediated enzyme replacement therapy targeting both lysosomal and cytoplasmic glycogen in Pompe disease. Haiqing Yi, Tao Sun, Dustin Armstrong, Chunyu Yang, Stephanie Austin, Priya S Kishnani, and Baodong Sun

Background & Hypothesis: In addition to lysosomal glycogen accumulation due to the lack of lysosomal enzyme acid α-glucosidase (GAA), cytoplasmic glycogen accumulation resulting from rupture or shearing of lysosomes also occurs with disease progression in Pompe disease. Traditional enzyme replacement therapy with recombinant human GAA (rhGAA) via mannose-6-phosphate receptor (M6PR) delivers the enzyme to lysosomes only and thus cannot clear cytoplasmic glycogen. Antibody 3E10 (and its Fab fragment) can penetrate living cells and delivers "cargo" proteins to the cytosol via equilibrative nucleoside transporter 2 (ENT2). We speculate that a fusion protein of the Fab fragment of 3E10 with human GAA (FabGAA) will target both lysosomal and cytoplasmic glycogen in Pompe disease.

Objective: To examined the cell penetration and intracellular processing of FabGAA, and its efficacy in reducing glycogen accumulation in Pompe disease (GAA-KO) mice.

Methods: Uptake of FabGAA was examined in L6 rat myoblasts and Pompe patient primary fibroblast cells to vindicate both the cytosolic (via ENT2) and lysosomal (via M6PR) delivery. To test in vivo efficacy, FabGAA (30 mg/kg) was weekly injected via tail vein for 4 weeks to GAA-KO mice. Tissues were collected 48h after the last injection. GAA activity and glycogen content were measured, and PAS stain and Western blotting were performed for major affected tissues.

Results: In cultured cells, immunostaining with an anti-Fab antibody indicates that FabGAA resides outside the lysosomes; in Western blots, the full-length (150-kDa) FabGAA protein can be detected using an anti-GAA antibody, but the majority of GAA was in lysosomal forms (95-kDa and 76-kDa). In GAA-KO mice, FabGAA had efficacy similar to rhGAA in clearing lysosomal glycogen in tissues, and the protein can be detected in tissues as mature lysosomal forms 48h after the last enzyme injection. In a short-term experiment, strong bands of the full-length FabGAA were observed at 3h after FabGAA injection but became much weaker at 9h in heart and skeletal muscles.

Conclusions: FabGAA can be delivered to cells through both ENT2-mediated route and M6PR-mediated route. FabGAA retains the ability of rhGAA to treat lysosomal glycogen accumulation, and has the beneficial potential over rhGAA to reduce cytoplasmic glycogen storage in Pompe disease.

#39 Radical and Partial Nephrectomy in Children and Young Adults: Equivalent Readmissions and Postoperative Complications Muhammad H. Alkazemi, MS, Ruiyang Jiang, MD, Steven Wolf, Gina Marie-Pomann, PhD, J. Todd Purves, MD, PhD, John S. Wiener, MD, and Jonathan C. Routh, MD, MPH

Background & Hypothesis: Radical nephrectomy (RN) is the standard of care for renal masses in children. There has been increasing interest in partial nephrectomy (PN), particularly for bilateral renal tumors and unilateral syndromic tumors. The use of PN for unilateral non-syndromic renal tumors remains controversial. There is little national surgical data characterizing outcomes from RN and PN.

Objective: The objective of this study was to characterize these surgical interventions on a nationally representative database stratified by cohort age.

Methods: The 2013 Nationwide Readmissions Database (NRD) was used to obtain RN and PN readmissions data. ICD-9-CM codes were used to identify children (<10 y), adolescents (10-19 y) and young adults (20-30 y) diagnosed with benign and malignant renal tumors who were treated with an RN or PN. The primary outcome of this study was the presence of a 30-day readmission, and the secondary outcome was the occurrence of postoperative complications as described in the National Surgical Quality Improvement Program (NSQIP).

Patients with a primary lesion elsewhere in the genitourinary system, or who underwent both an RN and PN, were excluded. Encounters in December were omitted to ensure a 30-day follow-up period. A weighted multivariate logistic regression model was used to adjust for gender, insurance type, income, hospital type, and comorbidity.

Results: There were 962 patients (638 RN, 324 PN) that met inclusion criteria: 47% were children, 11% were adolescents, and 42% were young adults. Children and adolescents were more likely to receive an RN whereas young adults were more likely to receive a PN (p<0.0001). Overall, there was no significant association between the type of nephrectomy performed and the presence of a 30-day readmission or postoperative NSQIP complication. However, adolescents who underwent an RN, rather than a PN, were less likely to have a 30-day inpatient readmission (OR 0.07; 95% CI: 0.013-0.353; p<0.002). This datum should be interpreted cautiously given the smaller adolescent sample size compared to the other age groups (n=105). There was no difference in 30-day readmissions between an RN and a PN in children or young adults. The occurrence of postoperative NSQIP complications did not differ between RN and PN in all age groups.

Conclusion: There was no significant difference between RN and PN in terms of postoperative readmissions or in-hospital complications.
#40 Title & Authors: “Race, ethnicity, and poverty influences on physical activity in youth from a nationally representative sample, 2007-2014” Sarah Armstrong, MD; Eliana Perrin, MD, MPH; Asheley Skinner, PhD

**Background & Hypothesis:** Physical activity improves health, quality of life, and reduces obesity among adolescents. Multiple studies have concluded that living in a neighborhood that is safe and supportive of physical activity is highly predictive of increased activity. However, national survey data have shown that adolescents from low-income and racially diverse groups are less likely to live in a neighborhood that supports physical activity. What is unknown is what levels of activity adolescents experience by income and race/ethnicity, and if that is confounded by weight status.

**Objective:** To describe physical activity duration and intensity of adolescents by income, race, and ethnicity using a nationally representative sample.

**Methods:** We used the National Health and Nutrition Examination Survey (NHANES) for years 2007-2014 with reported physical activity data for adolescents aged 12-18 years (n=8,849), and for years 2013-14 with reported school sport participation for children aged 5-15 years (n=2,257). We used statistical tests to examine the total amount of moderate and vigorous physical activity and school sport participation by race, ethnicity, and income. We examined weight as a covariate (right term?) of the relationship between race, ethnicity, income and physical activity.

**Results:** In all weight-status groups, Black and Hispanic adolescents were less likely to report moderate and vigorous levels of physical activity (numbers). Income was directly related to the amount of moderate and vigorous physical activity (numbers), and the relationships are linear. Higher income and White children are more likely to participate in school sports (numbers). When weight status was considered, only the relationship between income and physical activity remained.

**Conclusions:** In a large, nationally-representative sample, child moderate-to-vigorous physical activity and school sport participation are directly related to income, and the lower the income, the less likely the child is to be active. The relationship between activity and income was stronger than the relationship between activity and race, ethnicity, or weight status. Low-income neighborhoods are a potential high-yield target for future interventions to improve the support for safe physical activity for children and adolescents.

#41 Title & Authors: “Is the switch to low-fat milk making children more healthy? Associations between milk-fat type consumed and child obesity from the National Health and Nutrition Examination Survey (NHANES), 1999-2014” Sarah Armstrong, MD; Eliana Perrin, MD, MPH; Asheley Skinner, PhD

**Background:** Dietary guidelines recommend consumption of reduced (2%), low (1%), or nonfat milk in place of whole (4%) milk to reduce obesity. As of 2014, the Women, Infants and Children (WIC) program offers skim or 1% milk starting at 12 months. However, recent data suggest that switching to low-fat milk may not lower body mass index (BMI). It is not known if low-fat milk consumption is related to lower obesity prevalence.

**Objective:** To investigate the association between whole milk vs. low-fat milk consumption and child BMI among a nationally representative sample.

**Methods:** We used repeated cross-sections of NHANES, 1999-2014. First milk consumed after formula/breast was asked of parents of children ≤ 6 years (n=8,727). Current milk most commonly consumed was asked of those 2-20 years of age (n=32,824). We measured the association between milk type (whole, 2%, 1%, skim, other) and BMI percentile derived from measured height and weight. Categories of obesity using age- and sex-specific BMI percentiles, or BMI for those 18-20, were: healthy weight (5th – 85th; 18.5-25 ), overweight (>85th – 95th; >25-30), class I obesity (>95th - <120% of the 95th percentile; >30-35), class II obesity (120 - 140% of the 95th percentile; >35-40), and class III obesity (>140% of the 95th percentile; >40). We used logistic regression, controlling for race, age, income, and NHANES cycle.

**Results:** Children who were weaned from breast or bottle to whole milk were more likely to be a healthy weight (79% healthy vs. 73% obese, p=0.023) than those weaned to lower fat milk, even when controlling for demographic factors (aOR=0.81, p=0.056). Current whole milk consumption was also associated with lower odds of obesity (aOR=0.75, p<0.001). Time trends show a continuous decrease in consumption of whole milk, controlling for other factors.

**Conclusions:** Although low-fat milk is calorically less dense than whole milk, consumption of low-fat milk is associated with higher, not lower, BMI. Children with obesity may be more likely to drink low-fat milk as weight control. Future studies should utilize a design that allows for clear determination of causation. These data stress the need to focus weight-targeted recommendations on dietary behaviors with a stronger evidence base.
#42 PRKAG2 as a Mimicker of Pompe Disease

Stephanie L. Austin, MS1, Rachel D. Torok, MD2, Chanika Phornphutkul, MD3 Kathleen M. Rotondo, MD4, Anne F. Buckley, MD, PhD5, Gregory H. Tatum, MD2, Stephanie B. Wechsler, MD1,2, Priya S. Kishnani, MD1

1Divisions of Medical Genetics and 2Pediatric Cardiology, Department of Pediatrics, and 5Division of Pathology Clinical Services, Department of Pathology, Duke University Medical Center, Durham, North Carolina. 3Divisions of Human Genetics and 4Pediatric Cardiology, Department of Pediatrics, Warren Alpert Medical School of Brown University, Rhode Island Hospital, Providence Rhode Island.

**Background & Hypothesis:** PRKAG2 encodes the γ2 subunit of AMP-activated protein kinase (AMPK), which is an important regulator of cardiac metabolism. Mutations in PRKAG2 cause a cardiac syndrome comprised of ventricular hypertrophy, preexcitation, and progressive conduction system disease, which is typically not diagnosed until adolescence or young adulthood. However, significant variability exists in the presentation and outcomes of patients with PRKAG2 mutations.

**Methods:** We add three cases to the five previously described where patients with PRKAG2 mutations presented with symptoms in infancy.

**Results:** In all three of our cases, Pompe disease was the suspected diagnosis, with two going on to receive enzyme replacement therapy. However, Pompe disease was eventually ruled out, and a disease causing PRKAG2 mutation was identified in each case.

**Conclusions:** We highlight the potential for PRKAG2 mutations to mimic Pompe disease in infancy and the need for confirmatory testing when diagnosing Pompe disease.

#43 Diagnostic testing and novel approach to Fabry Disease screening in the US population ("the Program")

Deeksha Bali, Haoyue Zhang, Jian Dai, Patricia McCaw, James Beasley, Denise Peterson, Ashlee Stiles, Dwight Koeberl, Marie McDonald, Catherine Rehder, Sarah Young

**Background & Hypothesis:** Fabry disease is an X-linked lysosomal storage disorder associated with glycosphingolipid accumulation in all tissues. In males the disease manifests in childhood with acroparathesia, hypohidrosis, gastrointestinal involvement, angiokeratoma and corneal whoring. In adulthood the disease progresses to end stage renal disease, cardiomyopathy and other cardiac abnormalities, and stroke. It is considered an X-linked dominant disorder as a majority of heterozygotes are also affected. Its estimated incidence is 1 in 50,000 males, however recent data from newborn screening programs in Missouri, Illinois and Taiwan have suggested a much higher incidence. Diagnosis of Fabry disease is often delayed or missed, especially in females, due to variability in the presentation and phenotype, the nonspecific nature of some of the features, and a lack of awareness of Fabry disease. Timely diagnosis and institution of appropriate treatments such as enzyme replacement therapy before irreversible damage occurs is expected to improve outcomes for most patients. Diagnostic tests for Fabry disease include alpha-galactosidase A (alpha-GAL) activity in various tissues and GLA full gene sequencing. Recently, plasma lyso-globotriaosylceramide (lyso-Gb3) has been reported to be a sensitive biomarker for Fabry disease. **Objective:** Our objective was to compare the sensitivity of alpha-GAL activity, GLA gene sequencing and plasma lyso-Gb3 for diagnosing males and females with Fabry disease in high risk populations, and to determine the most cost effective and sensitive testing algorithm.

**Methods:** Whole blood samples sent to the Duke Biochemical Genetics lab were: 1) dried on filter paper (dried blood spot sample) for alpha-GAL activity measurement using a fluorimetric method, 2) isolated for DNA for GLA full gene sequencing by Sanger sequencing, and 3) separated for measurement of plasma lyso-Gb3 UPLC-MS/MS. **Results:** Over a 12 month period (January 2016-January 2017) blood samples from 390 patients (152 males and 238 females) were received for Fabry testing. The reasons for referral varied from a positive family history, and/or because of one or more combination of symptoms suggestive of Fabry disease. Using a combination of all three methods, 36 males (9.2%) and 39 females (10%) were confirmed to have Fabry disease. Alpha-GAL activity had the highest sensitivity as an initial screen for diagnosing male patients. Reflexing to gene sequencing and plasma lyso-Gb3 was useful for confirmation. In contrast females with Fabry disease were most efficiently diagnosed by plasma lyso-Gb3 measurements combined with full gene sequencing.

**Conclusions:** Fabry disease is underdiagnosed in both males and females affected with the disease. For many patients, the diagnostic delays places considerable burden on the families and delays treatment. We have instituted a cost effective and sensitive testing program to screen male and female populations at increased risk of Fabry disease.
#44 Title & Authors: Indications and Outcomes of Temporary Mechanical Circulatory Support in Pediatric Patients with Cardiac Failure.

David Bearl, MD, Robert D.B. Jaquiss, MD, Travis P. Vesel, MD

**Background & Hypothesis:** Temporary continuous-flow ventricular assist devices (cfVAD) have been approved for acute cardiogenic shock due to right heart failure, but are increasingly used for longer and for left heart failure. Prior to the availability of temporary VADs, extracorporeal membrane oxygen (ECMO) had been the exclusive temporary mechanical circulatory available. Despite the increase in pediatric temporary cfVAD use, the literature describing its use in pediatrics is limited. **Objective:** To describe and compare indications and outcomes of pediatric patients with cardiac failure who underwent ECMO cannulation or placement of temporary cfVADs.

**Methods:** Single-center retrospective review of temporary cfVAD and cohort of similar ECMO patients (based on pre-defined exclusion criteria) between January 1, 2011 and June 30, 2016 in patients < 19 years of age at the time of surgery. Pre-implantation demographics, laboratory and imaging data collected. The primary outcome was death, survival with decannulation, conversion to durable VAD or transplant. Multiple secondary outcomes were collected as well. Analysis was limited to descriptive elements given low patient numbers and heterogeneity of the patient characteristics. **Results:** Thirteen patients underwent temporary cfVAD during the study time period. After exclusion of 154 patients, eleven patients were identified who underwent ECMO cannulation for similar indications. cfVAD patients averaged 5.8 years old and 27 kg, while ECMO patients averaged 4.6 years and 24.4 kg. Diagnosis breakdown between cfVAD and ECMO was 2 vs 4 myocarditis, 6 vs 8 dilated cardiomyopathy, and 1 vs 3 with failed congenital heart disease palliation, respectively. Pre-cannulation data were not clearly different. Mean length of cfVAD support was 56.8 days (range 6 to 227), compared to 8.4 days (range 1 to 15) on ECMO. Primary outcome for cfVAD patients overall was 6 successfully transplanted, 6 died and 1 decannulated with recovery, compared to ECMO patients with 3 successfully transplanted, 3 died and 5 decannulated with recovery. Detailed description of cfVAD patient courses used to illustrate the heterogeneity and complexity of this group of patients. **Conclusions:** This review provides new evidence that temporary cfVAD use can be successfully used to support pediatric patients over longer periods of time, much longer than ECMO, with transition to durable VAD and transplant.

#45 Title & Authors: Multicenter cohort study of opioid use for neonates treated with therapeutic hypothermia for neonatal hypoxic-ischemic encephalopathy

Megan W. Berube, Rachel G. Greenberg, Monica E. Lemmon, Carolyn E. Pizoli, Margarita Bidegain, Reese H. Clark, C. Michael Cotton

**Background & Hypothesis:** Therapeutic hypothermia (TH) improves outcomes for term and near-term neonates with moderate to severe hypoxic-ischemic encephalopathy (HIE). While some preclinical research supports sedation and analgesia during TH for HIE, safety and efficacy data for this population is lacking. **Objective:** Determine the prevalence of opioid use for sedation and analgesia in a cohort of neonates treated with TH for HIE.

**Methods:** In this retrospective cohort study, we identified neonates with HIE who were treated with fentanyl or morphine during TH at Pediatrix Medical Group hospitals between 2007 and 2015. We compared characteristics of neonates who were exposed to opioids during TH with those who did not receive opioids during TH. We reported use of opioids over time and prevalence of use at centers that provided TH for ≥10 neonates. We also assessed prevalence of use of opioids in combination with dexmedetomidine, phenobarbital, benzodiazepines, or other antiepileptic drugs (AED). Differences in characteristics among groups were assessed using Fisher’s exact test.

**Results:** 2621 neonates underwent TH in 125 Pediatrix NICUs during the study period, with 64 centers providing TH for ≥10 neonates. Opioid use during TH increased from 33% to 68% over time. Exposure prevalence varied widely by center, ranging for <10% to 100% concurrent opioid use during TH. 874 (33%) neonates received morphine during TH, 938 (36%) received fentanyl, and 135 (5%) received both. 65% of neonates with Apgar score ≥7 at 10 minutes of life received opioids, compared to 58% of neonates with Apgar score < 4 (P=0.003). Dexmedetomidine was not given to any neonates during the study period. Neonates treated with opioids were more likely to receive benzodiazepines (63% vs. 24%, P<0.001). Neonates treated with opioids were less likely to receive phenobarbital (43% vs. 48%, P = 0.02) or other AED (3% vs. 6%, P = 0.005).

**Conclusions:** Opioid use during TH is prevalent but not uniform across sites. Benzodiazepine exposure was strongly associated with exposure to opioids, suggesting common use of benzodiazepines for sedation during TH. Safety and efficacy profiles of sedative and analgesic agents alone and in combination with other medications used during TH for HIE are needed to better inform practice.
Echocardiography use in patients meeting 'Rarely Appropriate' indicates is of little diagnostic utility and contributes to additional cost to the patient and healthcare system.
**Background & Hypothesis:** Outcomes can be challenging to predict in children with mild traumatic brain injury (initial GCS of 13-15). Transcranial Doppler (TCD) ultrasound has become an increasingly useful modality in adult and pediatric traumatic brain injury (TBI) by measuring blood flow velocities within the Circle of Willis. In children with moderate to severe TBI multiple studies have correlated abnormal TCD measurements and poor outcomes. Additionally, TCD has shown value in assessing adults with mild brain injury. To date there are no studies that correlate TCD findings and outcomes in children with mild TBI. We hypothesize that altered cerebral blood flow after mild TBI is associated with poor functional outcome using the Glasgow Outcome Scale-Extended, Pediatrics (GOS-E Peds).

**Objective:** Describe cerebral blood flow velocities in mildly brain injured children. Correlate abnormal flow velocities with outcome at discharge and follow-up

**Methods:** TCD was performed within 24 hours of admission on 60 patients at a tertiary level-1 children’s hospital. A secondary analysis was performed on the subgroup of mild TBI patients. TCD measures included mean middle cerebral artery flow velocity (Vmca). GOS-E Peds was measured at the time of hospital discharge and 4-6 weeks post-discharge.

**Results:** 28 patients were included in the analysis. 57% of patients were admitted to the ICU. Vmca did not show correlation with outcome. At discharge, the right sided Spearman’s correlation coefficient was +0.19 (p-value=0.33) and the left sided was +0.36 (p=0.06). At follow up the right sided coefficient was -0.25 (p=0.24), the left sided was -0.04 (p=0.84).

**Conclusions:** Though our data did not show correlation, it showed that the investigation could feasibly be done in pediatric patients with mild TBI. Our study was limited by relatively small sample size, sample heterogeneity and infrequent outcome of interest. Future studies may help define the potential role of TCD in the large population of mild pediatric TBI patients.

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**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. Desai AK1, Kazi ZB1, Martin RF2, Terry FE2, Martin WD2, De Groot AS2, Kishnani PS1

1 Division of Medical Genetics, Department of Pediatrics, Duke University Health System, Durham, NC. 2 EpiVax Inc., Providence, RI, United States of America.

**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 for all patients via chi-squared test.

**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.

Amy C. Gaultney, Maggie Bromberg, Mark Connelly, Tracy Spears, and Laura E. Schanberg

While previous research in juvenile idiopathic arthritis (JIA) has identified discrepancy between parent and child perception of disease-related symptoms such as pain, the significance and impact of this disagreement has not been characterized. We examined the extent to which parent-child discordance in JIA symptom ratings are associated with child functional outcomes. Linear regression and mixed effects models were used to test the effects of discrepancy in pain and fatigue ratings on functional outcomes in 65 dyads, consisting of youth with JIA and one parent. Results suggested that children reported increased activity limitations and negative mood when parent and child pain ratings were discrepant, with parent rated child pain much lower. Greater discrepancy in fatigue ratings was also associated with more negative mood, whereas children whose parent rated child fatigue as moderately lower than the child experienced decreased activity limitations relative to dyads who agreed closely on fatigue level. Our work suggests that disagreement in parent and child rating of pain and fatigue is associated with functional outcomes.

#51 Title & Authors: Deadspace to Tidal Volume Ratio as a Predictor of Extubation Success

Jonathan A Gehlbach, MD, Andrew G Miller, RRT-ACCS-NPS, Christoph P Hornik, MD, Ira M Cheifetz, MD

**Background & Hypothesis:** Extubation failure is associated with increased morbidity and a longer intensive care unit stay in pediatrics. Tools that predict extubation readiness may improve outcomes in mechanically ventilated pediatric patients. We hypothesized that the deadspace to tidal volume ratio (Vd/Vt) predicts extubation success.

**Objective:** Test the hypothesis that a lower Vd/Vt is predictive of extubation success.

**Methods:** We report an interim analysis of a prospective, observational cohort study of all patients <18 years old extubated in the pediatric ICU (PICU) and pediatric cardiac ICU (PCICU) at a university hospital. We excluded patients extubated as part of withdrawal of care, those with tracheostomies, and those without an arterial blood gas within 12 hours prior to extubation. Patients were followed for 48 hours after extubation to monitor for escalation in respiratory support and need for re-intubation.

**Results:** To date, we have analyzed data on 86 of our targeted 400 patients. Of these, 59% were male with an average age of 4 years. Sixty-three percent were PCICU patients. Eight subjects required re-intubation (9.3%). There was no significant difference between the Vd/Vt values at the time of extubation for patients who remained extubated compared to those who required re-intubation (0.27 ± 0.12 vs. 0.30 ± 0.09, p = 0.43). There was no difference in Vd/Vt trend at the time of extubation (i.e., improving deadspace fraction) between groups. On average, those who were re-intubated had a longer duration of mechanical ventilation (prior to initial extubation) than those who remained extubated (221 ± 143 hours vs. 111 ± 137 hours, p = 0.02). The causes of extubation failure are described in the table.

<table>
<thead>
<tr>
<th>Diagnostic Category for Extubation</th>
<th>n</th>
<th>Individual Vd/Vt Ratios at Time of Extubation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiovascular</td>
<td>4</td>
<td>0.30, 0.33, 0.38, 0.44</td>
</tr>
<tr>
<td>Upper Airway</td>
<td>2</td>
<td>0.16, 0.24</td>
</tr>
<tr>
<td>Procedure</td>
<td>1</td>
<td>0.33</td>
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<tr>
<td>Pulmonary</td>
<td>1</td>
<td>0.20</td>
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**Conclusions:** In this interim analysis, all patients who failed extubation did so with a relatively normal Vd/Vt. However, only 1 subject failed due to a primarily pulmonary etiology. Furthermore, this patient’s lung collapse was likely related to the removal of positive pressure, which would not have been predicted by the Vd/Vt. Reintubation indications other than parenchymal lung processes and the small number of re-intubations may be confounding our preliminary results. Therefore, analysis of the complete trial cohort of 400 subjects should be awaited prior to drawing definitive conclusions regarding the prognostic significance of the Vd/Vt.
#52 Title & Authors: HIV replication in infant tonsils is inhibited in presence of Hsp90 Inhibitor, Hs10.

Ria Goswami, Riley Mangan, Holly Heimsath, Joshua Eudailey, Philip Hughes, Guido Ferrari, Timothy Haystead, Barton Haynes and Sallie Permar.

**Background & Hypothesis:** Oral transmission of HIV-1 via breastfeeding accounts for more than half of the 150,000 pediatric infections occurring annually. Unlike transmission during delivery, HIV-1 transmission via breastfeeding is not readily diagnosed after infection, and ARV initiation is often delayed. Moreover, in spite of the clinical success of ART, it requires long time to attain virological control. Thus, targeting cellular pathways utilized by HIV can be an alternate antiviral therapy. Heat shock protein 90 (Hsp90) is a chaperone protein utilized by HIV for completion of its life cycle. We hypothesized that Hsp90 inhibitors can effectively reduce HIV replication in infant tonsils and other oral cavity-associated lymphoid tissues. **Objective:** Our aim was to develop an *in vitro* model of HIV infection in human tonsil tissues and investigate whether the Hsp90 inhibitor, Hs10 administered early after viral infection can inhibit viral replication. **Methods:** We isolated mononuclear cells from discarded bilateral tonsil tissues of children <10 years of age, and then infected them with CXCR4-tropic (HIVNLGI) or CCR5-tropic (HIVJRFL)-GFP-labeled strain of HIV-1. The infected tonsil mononuclear cells were then treated with either 1μM Hs10 or RPMI, 24 hr post infection. Tonsillar CD4+ T cells were then analyzed for productive infection (GFP+) 5 days after viral exposure. Naïve (CD28+ CD95-), central memory (CD28+ CD95+), transitional memory (CCR7- CD28+), effector memory (TCD28- CD95+), and activated (HLA-DR+, CD25+) CD4+ T cells were quantified by flow cytometry. **Results:** Our results indicate that a mean of 7% and 1% of tonsillar CD4+ T cells were infected by the HIVNLGI and HIVJRFL virus, respectively. HIV primarily infected central memory (Mean: 9.5% for HIVNLGI) populations as compared to naïve CD4+ T (Mean: 3.2% for HIVNLGI) cells. Treatment with Hs10, reduced the number of productively infected cells by 67% (mean), without promoting significant cell death. Specifically, Hs10 reduced the number of central memory (Mean: 77% reduction) and effector memory (Mean: 67% reduction) CD4+ T cells population, and resulted into reduced infection of naïve (mean: 45% reduction), central memory (Mean: 79% reduction), effector memory (Mean: 68% reduction) and activated populations (mean: 56% reduction) compared to mock treatment group. **Conclusions:** Hs10 has potency in blocking HIV replication in infant tonsils after an initial round of viral replication, suggesting that targeting Hsp90 is a potential antiviral strategy to impair the establishment of an oral viral reservoir in infants.

#53 Title & Authors: Safety of Diazoxide for Infants in the Neonatal Intensive Care Unit (NICU).

KG Gray, CM Cotten, RH Clark, RG Greenberg

**Background & Hypothesis:** Between 5-15% of all newborns develop hypoglycemia, but most cases resolve by 48 to 72 hours of life. Some infants with prolonged neonatal hypoglycemia are treated with diazoxide, but the incidence of diazoxide exposure and its association with adverse effects have not been well described. **Objective:** Describe demographic characteristics of infants requiring diazoxide in the NICU and incidence of diuretic use or increased respiratory support in infants during diazoxide treatment. **Methods:** This was a retrospective cohort study of all infants admitted to 1 of 382 NICUs from 1997 to 2015 using the Pediatrix database. We examined the incidence of diazoxide use and diagnosis of hypoglycemia over time and by center. Among infants with hypoglycemia, we compared demographic characteristics between those exposed and not exposed to diazoxide. Among diazoxide courses longer than one day, we reported the percentage of infants who required new exposure to diuretics during therapy and developed new ventilator or oxygen requirement during therapy. **Results:** A total of 990/1,158,789 (0.1%) infants were exposed to 1100 diazoxide courses, and 167,028/1,158,899 (14.4%) infants had a diagnosis of hypoglycemia. The percentage of infants exposed to diazoxide increased over time (P=0.002; Figure 1), and the percentage of infants with hypoglycemia exposed to diazoxide varied from 0%-15% among centers. Compared to infants with hypoglycemia who were not exposed to diazoxide, a notable percentage of those exposed to diazoxide were small or large for gestational age (P<0.001). The median total diazoxide exposure was 4 days (25th-75th percentile: 1-7), and 59/826 (7%) of infants exposed to diazoxide who were discharged home were receiving diazoxide at discharge. While 50/709 (7%) of diazoxide courses were started in infants already receiving diuretics, an additional 97/659 (14%) courses were associated with a new course of diuretics that began during diazoxide therapy. 140/689 (20%) diazoxide courses were started in infants on oxygen therapy at the start of their course, and an 64/549 (11%) courses were associated with new oxygen requirement during therapy. 60/699 (8.5%) of diazoxide courses were started in infants who required the ventilator at the start of therapy, and an additional 38/639 (6%) courses were associated with development of a new ventilator requirement during therapy. **Conclusions:** Diazoxide use has increased over time in infants hospitalized in the NICU. Infants exposed to diazoxide commonly require treatment with diuretics. Further prospective studies are needed to evaluate diazoxide safety.
**Title & Authors:** Pathogenesis of Type 2 Diabetes in Obese Adolescents: Metabolites of Serotonin and Mitochondrial Function in 24Hour Urine Samples  

Pinar Gumus Balikcioglu, MD, James Bain, PhD, Michael Muehlbauer, PhD, Thomas L O'Connell, PhD, Stuart Alan Chalew, MD and Michael Freemark, MD

**Background & Hypothesis:** Obesity and insulin resistance (IR) are the major determinants of risk for type 2 diabetes mellitus (T2D). Yet only onehalf of obese youth are insulin resistant and a relatively small proportion of these progress to T2D. To identify those at highest risk it is essential to characterize metabolic markers that predict the development of IR and glucose intolerance. In previous studies we used principal components analysis of fasting plasma samples to characterize metabolic markers of IR in obese adolescents. Here, we used nontargeted metabolic profiling of urine samples to characterize metabolic differences in obese youth with and without T2D. In contrast to single pointintime plasma analysis, urine metabolic profiling integrates differences in metabolic status over a 24 hour period. **Objective:** To identify key urinary metabolic signatures that distinguish obese youth with and without T2D.  

**Methods:** We recruited 33 obese AfricanAmerican youth with normal liver, renal, and thyroid functions from academic pediatric obesity and diabetes clinics. We excluded subjects taking medications other than metformin, insulin, or levothyroxine, and those with genetic syndromes. Patients on metformin discontinued the medication one day prior to study. 24-hour urine samples were analyzed by nontargeted, gas chromatography/mass spectrometry. Group differences were examined by Welch’s t-test.  

**Results:** Age, sex, and BMI were comparable between the groups. Among 187 metabolites identified in urine metabolic profiles, 3 metabolites were significantly higher in obese youth with T2D than in nondiabetic obese youth. Among these were the metabolites related to mitochondrial dysfunction and respiratory chain defects. In contrast, a single metabolite was strikingly lower (p=0.0007) in diabetic than in nondiabetic subjects; this was the level of 5hydroxyindoleacetic acid (5HIAA), the major metabolite of serotonin. **Conclusions:** Reductions in serotonin metabolism and mitochondrial dysfunction may contribute to diabetes pathogenesis through inhibition of insulin secretion. Validation of urine metabolomic profiling in future longitudinal studies could provide a new noninvasive approach to identification of biomarkers for metabolic risk in children as well as adults.

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**Title & Authors:** Outside the fiber: interstitial pathology of skeletal muscle in infantile Pompe disease  

Christine I Ha, Ankit K Desai, Justin Waterfield, Zoheb B Kazi, Stephanie L Austin, Edward H Bossen, Priya S Kishnani, Anne F Buckley

**Background & Hypothesis:** Pompe disease is an inherited, lysosomal disease in which deficiency of acid alpha glucosidase results in an accumulation of lysosomal glycogen. The outcome of infantile Pompe disease (IPD) has improved dramatically since the introduction of alglucosidase alfa enzyme replacement therapy (ERT). However, long-term survivors on ERT – even those treated from early infancy – demonstrate muscle weakness, dysphagia, motor speech deficits, and ptosis, indicating that current therapy is insufficient to prevent disease progression in skeletal muscle. Traversing the interstitium of skeletal muscle tissue is likely a key factor in the delivery of ERT to myocytes, but little is known about the details of interstitial pathology in IPD.  

**Objective:** The goal of this study is to characterize pathologic changes in capillaries and interstitium of skeletal muscle that could affect ERT efficacy and muscle function.  

**Methods:** Clinical muscle biopsies from IPD patients – taken before and during ERT – were reviewed via light microscopy using special stains and immunohistochemistry, and via electron microscopy.  

**Results:** We found that skeletal muscle biopsies from IPD patients frequently demonstrated expansion of the interstitial space, both before and during ERT. CD31 immunostaining and ultrastructural examination showed that even in skeletal muscle with markedly increased stroma, capillary presence and architecture was not qualitatively different from that of less-affected muscle. We did not often see inflammatory cells within the interstitium. Trichrome staining, reticulin staining, and type IV collagen immunostaining revealed that fibrosis was only one component of the stromal expansion. Glycogen was another component of the interstitium, which is unlike that of patients with other fibrotic myopathies.  

**Conclusions:** These newly-described skeletal muscle interstitial findings challenge some of our assumptions regarding the pathophysiology of IPD. Stromal changes could contribute to the limited efficacy of ERT in skeletal muscle and to skeletal muscle symptoms of long-term IPD survivors on ERT.
Title: Postoperative Venous Thromboembolism in Children is Increased in Setting of Cancer or Infection

Authors: Harold J. Leraas, M.A., Jina Kim, M.D., Zhifei Sun, M.D., Uttara P. Nag, M.D., Reed W. Kamyszek, B.S., Henry E. Rice, M.D., Obinna O. Adibe, M.D., Alexandra J Borst, MD, Jennifer A Rothman, MD, Jeffrey H. Lawson, M.D., Elisabeth T. Tracy, M.D.

Background: In adults postoperative venous thromboembolism (VTE) is associated with malignancy, trauma, and obesity. Although adult risk factors are often extrapolated to children, age related changes in hemostasis and lower rates of VTE may affect postoperative VTE risk in these patients. Therefore, we examined a national database to identify risks and outcomes of VTE in pediatric surgical patients. Objective: To identify factors predisposing children to postoperative VTE. We hypothesized that children would be more likely to experience postoperative VTE following major infections or in the presence of cancer. Methods: The 2012–2013 National Surgical Quality Improvement Program-Pediatric was queried to identify patients (ages 0-18) diagnosed with postoperative VTE. Perioperative outcomes were compared between propensity-matched patients who experienced VTE vs. those who did not using a 2:1 nearest neighbor algorithm. Univariate analysis was conducted using Kruskal-Wallis test for continuous variables and Pearson χ2 test for categorical variables. Risk factors for VTE were identified by multivariate analysis. Results: We identified 130 children who developed postoperative VTE. Patients developing VTE had increased median operative time (122.5 min vs. 79) and total length of stay (21.5 days vs. 4) compared to those who did not. In multivariate analysis major infections and active cancer significantly increased VTE risk. Specifically, pneumonia (odds ratio [OR] 1.73, 95% confidence interval [CI] 1.30–2.29, p<0.001), central line-associated bloodstream infection (OR 1.69, 95% CI 1.18–2.42, p<0.001), and sepsis (OR 1.47, 95% CI 1.18–1.82, p<0.001) demonstrated increased likelihood of VTE. Cancer also demonstrated significant risk for postoperative VTE (OR 1.30, 95% CI 1.08–1.58, p=0.01). Conclusions: Malignancy and systemic infection increase postoperative VTE risk in children. These findings should prompt consideration of prophylactic anticoagulation in the appropriate clinical setting. Further studies investigating the biology underlying VTE risk in children are needed.

Title & Authors: c.-32-13T>G mutation in late-onset Pompe disease and associated cardiac manifestations: implications for Newborn Screening

Authors: Mrudu Herbert, M.D, Priya S. Kishnani, M.D. Division of Pediatrics/Medical Genetics, Duke University

Background & Hypothesis: With the addition of Pompe disease (PD) to newborn screening programs (NBS) in USA, more and more infants are being diagnosed with PD. The “leaky” splice site mutation c.-32-13T>G is the most common mutation in late-onset PD (LOPD). It is reported that cardiac anomalies are rare in patients with this mutation. If it can be demonstrated that this mutation is not associated with severe hypertrophic or dilated cardiomyopathy (HCM, DCM), it will help to provide guidance for follow-up algorithms for new cases diagnosed through NBS. Objective: 1) to evaluate if HCM or DCM exists in association with the c.-32-13T>G mutation, as this will determine cardiac follow up2) To determine the age of onset of cardiac symptoms in LOPD patients Methods: We conducted 1) a chart review of our Duke Pompe LOPD cohort to examine how many had the c.-32-13T>G mutation and if it was associated with HCM; 2) review of mutation data of our infantile Pompe cohort to see if any had the IVS mutation 3) literature review to examine for genotype-cardiac phenotype in Pompe disease Results: Out of 383 patients in our Duke Pompe cohort, 133 had LOPD, and 253 had infantile PD (IPD), 76 LOPD patients had the c.-32-13T>G splice site mutation (57%). Twenty six of them (26/76, 34%) had cardiac abnormalities. Cardiac findings identified in LOPD patients with the c.-32-13T>G mutation include structural abnormalities such as mild to moderate LVH, left atrial enlargement, and mild valvular lesions; and conduction abnormalities such as SVT, 1st degree A-V block, RBBB, and prolonged QTc. 7 IPD patients had the c.-32-13T>G mutation; none had HCM. No HCM or DCM due to PD was seen in any of the patients in our cohort with the IVS1 mutation. Most structural anomalies could be attributed to risk factors such as hypertension, diabetes type II, hyperlipidemia or advancing age. Literature reviews corroborated that PD patients with severe cardiac involvement are unlikely to have the c.-32-13T>G mutation, and vice versa, patients with the c.-32-13T>G mutation are unlikely to have HCM. Conclusions: The c.-32-13T>G genotype is unlikely to be associated with HCM or DCM in patients with LOPD and atypical infantile PD. Children diagnosed through NBS who have this genotype can undergo cardiac follow-up with ECG and Echo at reduced frequencies than currently performed. This will also help to reduce health care costs and allay anxiety in patients and families of newly diagnosed Pompe patients who needs long term medical follow-up throughout their lives.
#58 Title & Authors: ADHD and High-Risk Sexual Behaviors and Consequences in Adolescence and Adulthood
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Background & Hypothesis: ADHD patients may be at higher risks for negative sexual health outcomes given key clinical characteristics of high impulsivity and sensation-seeking.

Objective: A comprehensive examination of childhood Attention Deficit Hyperactivity Disorder (ADHD) and sexual health in adolescence and adulthood is needed in a nationally representative sample.

Methods: Sample was drawn from the National Longitudinal Study of Adolescents to Adult Health (N=13,368) based on complete data on ADHD symptoms in Wave 1 and sexual health outcomes in Wave 1 (ages 12-19) and Wave 3 (ages 18-26). Multivariate regressions evaluated outcomes for age of sexual debut, sexually transmitted diseases (STD), pregnancy, abortion, paid sex, sexual victimization, lifetime sex partners, and unstable adult partnerships. Inconsistent contraception use and substance use were tested as mediators and sensitivity analyses were conducted for adult ADHD medication use. All models accounted for survey weights, age, sex, race, vocabulary-intelligence, Conduct Disorder, childhood single-parent household, and highest education achieved for mother (in adolescence) or respondent (in adulthood).

Results: In adolescence, ADHD youth had higher risks for vulnerable sexual debut <14 years (OR 1.33, p<.05) and experiencing forced sex (OR 1.72, p<.001). In adulthood, risks were higher for age of sexual initiation (Beta -0.25, p<.05), lifetime sex partners (Beta 1.19, p<.01), paid sex (OR 1.68, p<.01), STDs (OR 1.39, p<.05), and victimization (OR 1.71, p<.001). Medication use did not remove significance. Combined subtype drove the association for younger sexual debut; Inattentive, STDs; and Hyperactive-impulsive, lifetime partners, paid sex, and victimization. Developmentally-specific mediators included adolescent drinking, adult inconsistent contraception use, and adult drug use.

Conclusions: For ADHD patients, additional precautions/interventions to medication use should be considered to deter negative sexual health outcomes.

#59 Prophylactic immune modulation in infantile Pompe disease using low-dose methotrexate induction: A safe, inexpensive, widely accessible, and efficacious strategy
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Background & Hypothesis: Enzyme replacement therapy (ERT) with alglucosidase alfa (rhGAA) has improved clinical outcomes in Pompe disease. Irrespective of cross-reactive immunologic material (CRIM)-status, 89% of infantile Pompe disease (IPD) patients and 100% of late-onset Pompe disease (LOPD) patients seroconverted after ERT in clinical trials. Majority of CRIM-negative IPD, approximately 40% of CRIM-positive IPD, and 10-15% of LOPD develop high and sustained anti-rhGAA IgG antibody titers (HSAT: defined as titers of ≥51,200 at or beyond six months on ERT) with subsequent clinical decline. A prophylactic immune tolerance induction (ITI) with rituximab, methotrexate, and IVIG minimizes this immune response and achieves immune tolerance. Prophylactic ITI has become a standard practice in CRIM-negative IPD patients. However, the side effects and cost of rituximab have limited its use only to CRIM-negative IPD. There is a need for a safer immune modulation protocol for CRIM-positive and LOPD patients. Objective: We developed an immune modulation protocol using low-dose methotrexate (0.4 mg/kg/dose for a total of nine doses) induction for CRIM-positive IPD and LOPD. The purpose of this study was to assess its safety and efficacy in patients with Pompe disease.

Methods: Low-dose methotrexate protocol was administered along with the first ERT infusion in the newly diagnosed IPD and LOPD patients. Absolute neutrophil count (ANC), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) data were collected to assess safety and anti-rhGAA IgG antibody titers were collected to assess the efficacy of the protocol. The antibody titers from this cohort were compared to the historical cohort of CRIM-positive IPD and LOPD from the clinical trials who received ERT monotherapy to assess the efficacy. Results: This protocol has been administered to 16 Pompe patients (9 classic IPD, 5 atypical IPD, and 2 LOPD). Overall, the low-dose methotrexate protocol was safely tolerated. Three patients had an ANC less than 750 cells/mm3 around the time of methotrexate administration, which was reversible. AST greater than 3 times their baseline was seen in one patients and ALT greater than 3 times their baseline was seen in two patients. 32% of IPD and LOPD patients in the comparator group had titers ≥12,800. In contrast, only one out of 16 Pompe patients who received low-dose methotrexate protocol had titers ≥12,800 on two separate time points. Of note, this is the patient who did not receive the full protocol due to low ANC. Conclusions: All 16 patients safely tolerated the protocol without any serious adverse events. The antibody titer data suggests that low-dose methotrexate protocol achieved immune tolerance induction as observed by low/no antibody titers. It is a safe, inexpensive, widely accessible, and efficacious alternative to the current regimen. Further data in a larger cohort is needed to monitor the long-term outcomes in Pompe patients.
#60 Title & Authors: The Significance of Anti-FcεRI Antibodies in Chronic Idiopathic Urticaria: The Role of Autoimmunity in the Differential Response to Treatment. Julie J. Kim-Chang, MD1, Patricia L. Lugar, MD, MS1, Anjeni Keswani, MD2 1Duke University Medical Center, Durham, NC, 2George Washington University School of Medicine, Washington, DC

**Background:** Autoimmunity is thought to play a role in the disease process of chronic idiopathic urticaria (CIU) as 30-50% of patients with CIU have clinical autoimmune disease or circulating autoantibodies. However, the role of autoimmunity in the clinical course of CIU is unclear. **Objective:** This study assesses the differential response to CIU therapies as stratified by anti-FcεRI antibody status. **Methods:** A retrospective review of electronic health records of patients aged ≥12 with CIU evaluated for anti-FcεRI antibodies was conducted at Duke University Medical Center. Patients were characterized by response to treatment as well as clinical and laboratory characteristics. Univariate analysis followed by multiple logistic regression was performed. **Results:** Of the 230 patients identified, 50 (22%) tested positive for anti-FcεRI antibodies. There were no significant differences in age, gender, race, thyroid disease or autoimmune disease between those with positive vs negative anti-FcεRI antibodies. Mean monocyte count was significantly higher in patients with negative anti-FcεRI antibodies (529 vs 393 per mm3, p<0.0001). In patients with negative anti-FcεRI antibodies, there was a higher odds ratio of response to antihistamines (OR = 1.50 [95% CI 1.12 – 2.03]) and omalizumab (OR = 6.00[2.32 – 20.4]). Negative ANA (<1:160) and the absence of clinical autoimmune disease were associated with a higher odds ratio of response to omalizumab (OR = 9.50 [2.76 – 59.63], 5.25 [2.00 – 18.00], respectively). **Conclusions:** CIU patients with negative anti-FcεRI antibodies may respond more favorably to treatment with antihistamines and omalizumab than those with positive anti-FcεRI antibodies. CIU patients with evidence of autoimmunity may have more treatment resistant disease.

#61 Title & Authors: Peri-operative Hypersensitivity Reactions and Anaphylaxis: Outpatient Evaluation and Management. Renee Kleris M.D. and Patricia L. Lugar M.D., M.S.

**Background & Hypothesis:** Beyond acute management, the challenge of evaluating peri-operative anaphylaxis rests not only on number of possible exposures but also designing testing protocols. We serve a large area in the Southeast where referrals for peri-operative anaphylaxis are not uncommon. **Objective:** We reviewed our outpatient testing protocols, testing outcomes and safety in this setting. **Methods:** A retrospective chart review identified patients who completed outpatient evaluation and testing for peri-operative anaphylaxis during 1/2010-2/2017. We recorded demographics, reported allergic reaction, skin testing results (skin prick test-SPT and intradermal-ID), and where appropriate outcome of drug challenge, serum specific IgE, serum tryptase, subsequent use of medications and whether the allergy list was updated or revised in the electronic medical record. **Results:** Complete testing data was available for 24 patients comprising a total of 64 testing procedures. The patient population included one pediatric patient. The remaining 23 patients were adults. The medications tested included local anesthetics (28), neuromuscular blocking agents (20), general anesthetics (5), opioid analgesic agents (1), antibiotics (3), and other (7). Four patients had positive skin prick (SPT) test/intradermal (ID) to cisatracurium, followed by vecuronium (1), tetracaine (1), alcaine (1), versed (1), fentanyl (1), and amoxicillin (1). One patient had an elevated tryptase suggestive of a mast cell disorder. **Conclusions:** Peri-operative anaphylaxis is underreported with estimates of 100 in one million surgical procedures. Generally testing is limited to evaluation at academic centers. A literature review identifies neuromuscular blocking agents as the most common cause for anaphylaxis in this setting.1 In this review we identified the cause for anaphylaxis in seven of the twenty-four patients tested. In our series, we found neuromuscular blocking agents to be the most common cause of peri-operative hypersensitivity reactions and anaphylaxis. 1.Mertes PM, Alla F, Trechot P, Auroy Y, Jouglé E. Anaphylaxis during anesthesia in France: an 8-year national survey. J Allergy Clin Immunol 2011; 128:366-73.
#62 Title & Authors: Basement membrane nephropathy like phenotype in a family with an ARHGAP24 mutation known to cause familial FSGS
Elizabeth Kotzen, Gentzon Hall, Megan Chryst-Ladd, Guanghong Wu, Brandon Lane, Shashi Nagaraj, John Foreman, Michael Randles, Rachel Lennon, David Howell, Rasheed Gbadegesin.

Background & Hypothesis: Alport Syndrome (AS), other glomerular basement membrane (GBM) disorders, and focal segmental glomerulosclerosis (FSGS) are major causes of glomerular disease worldwide. The pathogenesis and phenotypic spectrum of these conditions is not completely known, however recent genomic discoveries have demonstrated significant phenotypic overlap.

Objective: In this study, we report a family with a known mutation in the FSGS gene ARHGAP24 that was associated with predominant GBM defects on renal biopsy.

Methods: We identified a 3-year-old Hispanic female with persistent microscopic hematuria and proteinuria of unclear etiology but normal audiologic and ophthalmologic findings. Renal biopsy, targeted sequencing of the COL4A3, COL4A4, COL4A5, and MYH9 genes and whole exome sequencing (WES) were performed to establish the molecular cause(s) of the disease.

Results: Light microscopy showed unremarkable glomeruli with no hypercellularity, sclerosis, or significant GBM thickening or duplication. Immunofluorescence staining showed patchy, discontinuous staining for alpha-3 and -5 chains of type IV collagen and electron microscopy revealed alternating areas of thinning and thickening suggesting an AS phenotype. Targeted sequencing and copy number variation analysis of COL4A genes and MYH9 were normal. WES revealed the Q158R mutation in ARHGAP24. Q158R was previously reported as a cause of hereditary FSGS in a Hispanic kindred. We did not find a mutation in any of the known >50 FSGS genes, nor in COL4A genes including the intronic sequences captured by WES. Direct sequencing of the family showed that the father, who had 1+ proteinuria, also carried the same mutation. In-silico analyses showed that ARHGAP24 interacts with TIAM1, known to play a role in cell-matrix interactions.

Conclusions: Rare variants in ARHGAP24 may mimic GBM disorders such as AS. In-silico analysis of the ARHGAP24 interactome suggests that the mutation may disrupt podocyte-matrix interactions at the GBM. Alternatively the disease in this family may represent a digenic inheritance of ARHGAP24 and an unidentified functional deep intronic sequence variant in a COL4A gene. These findings emphasize the need for a multifaceted approach to glomerular diseases classification that integrates clinical, morphologic, and genomic data.

#63 Title & Authors: Effects of breast milk-derived non-broadly neutralizing antibodies on oral SHIV acquisition and viral reservoir establishment in infant rhesus macaques
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Background & Hypothesis: While breast feeding is the predominant mode of vertical HIV acquisition, breast milk transmission of HIV is highly inefficient, suggesting the presence of naturally occurring protective factors in breast milk. In this study, we investigated whether breast-milk derived non-broadly neutralizing antibodies isolated from the colostrum of HIV-infected women could impede oral SHIV acquisition and the establishment of the virus reservoir in SHIV-exposed infant rhesus monkeys (RM).

Objective: We evaluated the impact of DH378 (a CD4 binding site monoclonal antibody (mAb) with weak tier 2 HIV-neutralizing capability) as well as a cocktail of DH378, DH377 (a tier 1 HIV-neutralizing V3-specific mAb), and DH382 (an ADCC-mediating C1-specific mAb) on both the vertical transmission rate and virus reservoir establishment in an infant RM model of oral SHIV exposure.

Methods: Infant RMs were intravenously infused with either DH378 (n=6); DH377, DH378, and DH382 (n=6); or CH65 (anti-influenza mAb; n=8). Antibody infusion was followed by repeated low dose oral SHIV inoculation premixed in formula feed with the respective mAbs. Lymphoid and intestinal tissues were harvested 8 weeks post-challenge. Plasma viral load and cell-associated proviral loads were measured to assess SHIV acquisition. SHIV-1157ip3N4 variant diversity was assessed by single genome amplification.

Results: While no significant difference was observed in vertical transmission rates between treatment groups (3/6 DH378-, 6/8 CH65-, and 2/6 Tri-mAb cocktail-treated infected; p=0.55), tri-mAb cocktail-treated RMs exhibited significantly fewer plasma SHIV variants. Additionally, one of the two infected tri-mAb cocktail-treated RMs demonstrated lower plasma and infectious tissue-associated viral loads, as well as no detectable tissue-associated provirus, suggesting that mAbs with diverse functionality may partially influence oral SHIV acquisition.

Conclusions: These findings suggest that breast milk non-broadly neutralizing antibodies may minimally reduce oral SHIV acquisition, but can impact virus evolution and viral reservoir establishment, suggesting that breast milk antibodies may influence the pathogenesis of HIV-1 after breast milk transmission.
## #64 Title and Authors: Type 2 Diabetes Is Associated with Reduced Metabolic Inactivation of Cortisol due to Increased Whole body 11β Hydroxy Steroid Dehydrogenase Activity in Obese African-American Children

Mark Miller, MD, Pinar Gumus Balikcioglu, MD, James Bain, PhD, Michael Muehlbauer, PhD, Thomas L O’Connell, PhD, Stuart Alan Chalew, MD and Michael Freemark, MD.

**Background & Hypothesis:** Increased levels of circulating cortisol lead to central obesity, impaired glucose tolerance, hypertension, dyslipidemia and inability to lose weight. These clinical manifestations of hypercortisolism are consistent with the manifestations of the metabolic syndrome. Obesity may lead to altered cortisol metabolism potentially interfering with glycemic control in children. We hypothesize that cortisol metabolism would differ between obese patients with and without Type 2 diabetes (T2D). **Objective:** To identify cortisol metabolome signatures that distinguish obese youth with and without T2D. **Methods:** We previously collected 24-hour urines from obese African-American (AA) children with (n=14) and without (n=14) T2D for our pilot study. Now we are collecting 24-hour urine samples from obese youth with and without T2D, and age, sex, and puberty matched lean controls (50 subjects in each group). In our pilot study, Tetrahydrocortisone (THE), Tetrahydrocortisol (5β), Allo-THF (5α), α-cortolone, β-cortolone + β-cortol, α-cortol were assayed using gas chromatography with mass spectrometry. 11-βHSD activity was calculated as the ratio of [THF + 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 between the groups were assessed using linear regression analysis adjusting for age and gender. Log transformation for non-normally distributed data was performed. Statistical significance was accepted at p≤0.05 **Results:** We continue to recruit subjects for our current prospective cohort. We will summarize our findings from our pilot study. Age, gender distribution, and BMI-z scores as well as THE, 5β THF, Allo-THF (5α), α-cortolone, β-cortolone + β-cortol, α-cortol were not different between the groups. 5α reductase activity was lower in the T2D patients with 1.61 ± 0.22 compared to obese non-diabetic youth with 2.12 ± 0.13 (p=0.0211). There was an interaction effect of gender and diabetes status on 11β HSD activity with highest levels in males with T2D (p=0.0250). **Conclusions:** Obese patients with T2D appear to have impaired metabolism of cortisol to inactive metabolites which potentially exacerbates impaired glycemic control. Our larger prospective cohort with lean controls will help us to further delineate cortisol metabolism in obese children and identify novel biomarkers for development of T2D.

## #65 Title & Authors: β-cell sensitivity to incretins and glucose in healthy men and women.

Mark Miller, Cris Slenz, David D’Alessio

**Background & Hypothesis:** The incretins glucagon-like peptide 1 (GLP-1) and glucose-dependent insulintropic polypeptide (GIP) stimulate insulin secretion in response to enteral glucose. Animal studies have shown that deletion of the receptor for one incretin leads to a compensatory increase in β-cell sensitivity to the other. Based on these findings we hypothesized an interaction between the incretins such that one or the other would be dominant and the combination of GLP-1 and GIP sensitivity would be relatively constant.

**Objective:** Determine relationship of GLP-1 and GIP sensitivity in healthy young adults.

**Methods:** To test this hypothesis, we performed paired hyperglycemic clamps that started with a 90-minute glucose ramp to target glycemia, followed by a step-wise graded infusion of GIP or GLP-1 over 120 minutes. The slope of plasma insulin to blood glucose determined β-cell sensitivity to glucose; the slope of insulin secretion across the GIP/GLP-1 infusions determined incretin sensitivity.

**Results:** β-cell glucose sensitivity ranged from 3.8 to 19.8 in this cohort of healthy subjects, while β-cell sensitivity to GIP ranged from 3.4 to 30, and to GLP-1 22 to 59. There was no relationship between the β-cell sensitivity to glucose and the sensitivity to each incretin. However, there was a positive relationship between GLP-1 sensitivity and GIP sensitivity (r = 0.51, p < 0.05).

**Conclusions:** These findings indicate that β-cell sensitivity to glucose, GIP and GLP-1 varies widely across a group of healthy young adults, but that subjects who respond strongly to one incretin are also sensitive to the other. These results suggest that the actions of the incretins to promote insulin secretion are additive rather than compensatory.
Background & Hypothesis: Vascular injuries in children, though considered uncommon, have the potential for lifelong disability and present unique challenges in diagnosis and management due to anatomic and physiologic differences that vary with age. While vascular trauma is well studied in adult populations, data describing epidemiology in pediatric populations are sparse.

Objective: The purpose of this study is to characterize incidence, patterns of injury, and outcomes using a multiinstitutional national database.

Methods: The National Trauma Data Bank was queried for the years 2007-2014 to identify children (ages 0-18 years) who sustained major vascular injury. Patients were stratified by age and mechanism of injury. Patient characteristics, injury distribution, severity, and inpatient outcomes were compared using Pearson χ² tests for categorical variables and Wilcoxon rank-sum tests for continuous variables.

Results: Of 913,861 children presenting with trauma during the study period, 7,482 (0.8%) were diagnosed with one or more vascular injuries. Blunt trauma was more frequent, accounting for 69.1% of injuries vs. 30.9% due to penetrating trauma. Infants (age 0-1y) were most severely injured, with the lowest median GCS (10 [95% CI 3,15] for age 0-1y vs. 15 [95% CI 3,15] overall, p<0.001), and highest mean ISS (23 [95% CI 14,30] age 0-1y vs. 18 [95% CI 9,30] overall, p<0.001). Infants were disproportionately affected by cerebrovascular injury (51.7% age 0-1y vs 31.2% overall, p<0.001). Extremity (18.6% age 0-1 vs 30.7% age 16-18, p<0.001) and intra-thoracic vascular (8.3% age 0-1 vs 16.3% age 16-18, p<0.001) injuries increased with increasing age; whereas, intra-abdominal vascular injuries and overall aortic injuries were consistent across age groups (22.9% and 3.9% overall). Both initial and overall mortality were highest in infants, 8.3% and 21.4% respectively vs. 5.1% and 10.9% overall, p<0.001. Thromboembolic complications were infrequent (4.3% overall), but significantly increased in infants (7.6%, p = 0.045).

Conclusions: This study, the largest national study of pediatric vascular trauma to date, confirms that although pediatric vascular trauma is rare, it is associated with considerable morbidity and mortality. Patterns of injury and outcomes vary with age, with the highest rates of cerebrovascular injury, thromboembolic complications, and death occurring among infants. Further studies are needed to assess optimal diagnostic and treatment strategies among the youngest traumatically injured patients.
#68 Title & Authors: The Use of Echocardiography for ‘Rarely Appropriate’ Indications in Pediatric Patients Presenting with Syncope is associated with Increased Cost with Minimal Diagnostic Yield: A Retrospective Cohort Study.

Jonathan H. Pelletier, Sarah Blanchard, Reid C. Chamberlain, Christoph P. Hornik, Kevin D. Hill, Michael J. Campbell

**Background & Hypothesis:** The American College of Cardiology recently published appropriate use criteria (AUC) to guide use of echocardiography for syncope and other complaints in children and adolescents. These guidelines specifically define clinical encounters where echocardiography is ‘Rarely Appropriate.’ We hypothesized that adoption of these guidelines in clinical practice was variable, and that improved guideline adherence had the potential for significant cost savings.

**Objective:** To evaluate frequency, yield, and cost of echocardiograms meeting ‘Rarely Appropriate’ criteria.

**Methods:** Retrospective, single-center study of patients ages 0-18 years presenting with syncope. Patients were categorized according to the AUC and based upon location of care (emergency department only [ED], primary care setting only [PCP] or referred to a pediatric cardiologist). Multivariate regression was used to determine factors associated with performance of a ‘Rarely Appropriate’ echocardiogram. Costs were calculated using fair market values from the Healthcare Bluebook.

**Results:** The cohort included 637 patients presenting with syncope during the one-year study. Echocardiograms were ordered for 127/637 (20.1%) including 0/328 ED patients, 1/66 (1.5%) PCP patients and 127/243 (52.3%) pediatric cardiology referrals. Use of echocardiography by pediatric cardiologists was categorized as ‘Appropriate’ in 92/127(72.4%), ‘Maybe Appropriate’ in 6/127 (4.7%), and ‘Rarely Appropriate’ in 29/127 (22.8%). Abnormal findings were seen in 6/128 (4.7%) echocardiograms but in none of the ‘Rarely Appropriate’ studies. In multivariate analysis, female gender and younger age were the only factors associated with performance of a ‘Rarely Appropriate’ echocardiogram. Patients receiving a ‘Rarely Appropriate’ echocardiogram were more likely to receive cardiology follow-up compared to patients with ‘Rarely Appropriate’ symptoms that did not receive an echocardiogram (25/29 versus 17/90, p<0.01). ‘Rarely Appropriate’ echocardiograms cost an estimated $22,156 annually.

**Conclusions:** ‘Rarely Appropriate’ echocardiograms performed for syncope do not contribute management changing diagnostic information. However they burden patients with additional cost and perhaps contribute to increased need for follow-up.

#69 Title & Authors: The Duke Experience with Participant Evaluations for the Undiagnosed Diseases Network

Loren DM Pena, Kelly Schoch, Rebecca Spillmann, Yong Hui Jiang, Allyn McConkie-Rosell, Nicole Walley, Jennifer Sullivan, Camilla Sanders, Slave Petrovski, Nicholas Stong, David Goldstein, Vandana Shashi, Members of UDN

**Background & Hypothesis:** After successful implementation of the NIH’s Undiagnosed Diseases Program, the Undiagnosed Diseases Network (UDN) opened for applications in September 2015 as an extension of the UDP. Duke University Medical Center is one of the seven clinical sites evaluating patients as part of this network, in addition to two genome sequencing cores, a coordinating center, a central biorepository, a metabolomics core, and a model organisms screening center. The Duke UDN site has been successful in incorporating clinical and research evaluations to reach a diagnosis in a number of patients.

**Objective:** To review the Duke experience as a clinical site in the UDN

**Methods:** Retrospective chart review

**Results:** As of November 2016, the Duke site has evaluated 21 patients and reached a diagnosis in seven by a combination of whole exome sequencing or targeted testing. Two new disease genes were identified as part of the evaluation. Careful examination of the participants allowed for clinical diagnoses and development of an extensive phenotypic profile in new diseases.

**Conclusions:** Patient evaluations through the Duke site of the UDN offer extensive tools such as metabolomics and model organisms that allow us to reach a diagnosis for patients with rare diseases.
#70 Title & Authors: Infantile systemic hyalinosis, an ultra-rare condition with a well delineated phenotype but no pathophysiologic understanding or treatment
Loren DM Pena, Rebecca Spillmann, Kelly Schoch, Nicole Walley, Vandana Shashi, Members of UDN

Background & Hypothesis: Infantile systemic hyalinosis (ISH) is a rare autosomal recessive condition caused by mutations in the ANTXR2 gene on chromosome 4q21. The condition has a very characteristic phenotype, with accumulation of an amorphous hyaline substance throughout the body and leading to painful joint contractures, gingival hyperplasia, tumor-like growths, and other cutaneous findings. We present a case with a clinical diagnosis but no molecular confirmation, which was solved at Duke.

Objective: To review phenotypic and histological findings in a case of ISH.

Methods: Retrospective chart review

Results: The participant's clinical diagnosis was confirmed by Sanger sequencing of the ANTXR2 gene. A known pathogenic mutation, c.1073dupC, was detected in the homozygous state. The mutation was not previously detected via next generation sequencing because it occurs in a homopolymer and was missed by analysis software. We review known histological information about the disease mechanism and potential treatment approaches.

Conclusions: Patient evaluations through the Duke site of the UDN offer extensive tools such as exome reanalysis and extensive phenotypic characterization that allow us to reach a diagnosis for patients with rare diseases.

#71 Title & Authors: Safety of Off-label use of Caffeine Citrate in Premature Infants
M Puia Dumitrescu, PB Smith, J Zhao, A Soriano, M Morris, E Bendel-Stenzl, F Moya, R Chhabra, M Laughon, K Wade
Pharmaceuticals for Children Act–Pediatric Trials Network Steering Committee

Background & Hypothesis: Caffeine citrate is labeled by the FDA for short-term treatment of apnea of prematurity (AOP) for infants born between 28 and 33 weeks gestational age (GA). The label includes an association with necrotizing enterocolitis (NEC) based on a trial of 45 infants exposed to the drug. Caffeine citrate is often used for long-term treatment of infants born <28 weeks GA, and NEC has not been observed with caffeine citrate use in subsequent larger trials.

Objective: Characterize the safety of caffeine citrate in premature infants receiving caffeine off-label.

Methods: We used Electronic Health Records (EHR) from 2005-13 from four neonatal intensive care units (NICUs) to identify all infants <28 weeks GA, <120 days of age, without major congenital anomalies exposed to caffeine citrate. Safety outcomes of interest included death, NEC (either medical or surgical), spontaneous perforation (SIP) and laboratory abnormalities. We used logistic regression models controlling for GA, birth weight, caffeine dose, and duration of caffeine citrate therapy to evaluate the association of caffeine exposure with NEC. NEC was evaluated through logistic regression model by whether the event occurred or did not occur on a day of caffeine dosing at infant level. The independent factors included site, GA (weeks), birth weight (by units of 100 grams), mean study dose (mg/kg/day), duration of therapy (day) and concomitant medications by classification.

Results: The cohort comprised 410 infants with median (min, max) GA of 26 weeks (22, 28) and BW 800 g (340, 1460); 90% were exposed to antenatal steroids; Outcomes of interest in table. 94% received caffeine citrate for >13 days (median 60 days; range 1-144). The median daily and cumulative study dose per kg of participant dosing weight were 8 mg/kg (4 – 25) and 455 mg/kg (20 – 1231), respectively. Increasing caffeine citrate dose was associated with lower risk of NEC events (medical or surgical), odds ratio = 0.78 (0.63, 0.92), and increased caffeine citrate duration was associated with lower risk of NEC events (medical or surgical), odds ratio=0.93 (0.91, 0.96).

Conclusions: In this cohort of infants <28 weeks GA, increased exposure to caffeine (dose or duration) was not associated with increased risk of NEC. This study adds significant data for the safety of caffeine use in clinical practice in premature infants born < 28 weeks. The results from this research are being submitted to FDA for review and possible labeling change.

<table>
<thead>
<tr>
<th>Outcomes</th>
<th>N = 410 (%)</th>
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<tbody>
<tr>
<td>Death</td>
<td>2%</td>
</tr>
<tr>
<td>NEC (either medical or surgical)</td>
<td>9%</td>
</tr>
<tr>
<td>Medical NEC</td>
<td>5%</td>
</tr>
<tr>
<td>Surgical NEC</td>
<td>4%</td>
</tr>
</tbody>
</table>
#72 Title & Authors: Evaluation of Gentamicin-Induced Ototoxicity in Hospitalized Infants
M Puia Dumitrescu, DK Benjamin, PB Smith, R Clark, D Gonzalez, CP Hornik

Background: Gentamicin is the second most commonly used drug in U.S. neonatal intensive care units (NICUs). This frequent use persists despite a known risk of irreversible ototoxicity found in prior clinical trials. How this risk translates to routine neonatology practice is largely unknown. Objective: Characterize the association between gentamicin dosing, duration of treatment and ototoxicity in hospitalized infants. Methods: We included inborn infants without major congenital anomalies who had available hearing test results at the time of discharge from 330 NICUs between 2002 and 2014. We excluded infants with incomplete gentamicin dosing data. Our primary outcome was the final hearing test result performed during hospitalization, defined as a binary variable: abnormal (failed or referred), or normal (passed). Gentamicin exposure measures: highest daily dose, average daily dose, cumulative dose, and cumulative duration of exposure. For each of these, we fit separate multivariable logistic regression models adjusted for demographics (gestational age (GA), small for gestational age status, gender, and prenatal steroids), comorbidities diagnosed at any time during hospitalization (PDA, HIE, IVH grade 3 or 4, NEC, or BPD) and clinical events prior to the hearing test result (positive blood or cerebrospinal fluid culture, cytomegalovirus or other TORCH infections, total serum bilirubin ≥15 mg/dL, exposure to inotropes or ototoxic drugs (tobramycin, vancomycin, furosemide, bumetanide)). Results: 76,176 infants met our inclusion/exclusion criteria and received gentamicin. Their median (25th, 75th percentile) GA and birth weight (BW) were 35 weeks (32, 38) and 2415 g (1754, 3130) respectively. The median (25th, 75th percentile) highest daily dose, average daily dose, and cumulative dose received during hospitalization were 3.9 mg/kg/day (3.0, 4.0), 3.7 mg/kg/day (3.0, 4.0), and 12.1 mg/kg (9.1, 20.7) respectively. The median (25th, 75th percentile) cumulative duration of exposure was 4 days (3, 6). Failed hearing test results occurred in 3044 (4.0%) infants. We observed no association between our gentamicin exposure measures and the occurrence of a failed hearing test (Table). Conclusions: In this cohort of hospitalized infants, gentamicin dosing and duration of treatment were not associated with increased odds of a failed hearing test. Table: Median (25th, 75th %ile) of exposure variables, by hearing test result, and adjusted odds ratios for abnormal hearing test.

<table>
<thead>
<tr>
<th>Hearing Test</th>
<th>Normal Test (N = 73,132)</th>
<th>Abnormal Test (N = 3,044)</th>
<th>p-value*</th>
<th>Adjusted odds ratio (95% CI) for abnormal hearing test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average daily dose (mg/kg/day)</td>
<td>3.8 (3.0, 4.0)</td>
<td>3.7 (2.9, 4.0)</td>
<td>&lt;0.01</td>
<td>0.99 (0.98, 1.00)</td>
</tr>
<tr>
<td>Cumulative dose (mg/kg)</td>
<td>12.1 (9.1, 20.5)</td>
<td>15.6 (10.0, 25.4)</td>
<td>&lt;0.01</td>
<td>1.00 (1.00, 1.00)</td>
</tr>
<tr>
<td>Highest daily dose (mg/kg/day)</td>
<td>3.9 (3.0, 4.0)</td>
<td>3.9 (3.0, 4.0)</td>
<td>0.26</td>
<td>0.99 (0.98, 1.01)</td>
</tr>
<tr>
<td>Cumulative duration of exposure (days)</td>
<td>4 (3, 6)</td>
<td>4 (3, 8)</td>
<td>&lt;0.01</td>
<td>1.00 (0.99, 1.01)</td>
</tr>
</tbody>
</table>

*p-values from Wilcoxon rank sum tests comparing unadjusted distribution of dosing variables between infants with normal vs. abnormal hearing tests.

#73 Title & Authors: Impact of gastrostomy tube placement on weight gain in hospitalized premature infants
M Puia Dumitrescu, PB Smith, DK Benjamin, R Greenberg, N Abuzaid, W Andrews, K Chellani, A Gupta, D Price, C Williams, R Clark, K Zimmerman

Background & Hypothesis: Gastrostomy tubes (G-tubes) are commonly used to support growth in premature infants with feeding difficulties in the neonatal intensive care unit (NICU). Although the use of G-tubes in the NICU has been increasing, there are no published studies evaluating weight gain in infants who receive G-tubes prior to discharge from the NICU.

Objective: Describe weight gain in hospitalized, premature infants before and after G-tube placement and compare weight gain between G-tube infants and controls.

Methods: We included all infants <37 weeks gestation without major congenital anomalies discharged from 327 NICUs in the US from 2004 to 2013. We compared weight change for G-tube infants for up to 30 days pre- and post-procedure, excluding the weight changes in the first 7 days post G-tube placement. We also matched these infants 1:1 with infants without G-tubes (controls) using propensity scores based on birth weight (BW), small for gestational age (SGA) status, length of stay in NICU, race, sex, necrotizing enterocolitis (NEC), patent ductus arteriosus ligatoin (PDA), grade 3 or 4 intra-ventricular hemorrhage (IVH), hypoxic ischemic encephalopathy (HIE) and chronic lung disease (CLD). We compared the weight change 7 days prior to discharge home between the matched and control groups.

Results: We identified 1393 infants who received a G-tube with a median (25th-75th percentile) BW of 872 g (660, 1355) and GA of 27 weeks (25, 30). Daily weight gain between day 7-14 after G-tube placement was significantly less than observed during the 14 days prior to placement (18 vs 28 g/day, p<0.001). There was no significant difference in weight gain between the 30 days pre- and post-procedure, (26 vs 24 g/day, p=0.20). We matched 1111 infants with G-tubes to controls. Weight gain during the 7 days prior to discharge was 21 g/day in infants with G-tubes vs. 24 g/day in controls (p <0.01).

Conclusions: In hospitalized infants, G-tube placement did not improve weight gain prior to discharge home.
#74 Title & Authors: Clinical approach towards the management of infants with late onset Pompe disease identified by newborn screening: Mugdha Rainkar MBBS, DCH; Lauren A. Bailey MS, CGC; Kathryn L. Berrier MS, CGC; Ankit Desai MBBS; Zoheb B. Kaz MBBS; Laura E. Case DPT, PCS; Priya S. Kishnani, MD

**Background & Hypothesis:** Late onset Pompe Disease (LOPD) is highly variable with presentation up to 6th decade of life, typically associated with long diagnostic odyssey of up to 20 years and prominent musculoskeletal deficits. IVS c.-32-13T>G mutation is the most common mutation seen in 68 to 90% of LOPD patients. Published literature suggests that LOPD patients with IVS mutation have no clinical symptoms until adulthood and are treated with enzyme replacement therapy (ERT) only after overt symptoms develop. Early initiation of ERT with alglucosidase alfa, the only FDA approved treatment till date, has shown evidently dramatic improvements and favorable outcome in LOPD. Pompe disease has been added to the recommended uniform screening panel (RUSP) for newborn screening (NBS) thus facilitating identification of newborns with LOPD. In our experience infants and children with LOPD having IVS mutation have early musculoskeletal involvement adversely affecting their growth, development and long-term clinical outcome. NBS thus makes it important to identify probable early signs and symptoms, if any in young infants and determine their optimum management and timely ERT initiation.

**Objective:** To identify if patients with LOPD having IVS mutation identified by NBS exhibit an infantile phenotype.

**Methods:** Review of LOPD cases referred by NBS programs from multiple states nationwide to Duke as a tertiary referral center for rare genetic diseases. These cases were evaluated by laboratory (CK, urinary Glc4, metabolic profile) and physical therapy (PT) assessments along with genetic and cardiac evaluations (ECG and ECHO).

**Results:** All the cases exhibited subtle symptoms upon vigilant clinical evaluation that were consistent with LOPD and had disease onset in initial months of life. Genetic and PT evaluation revealed features like feeding difficulties, failure to thrive, motor developmental delay, milestone regression, and motor dysfunction. None had significant cardiac involvement. Urinary glc4 was within normal limit and may not be an appropriate screening tool in this situation. Detailed PT assessment especially looking for limb-girdle muscle involvement was often the mode of symptom ascertainment and an essential part of the clinical workup determining the need for ERT.

**Conclusions:** This cohort of LOPD by NBS presenting early provides a valuable new contribution to literature. All of them exhibit the typical limb-girdle muscle involvement in the first months of life with a need to initiate ERT in some and a close follow-up in others. This previously uncharacterized presentation provides a novel insight into the course of LOPD and highlights initiation of ERT based on the often overlooked subtle clinical symptoms prior to extensive and often irreversible skeletal muscle damage. This could serve as a paradigm to addition of other genetic disorders to RUSP and establish the benefits of NBS for ultra-orphan rare disorders like Pompe disease.

#75 Title & Authors: Modality of Primary HIV Disclosure and Association with Mental Health, Stigma, and Antiretroviral Therapy Adherence in HIV-Infected Tanzanian Youth. Julia Ramos BS, Leonia Laureana BA, Severa Luhanga MPH, Blandina Mmbaga MD PhD, Dorothy Dow MD, MSc-GH.

**Background & Hypothesis:** HIV disclosure is a major challenge for families and health care providers caring for HIV-infected children. Published research focuses on caregiver perspective of disclosure, though capturing disclosure information and narratives from youth is rare. We hypothesized that the way in which a youth is disclosed to impacts their mental health, stigma, and adherence. **Objective:** This study evaluated how self-discovery versus purposeful disclosure of HIV-status later impacts mental health and HIV outcomes of youth living with HIV. **Methods:** This was a mixed-methods study that included adolescents aged 12-24 years attending two adolescent HIV clinics in Moshi, Tanzania. Adolescents answered questions including when and how they found out they had HIV, who is their caregiver, mental health surveys (PHQ-9, depressive symptoms score >10; SDQ, behavioral/emotional symptoms score > 17; and modified UCLA trauma screen, post traumatic stress symptoms score > 18), modified Berger’s stigma scale, and self-reported adherence. HIV-1 RNA and latest CD4 were obtained. In-depth interviews were conducted using a convenience sample from the referral clinic site. **Results:** The majority of youth report they found out their HIV status on their own (80%). Youth attending the government site were less likely to be purposefully told their HIV status compared to those attending the referral site (p<0.01). Depressive and emotional/behavioral symptoms (dichotomous variables) and incomplete adherence were significantly more likely among those who figured out their HIV status on their own as compared to those who were purposefully told. Mental health symptoms PHQ9, SDQ, UCLA Trauma (continuous variables) and incomplete adherence were significantly increased for those who found out on their own compared to those who were purposefully told. Youth discussed how they needed to understand why they took medication during in-depth interviews. They described caregiver avoidance of answering this question and anger thinking they were lied to when they figured it out on their own. **Conclusions:** These findings demonstrate that youth who figured out their HIV status on their own had increased mental health symptoms and worse adherence to ART. It is imperative to develop and further implement disclosure protocols for HIV-infected children to reduce mental health difficulties and stigma and promote ART adherence. In addition, mental health programming for youth living with HIV should support youth as they work through their complex disclosure narratives.
**#76 Title & Authors:** “Electrical Status Epilepticus of Sleep, Continuous Myoclonus and Superrefractory Status Epilepticus in Patients with KCNA2 Mutations.”
Monisha Sachdev, Marina Gainza-Lein, Dmitry Tchapyjnikov, Tobias Loddenkeper and Mohamad A. Mikati.

**Background & Hypothesis:** The KCNA2 gene codes for the Kv1.2 potassium channel, and mutations in this gene have been implicated in the development of epileptic encephalopathies. To our knowledge, there are only ten cases of KCNA2 related epileptic encephalopathies. The spectrum of presentation of these encephalopathies has not been fully defined. Here we report novel clinical characteristics in three previously unreported cases that expand the phenotype.

**Objective:** To characterize the range of clinical manifestations of KCNA2 mutation related epileptic encephalopathies.

**Methods:** Blood samples were sent for whole exome sequencing by GeneDx. Seizure types were characterized by clinical exam, history taking and EEG recording.

**Results:** Through whole exome sequencing we identified 3 patients with KCNA2 mutations. Patient 1 is a 5-year-old male with a c.1214 C>T (p.P405L) mutation, previously reported as a disease-associated mutation. In addition to experiencing several seizure types and multifocal areas of epileptogenic activity on EEG, Patient 1 was noted to have electrical status epilepticus of sleep, which has never been reported in patients with KCNA2 mutations. Patient 2 is a 7-year-old female with a novel c.1195 G>A (p.V399M) mutation not previously documented in the literature, however is predicted to be disease causing based on in silico analysis. Patient 2 presented with continuous polymyoclonus and limb myoclonus, another new phenotype in patients with KCNA2 mutations. Patient 3 is a 23-year-old male who has a c.899C>T (p.A297T) mutation, which is a location previously reported however a novel amino acid substitution. Patient 3 presented with superrefractory status epilepticus, yet another novel seizure type for patients with KCNA2 mutations.

**Conclusions:** We have identified 3 new patients with KCNA2 mutations with novel characteristics as compared to previously reported cases, including electrical status epilepticus of sleep, continuous myoclonus and status epilepticus. These results expand the spectrum of epileptic manifestations of KCNA2 mutations.

**#77 Title & Authors:** Rare and Undiagnosed Diseases: The Value of Thinking Beyond the Exome.
Kelly Schoch, Rebecca Spillmann, Allyn McConkie-Rosell, Loren Pena, Yong-Hui Jiang, Nicole Walley, Jennifer A. Sullivan, Camilla Sanders, Vandana Shashi

**Background & Hypothesis:** Whole exome sequencing (WES) is increasingly utilized in clinical genetics practices for individuals whose phenotype-driven first tier genetic testing has been non-diagnostic or when the clinical presentation is nonspecific or heterogeneous. Diagnostic rates using whole exome sequencing in general genetic clinic patients are consistently reported at 25-40%. Although limitations of WES are appreciated by clinicians, patients with negative WES from well-established clinical labs are often left without a confirmed diagnosis and the perception that there are no further diagnostic options. **Objective:** Here we present three cases in which whole exome sequencing was non-diagnostic, but further phenotype-driven molecular testing provided a diagnosis. **Results:** Case 1 is a 19 month old female with progressively worsening joint contractures beginning at 2 weeks of age, nodules on the gums, hyperpigmented discoloration over the joints, raised perianal skin lesions, protein losing enteropathy, failure to thrive, and motor and speech developmental delays, consistent with infantile systemic hyalinosis. Proband-only clinical WES was done, with deliberate and detailed analysis of ANTXR2, the only gene known to cause this condition, and was nondiagnostic. Sanger sequencing of ANTXR2 was performed, revealing a homozygous pathogenic variant (c.1073dupC) in exon 13, a previously described mutation in affected individuals that confirmed the diagnosis of infantile systemic hyalinosis. Case 2 is a 4 year old female with developmental regression beginning at 22 months and seizures developing at 3 years, 4 months. Metabolic screening, chromosomal microarray and trio clinical WES were negative. A subsequent exon-level oligo array CGH completed as part of a clinical 70 gene epilepsy panel identified a 44 base pair deletion in MECP2. Case 3 is a 3½ girl of a consanguineous union with developmental regression beginning at 16 months, white matter volume loss of the vermis and cerebellar hemispheres, bilateral optic atrophy and profound hypotonia. Clinical assessment of the genes in the regions of ROH on CMA identified 5 genes of interest. Sanger sequencing and multiplex ligation-dependent probe amplification for deletions and duplications detected a novel homozygous deletion involving the noncoding exon 1 of PLA2G6, extending into the promoter region, likely disrupting the transcription and resulting in a loss of function. Further clinical evaluation supported a diagnosis of PLA2G2-Associated Neurodegeneration, with progressive weakness, tongue fasciculations and decreased reflexes. **Conclusions:** This case series highlights the impact that limitations to current next generation sequencing methodology used for WES and many gene panels can have upon the diagnostic process and emphasizes the importance of clinicians looking beyond the exome when indicated by a specific phenotype.
#78 Title & Authors: A Recurrent de novo Variant in NACC1 Causes a Syndrome Characterized by Infantile Epilepsy, Cataracts and Profound Developmental Delay
Kelly Schoch, Linyan Meng, Szabolcs Szeling, David R. Bearden, Members of the UDN, Stanley Nelson, Julian A. Martinez-Agosto, Michael F. Wangler, Loren Pena, Yong-hui Jiang, Rebecca Spillmann, Allyn McConnkie-Rosell, Marie McDonald, Stephanie Burns Wechsler, Peter G. Kranz, Joan Jasien, Michael Freemark, Sujay Kansagra, Sharon Freedman, Deeksha Bali, Vandana Shashi

Background & Hypothesis: Whole exome sequencing (WES) has increasingly enabled new disease gene identification for undiagnosed neurodevelopmental disorders and provided insights into both gene function and disease biology. Objective: Here, we describe seven children with a neurodevelopmental disorder characterized by microcephaly, profound developmental delays and/or intellectual disability, cataracts, severe epilepsy including infantile spasms, irritability, failure to thrive and stereotypic hand movements. Methods: Whole exome sequencing and detailed clinical evaluations were performed for each individual, and individual 1 underwent a comprehensive evaluation through the Undiagnosed Diseases Network at Duke Clinical Site. Clinicians were connected via a web-based tool called GeneMatcher, database queries at collaborating labs and a UDN patient webpage designed to connect individuals with overlapping clinical features and candidate genes. Results: We observed an identical recurrent de novo heterozygous c.892 C>T (p.Arg298Trp) variant in the nucleus accumbens associated 1 (NACC1) gene in six affected individuals. The last individual was mosaic for this variant. NACC1 encodes a transcriptional repressor implicated in gene expression and has not previously been associated with germline disorders. The probability of finding the same missense NACC1 variant by chance in seven out of 17,228 individuals who underwent WES for diagnoses of neurodevelopmental phenotypes is extremely small and achieves genome-wide significance (p= 1.25e-14). Selective constraint against missense variants in NACC1 makes this excess of an identical missense variant in all seven individuals more remarkable. Conclusions: Our findings are consistent with a germline recurrent mutational hotspot associated with an allele-specific neurodevelopmental phenotype in NACC1.

#79 Title & Authors: A window into living with an undiagnosed disease: illness narratives from the Undiagnosed Diseases Network. Rebecca C. Spillmann, Allyn McConnkie-Rosell, Loren Pena Yong-Hui Jiang, Kelly Schoch, Nicole Walley, Camilla Sanders, Stephen R. Hooper, Jennifer Sullivan, Vandana Shashi

Background & Hypothesis: Patients’ stories of their illnesses help bridge the divide between patients and providers, facilitating more humane medical care. Illness narratives have been classified into three types: restitution (expectation of recovery), chaos (suffering and loss), and quest (acceptance of illness). Undiagnosed patients have unique illness experiences and obtaining their narratives would provide insights into the medical and emotional impact of living with an undiagnosed illness. Adults and children with undiagnosed diseases apply to be evaluated by the NIH Undiagnosed Diseases Network (UDN) at several clinical sites. Written illness narratives from 40 UDN applicants, including 20 adult probands who applied for themselves and 20 parents who applied for their children, were analyzed for: 1) narrative content and 2) narrative type. Methods: Using conventional content analysis, 40 consecutive narratives were coded and key concepts identified. The narratives were then grouped based on whether it was written by the proband (n= 20) or if it was written by a parent (n = 20) of a proband and examined to determine if there were differences related to the concepts identified. Results: Narrative content: could be grouped into three themes: 1) Expectations of the UDN: the majority had no further healthcare options and hoped the UDN would provide them with a diagnosis, with the adults expecting to return to their previously healthy life and the parents wanting information to manage their child’s health. 2) Personal medical information: the narratives reported worsening of symptoms and some offered opinions regarding the cause of their illness. The proband narratives had few objective findings, while parental narratives had detailed objective information. 3) Experiences related to living with their undiagnosed illness: frustration at being undiagnosed was expressed. The adults felt they had to provide validation of their symptoms to providers, given the lack of objective findings. The parents worried that something relevant to their child’s management was being overlooked. Narrative type: All the narratives were of the chaos type, but for different reasons, with the probands describing loss and suffering and the parents expressing fear for their child’s future. The parental narratives also had elements of restitution and quest, with acceptance of “a new normal”, and an emphasis on the positive aspects of their child’s illness; these were not as evident in the proband narratives. Conclusions: These narratives illustrate the chaos that coexists with being undiagnosed. The differences between the proband and parental narratives suggest that these two groups have different needs that need to be considered during their further evaluation and management.
#80 Title & Authors: Under-Dosing of Lorazepam as a First-Line Anti-Epileptic Drug (AED) is Associated with Increased Seizure Duration in Pediatric Refractory Status Epilepticus

Dmitry Tchapyjnikov, Lydia Feinstein, Marina Gainza Lein, Tobias Loddenkemper, Mohamad A. Mikati and the Pediatric Status Epilepticus Research Group (pSERG).

**Background & Hypothesis:** There is high variability in lorazepam (LZP) dosing when used in the treatment of status epilepticus (SE), but little is known about how this dosing variability affects seizure duration.

**Objective:** Using data from a multicenter prospective observational cohort of pediatric patients admitted with refractory SE between 2011-2016 in whom SE did not resolve after ≥2 AEDs, we assessed if a lower first dose of IV LZP when used as a first line AED was associated with increased seizure duration.

**Methods:** Three dosing groups were created with cutoffs based on current guidelines: lower dose (<0.05 mg/kg), medium dose (0.05 to <0.1 mg/kg), and higher dose (≥0.1 mg/kg). We used Cox proportional hazards regression to quantify the association between the first dose of LZP and subsequent time to seizure resolution. Models accounted for potential clustering by study site and were adjusted for age, sex, presumed underlying seizure cause, seizure duration prior to LZP administration, home AED use, prior neurological conditions, and location of LZP administration. Direct adjusted survival curves were used to obtain median seizure durations for each dosing group accounting for covariates.

**Results:** 103 patients were included in the analysis. Patients were a median of 4.5 years of age, 49/103 (48%) were female. LZP was administered at a median of 20 minutes following seizure onset, with 29/103 (28%) receiving a lower dose, 44/103 (43%) a medium dose, and 30/103 (29%) a higher dose. Individuals in the higher dose group were more likely to experience seizure resolution sooner than those in the medium and lower dose groups, with a hazard ratio of 1.62 (95% CI: 1.04, 2.53; p=0.0333) and 2.49 (95% CI: 1.43, 4.32; p=0.0012), respectively. Median time to seizure resolution following LZP administration was 93 min (IQR: 56-300 min) in the higher dose group, 160 min (IQR: 69-1664 min) in the medium dose group, and 350 min (IQR: 93-5580 min) in the lower dose group. 81/103 (79%) of patients required intubation during hospitalization, with no statistically significant difference between the dosing groups (p=0.6905).

**Conclusions:** The first dose of LZP, when administered as a first-line AED for pediatric refractory SE, is underdosed in the majority of pediatric patients. Doses lower than 0.1 mg/kg are associated with an increase in seizure duration, emphasizing the importance of following American Epilepsy Society SE guidelines for lorazepam dosing (0.1 mg/kg).

#81 Characteristics of Epilepsy in Alternating Hemiplegia of Childhood Authors: Julie Uchitel1, Lyndsay Prange1, Melissa McLean1, Jeffrey Wuchich1, Erin Heinzen2, David Goldstein2, Mohamad A. Mikati1 1Duke University Medical Center and Cure AHC Foundation, Durham, NC, USA 2Columbia University, New York, NY, USA

**Background and Hypothesis:** About half of AHC patients have epilepsy and some have SE. The characteristics of epilepsy and the long term outcome of SE in these patients have yet to be characterized. **Objective:** Describe the characteristics of epilepsy and status epilepticus (SE) in Alternating Hemiplegia of Childhood (AHC).

**Methods:** We analyzed a cohort of 25 patients seen in our center who fulfilled the AHC diagnostic criteria.

**Results:** 14/25 had epilepsy starting at the mean age of 3.57±3.94 years (0-14 years, median 2). Six had focal and secondary generalized semiology, 3 had focal and generalized semiology, 3 had generalized semiology, and 2 had focal semiology. Seven had generalized and focal seizures with impaired awareness, 4 had generalized, 2 had focal with impaired awareness, and 1 had focal without impaired awareness. Three patients were controlled on 1.5 ± 0.5 medications and 10 had drug resistant epilepsy on 3±1.71 medications; none appeared to be more effective than others. Seven had SE (>30 min): mean duration 7.64±11.96 hours (30 min-36), age of occurrence 6.18±7.15 years (0-23, median 4). Follow up was on average 2.29±1.91 years after first SE episode. Five had 3 episodes, 1 had two, and 1 had one. All episodes were refractory (failing to stop after a benzodiazepine and another medication); one was super-refractory (>24 hours). Intubation was required in 3/15 events (2/6 patients). All episodes were secondary generalized tonic-clonic SE. 2/7 had regression: One had regression after each of 3 status episodes: gross motor; swallowing, feeding, fine motor and sign language. Another had poor coordination and lost babbling after an episode. The first regained prior function after periods of few weeks after each episode and the second after few days. **Conclusions:** Focal and generalized semiology, high incidence of drug resistance, high incidence of status epilepticus, and RCS are features of epilepsy in AHC. Long term outcome after status epilepticus in AHC patients ranges from no regression to severe motor and linguistic regression. Regression may occur and may be reversible, but clinicians should be aware to treat early.
#82 Title & Authors: Characterizing Patient Applications Not Accepted to the Undiagnosed Diseases Network (UDN). Walley N, Peña LD, Schoch K, Spillmann R, Strong K, Shashi V. Background & Hypothesis: The UDN is a multi-site research program launched in 2015 to resolve the most difficult to solve undiagnosed cases. Applicants to the UDN complete an online questionnaire and submit a referral letter from their healthcare provider that summarizes their medical history. After a review of the application, referral letter, and comprehensive medical records, decisions regarding acceptance are communicated by letter. Inclusion criteria for the network are intentionally broad, therefore there are no pre-defined criteria for accepting (or not) applicants into the UDN. We are interested in an assessment of patterns in the clinical presentation of the patients who are not accepted to the UDN. We hypothesize that these applications will differ significantly from applications that are accepted in demographic make-up, with accepted subjects being much younger at the age of applications and also in symptomology of applicants. Objective: The aim of the study is to describe applications that are not accepted into the UDN so that we can (a) better inform the medical and patient communities regarding the types of patients that would most benefit from a UDN referral, and b) enable the network and medical community to better formulate specific recommendations that could be made for patients who are not suited to the UDN. Methods: We reviewed applications, referral and decision letters for applicants that were (N=50) and were not accepted (N=151) for inclusion in the UDN. We compared the demographics, application metrics and common symptoms reported in applications and referral letters as well as the outcomes of the application. Results: Applicants were equally comprised of males (47.8%) and females (52.2%) and there were no racial or ethnic disparities between Accepted/Not Accepted applicants. The median age applicants differed significantly between those Not Accepted and Accepted (37.7 yrs vs. 15.1 yrs, P<0.05). Sources of referral letters also differed significantly between applicants that were and were not accepted (Primary Care/Family Physicians referred 55.6% of Not Accepted applications vs. 8.00% of Accepted applications, P<0.05). Although 75.5% of Not Accepted applicants had at least one objective clinical finding documented by their referring physician, the most common symptoms endorsed in the referral letters were non-specific, vague complaints. Conclusions: We identified significant differences between applicants that ultimately are/are not accepted into the UDN. These differences can be used to guide future referral/recruitment efforts. We also highlight patterns of common symptoms that are not amenable to concrete diagnosis by current medical standards and are also not well-suited to the efforts of the UDN. Paths forward for diagnosis/treatment for these patients remain unclear.

#83 Diagnosis and Evaluation of Treatments for the Mucopolysaccharidoses by Analysis of Glycosaminoglycans in Various Tissues by UPLC/MS/MS. Haoyue Zhang, James Beasley, Patricia McCaw, Ashlee Stiles, Deeksha Bali, Dwight Koeberl, David Millington, Sarah Young.

Background & Hypothesis: Mucopolysaccharidoses (MPS) are lysosomal storage disorders caused by a deficiency of one of 11 different enzymes required for glycosaminoglycan (GAG) degradation. The resulting accumulation of GAGs in tissues causes a multisystem progressive disorder characterized by coarse features, skeletal abnormalities, hepatosplenomegaly, cardiac valve abnormalities, corneal clouding, hearing loss, and neurological impairment in some. Treatment options include intravenous and intrathecal enzyme replacement therapies and hematopoietic stem cell transplantation for some of the MPS disorders. Other treatments such as gene therapy and small molecule therapies are under development. Urinary GAG analysis is used as a first tier diagnostic test for the MPSs, and has traditionally been performed using a dye-binding assay to quantify the total GAG concentration. If elevated, a qualitative analysis by electrophoresis is used to determine which GAG species diagnostic test for the MPSs, and has traditionally been performed using a dye-binding assay to quantify the total GAG concentration. Objective: The aim of the study is to describe applications that are not accepted into the UDN so that we can (a) better inform the medical and patient communities regarding the types of patients that would most benefit from a UDN referral, and b) enable the network and medical community to better formulate specific recommendations that could be made for patients who are not suited to the UDN. Methods: We reviewed applications, referral and decision letters for applicants that were (N=50) and were not accepted (N=151) for inclusion in the UDN. We compared the demographics, application metrics and common symptoms reported in applications and referral letters as well as the outcomes of the application. Results: Applicants were equally comprised of males (47.8%) and females (52.2%) and there were no racial or ethnic disparities between Accepted/Not Accepted applicants. The median age applicants differed significantly between those Not Accepted and Accepted (37.7 yrs vs. 15.1 yrs, P<0.05). Sources of referral letters also differed significantly between applicants that were and were not accepted (Primary Care/Family Physicians referred 55.6% of Not Accepted applications vs. 8.00% of Accepted applications, P<0.05). Although 75.5% of Not Accepted applicants had at least one objective clinical finding documented by their referring physician, the most common symptoms endorsed in the referral letters were non-specific, vague complaints. Conclusions: We identified significant differences between applicants that ultimately are/are not accepted into the UDN. These differences can be used to guide future referral/recruitment efforts. We also highlight patterns of common symptoms that are not amenable to concrete diagnosis by current medical standards and are also not well-suited to the efforts of the UDN. Paths forward for diagnosis/treatment for these patients remain unclear.

Objective: Application of a UPLC-MS/MS method for CS, DS, HS measurements in CSF, serum, urine. When performed in conjunction with a UPLC-MS/MS method for measuring keratan sulfate, this method can be used as a combined qualitative and quantitative GAG screen, with superior performance compared with the traditional approach. Objective: Application of a UPLC-MS/MS method for CS, DS, HS measurements in CSF, serum, urine, dried urine spots (DUS) and cartilage homogenates. Methods: Aliquots of urine, serum, CSF, DUS extracts, or cartilage homogenates were methanolyzed, mixed with internal standards, and analyzed by UPLC-MS/MS using a published method. Positive control samples were obtained from patients and animal models of MPS type I. Control urine, serum and CSF samples were obtained from anonymized pediatric and adult patients with no known diagnosis. Results: Analysis of CS, DS and HS in various samples types showed good assay performance characteristics. Samples from untreated patients with MPS I and animal models had elevated DS and HS compared with controls. Conclusions: UPLC-MS/MS analysis of CS, DS, HS by a chemical degradation method that targets specific de-sulfated dimer products is versatile and can be reliably applied to various tissue types. This method can be used to monitor treatments in humans and animal models of MPS.
# Title & Authors: QI Project to Increase the Frequency of Diagnostic Documentation of Overweight and Obesity in the General Pediatrics Outpatient Clinic
Sarah Blanchard, MD; Andrew Dodgen, MD; Caren Mangarelli, MD

**Background & Hypothesis:** Obesity is an increasingly prevalent problem among pediatric patients, and the impact of obesity on health has been well studied. Previous studies have shown that, despite this knowledge, the diagnosis of overweight and obesity often goes unmade. Failing to establish this diagnosis can result in failure of provision of indicated lifestyle and clinical interventions, which can lead to poor health outcomes in both the short and long term.

**Objective:** The initial aim of our quality improvement project is to increase the frequency of appropriate diagnosis and coding of overweight and obesity at well child checks in the general pediatrics outpatient clinic. Once accurate diagnosis is improved we hope to increase the frequency of appropriate assessment of related cardiovascular risk factors and institution of indicated interventions.

**Methods:** The first step will be to establish a baseline rate of accurate diagnosis of overweight and obesity by doing an approximately three months-worth retrospective chart review of all resident well child visits at the general pediatrics Roxboro Rd clinic. A survey will be distributed to clinic personnel to elicit perceived barriers. Interventions will then be implemented, including education for personnel, reminder fliers in the clinic and updating the note template for easier documentation of BMI. Continuous data will be obtained through twice-monthly evaluation of documentation rates to provide a better understanding of the effect of each intervention.

**Results/Conclusions:** We estimate an initial rate of accurate diagnostic documentation to be 65-75%. Our aim is to increase that rate by 10% with our interventions.

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# Title & Authors: Improving Ocular Safety in the Pediatric Cardiac Catheterization Lab Using a Multimodal Series of Targeted Interventions
Andrew L Dodgen, MD, Jennifer Roark, NP, Heather McClean, MD, Michael J Campbell, MD, Greg Fleming, MD, MSCI

**Introduction:** Cardiac catheterization procedures are associated with a number of ocular hazards, including exposure to ionizing radiation, irritant materials, and potentially infectious bodily fluids. This safety improvement initiative sought to increase the rate of eye protection compliance among pediatric catheterization lab staff by at least 50% from the observed baseline compliance rate of 33%.

**Methods:** Through a series of Plan-Do-Study-Act (PDSA) cycles the authors implemented a modified time-out procedure which included a staff safety element; improved education among staff members regarding ocular hazards in the catheterization lab; and improved access to appropriate personal eye protection equipment. Post-intervention compliance rates were determined through direct observation.

**Results:** Baseline compliance rates were found to be low, with rates of 31%, 39% and 33% for scrubbed staff, non-scrubbed staff and all staff, respectively. After implementation of our interventions, an improvement in the baseline eye protection compliance rates to 98%, 83% and 90% among scrubbed staff, non-scrubbed staff and all staff, respectively, was observed. Additionally, key processes became more reliable: 100% of pre-procedural timeouts performed during the post-intervention study period included the newly introduced staff safety element and provisions were made to insure consistent and easy access to appropriate personal eye protection equipment.

**Conclusions:** A variety of barriers exist to the consistent use of personal eye protection equipment in the pediatric cardiac catheterization lab. Through a multimodal series of interventions aimed at reducing or eliminating these barriers, compliance with eye protection recommendations can be increased and safety of the cardiac catheterization lab can be improved.
Title & Authors: Implementation and evaluation of a preoperative huddle procedures to increase operating room efficiency, increase safety, and improve patient outcomes.
Authors: Harold J. Leraas, M.A., Krisian Becker, B.S., Dulce Perez Lessard R.N., Uttara P. Nag M.D., Reed W. Kamyszek, B.S., Henry E. Rice, M.D., Elisabeth T. Tracy, M.D., Bradley M. Taicher D.O.

Background: Several studies have examined the implementation of preoperative planning in improving patient safety and postoperative outcomes. However, these interventions are typically focused around the needs of adult surgical patients and exclude many needs and pathologies unique to children. Hypothesis: Implementation of a daily peroperative huddle in pediatric operating rooms is feasible and will improve delays, intraoperative complications, and short-term outcomes. Methods: Pediatric operating rooms in our institution are currently conducting daily preoperative huddles. The daily huddle reviews all cases for the day before beginning the first operation or procedure. Each case is discussed using the acronym PREPARE, which highlights topics pertinent to patient safety and disposition following the procedure (Figure). Operating room staff are being asked to complete evaluations of OR workflow on a daily basis for one month before implementation of the daily huddle and for three months following huddle implementation. Evaluations consist of questions identifying content of the daily huddle and improvement in workflow, operative safety, and safety culture in the OR. Results: Results for this study are currently pending as huddle evaluations are being collected. Huddle evaluations and 30-day outcomes will be collected and reported at the conclusion of this study. Five of six pediatric operating rooms are currently conducting daily huddles using the PREPARE acronym. Conclusions: This study is currently collecting data from daily operative flow and short-term patient outcomes. At this point, most pediatric operating rooms are currently conducting daily huddle.

Figure: The PREPARE acronym used during daily huddles with goals of improving workflow and safety.

PREPARE for Pediatric Huddles:
- Personnel in OR, Patient and Procedure to be performed
- Requirements for patient positioning
- Equipment needed and available for the operation
- Prophylactic antibiotics
- Anesthesia plan (including airway, invasive monitoring, regional anesthesia)
- Red blood cells, FFP, platelets or other transfusion needs, including T&S
- End of case disposition (destination, bed availability, planned ventilation)

Title & Authors: Outcome of Penicillin Skin Testing Results Fails to Affect Clinical Practice
Renee Kleris, MD and Patricia Lugar, MD MS

Background & Hypothesis: Drug allergy represents a challenge for patients and practitioners alike. Penicillin skin testing revolutionized the question of penicillin drug allergy. Penicillin skin testing is safe and is an effective tool used to evaluate for penicillin drug allergy.

Objective: We reviewed penicillin skin testing in pediatric and adult allergy clinics in a large academic medical center to establish the safety, efficacy, and utility.

Methods: A retrospective chart review identified 150 patients who completed outpatient penicillin allergy testing with Pre-Pen and Pen-G during 1/2010-2/2017. We recorded demographics, reported allergic reaction, skin testing results (skin prick test-SPT and intradermal-ID), outcome of penicillin/derivative challenge, serum specific IgE, subsequent penicillin/derivative use, and updated drug allergy in the electronic medical record (EMR).

Results: The majority of reported reactions were grade I/II hypersensitivity reactions (120/80%) followed by grade III/IV (11/7%). Other reactions (20/13%) included unknown and non-IgE-mediated reactions. Pediatric patients comprised 57/80%, and 5/9% had positive SPT or ID. 36/63% did not complete penicillin/derivative challenge despite 51/89% having negative skin test results. In 20/35% penicillin allergy remained (EMR) after negative testing. Adult patients comprised 93/63%, 3/3% had positive SPT or ID, and 59/63% completed penicillin/derivative oral challenge. 21/23% did not have updated allergy (EMR) after negative testing. 32/21% of all patients were prescribed a penicillin/derivative antibiotic after negative testing at subsequent encounters.

Conclusions: Penicillin skin testing is safe and our results are similar to reports in the literature. A number of unexpected findings included infrequent use of penicillin/derivative medications after negative testing, discrepancies between pediatric and adult clinics, maintenance of penicillin allergy in the record, and incomplete testing without dose challenge. Further education can ensure penicillin skin testing is utilized fully to address the public health concern of penicillin allergy.
#88 Title & Authors: “Mind the Gap: Helping Physician Trainees Learn to Guide Young Adults with Chronic Illness across the Chasm from Pediatrics to Adult Medical Care” RE Sadun, MD, PhD; A Rozycki, MSW; S Brotkin, BS; MD Pollack, PhD; LG Criscione-Schreiber, MD, MEd, RJ Chung, MD; GR Maslow, MD, MPH

**Background & Hypothesis:** The transition from pediatric to adult healthcare is a vulnerable time for adolescents and young adults (AYA) with chronic conditions, and most US physicians do not feel equipped to help AYA transition. This study sought to determine resident and fellow baseline preparedness to offer AYA transition care and hypothesized that educational interventions would improve trainee transition care skills. **Objective:** The objective of this QI project was to enhance – by a standard of statistical significance – Duke resident and fellow preparedness to provide transition best practices to AYA with chronic illnesses. **Methods:** Survey: All Duke physician trainees (n=985) were surveyed in May of 2015 about their educational experiences with core transition skills. **Clinic:** A Transition Collaboration Clinic was established, bringing together dyads of rotating pediatric and adult residents (n=46) to work together with AYA with chronic illness. Paired pre & post surveys were used to assess trainees’ confidence in their ability to deliver transition care. **Curriculum:** A 1-hour workshop on transition best practices was developed alongside an objective standardized clinical examination (OSCE) station in which trainees were asked to welcome a young adult and her family to a first visit in an adult clinic. Adult hemato-oncology fellows (n=22) from 4 institutions were evaluated with the OSCE, half having already experienced the transition workshop. We analyzed aggregated OSCE scores and pre & post surveys assessing trainee confidence with transition care skills and attitudes. **Results:** At baseline, 25% of Duke physician trainees rated themselves as “not at all prepared” to speak with a counterpart provider about a transferring patient or to speak with a patient and family about transition. Participation in the Transition Collaboration Clinic raised the transition skill confidence of Duke internal medicine residents from 1.6 to 2.9 (p<0.005), while Duke pediatric and Med/Peds resident transition skill confidence was raised from 2.2 to 3 (p<0.005). Fellow participants in the transition workshop demonstrated a statistically significant increase in their reported transition skill confidence, the importance they placed on the physician’s role in AYA transition, and their OSCE performance for several key transition skills. **Conclusions:** Resident and fellow transition skills were low at baseline, with trainees rating themselves as ill-prepared to help AYA transition and neglecting fundamental skills assessed by OSCE. However, transition skills can be learned, and transition clinic or workshop experience improved trainee confidence in transition skills. Additionally, and the workshop enhanced transition care performance in an OSCE assessing transition skills.

#89 Title & Authors: “Improving the Medical Care of Children in Foster Care: An Academic-Community QI Collaborative” Lindsay Terrell, MD; Beth Herold, DNP CPNP; Karen St. Claire, MD; Aditee Narayan, MD MPH

**Background & Hypothesis:** It is well established that children in foster care [FC] have increased health care needs. The AAP recommended per expert opinion that FC children receive an initial medical evaluation [IE] within 72 hours after placement. The Duke FC Clinic was started in 2013 and is staffed by four Child Abuse and Neglect specialty providers. In Durham County, effective medical care for children in FC has required collaboration among Child Welfare, Medicaid Coordinators, the Duke Child Abuse and Neglect Clinic staff and nursing to create a FC Collaboration. The average IE takes 1 hour. Despite AAP recommendations, NC Child Welfare policy states that FC children receive an IE within 7 days of placement into FC. Despite community collaboration and the current state policy, the median time to initial evaluation [TTIE] for a child in foster care in Durham County was 33 days (mean: 32 days). Understanding this high risk population with medical and social complexities, a large scale QI project was started. This QI project has required collaboration between multiple systems within and outside of medical clinic in order to improve the TTIE for children in FC.

**Objective:** Improve the mean TTIE from 32 days to less than 7 days within 12 months (Jan-Dec 2016) for children in FC in Durham County.

**Methods:** FC Collaboration was assembled, led by CAN fellow. Barriers were identified utilizing a process map and key driver diagrams. FC Clinic staff and providers underwent two reviews of current AAP recommendations and state policy compared to current TTIE. FC Clinic appointments were adjusted to accommodate urgent IE’s. A manual schedule tracker was utilized for real time tracking of failures and reason for delayed TTIE. The fellow performed weekly check ins with scheduler and staff and was used as one point of contact if urgent IE not available. The fellow hosted a Duke and Durham Child Welfare Conference and performed a focused interview with Child Welfare Social workers. An acceptable pathway was created to share data across medical and community entities. Monthly feedback was provided to Durham Child Welfare of current average TTIE’s. Balance measures assessed.

**Results:** TTIE improved from 32 days to as low as 4 days from Aug-Nov. December’s TTIE was elevated (12 days) due to delayed referrals and appointment availability secondary to provider and Child Welfare holiday breaks.

**Conclusions:** A QI project focused on a complex population and multiple community entities poses specific, unique challenges. This QI project identified several strategies to decrease TTIE that can be applied to other foster care clinics within and outside of NC.
#90 Title & Authors: Burnout Status and Milestone Performance in Pediatric Residents

**Background & Hypothesis:** Burnout in trainees has gained attention because of its potential relationship to depression and negative impact on patients. Milestones have been utilized to measure the competency of residents in training.

**Objective:** To compare the mean Milestone scores between pediatric residents who met criteria for burnout and those who did not by level of training.

**Methods:** This work was completed as part of the Pediatric Burnout and Resilience Consortium (PBRSC), a consortium of 34 programs, with the support of the Association of Pediatric Program Directors Longitudinal Education Assessment Research Network. PBRSC conducted a confidential online survey of its members’ residents in April – June, 2016, which included the Maslach Burnout Inventory (MBI). In addition, programs submitted their assessment of residents’ milestones. Burnout was defined as high range for either emotional exhaustion or depersonalization domains of the MBI. We examined the relationship between burnout and performance as assessed by milestones by domain of competence and stratified by post-graduate year.

**Results:** 1494 residents at 31 programs completed the MBI and had milestone data submitted. While residents who met criteria for burnout scored lower on all 21 Milestones compared with those without burnout, when PGY2 and PGY3 residents were examined the association between burnout status and milestone performance was not statistically significant. However, in the PGY1 cohort (n=507), those who screened positive for burnout had lower milestones in the following domains: patient care (2.97 vs 2.76, -0.21, p=0.001), systems based practice (2.86 vs 2.68, -0.18, p=0.004), problem based learning and improvement (2.93 vs 2.74, -0.19, p=0.002), professionalism (3.24 vs 3.07, -0.17, p=0.007), and interpersonal and communication skills (3.12 vs 2.93, -0.19, p=0.011) but not in medical knowledge.

**Conclusions:** Burnout status is most closely associated with decreased milestone for PGY1s in every domain of competence except medical knowledge. Future research needs to address whether strategies to mitigate burnout results in improved PGY1 performance.
Reviewers

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