Dear Friends and Colleagues,

On behalf of the UC San Diego/Rady Children’s Hospital San Diego Academic Council, it is my honor and privilege to present to you the Winter 2022 Scientific Discoveries report pertaining to Child Health from UC San Diego Health Sciences (UCSD) and Rady Children’s Hospital (RCHSD) with contributions from UCSD School of Medicine, UC Skaggs School of Pharmacy & Pharmaceutical Sciences and Wertheim School of Public Health & Human Longevity Science.

We are very proud of the achievements of our clinicians, physician-investigators and scientists. The work highlighted here illustrates that, in spite of enormous stress on our faculty in the past few years, our faculty performed admirably. This compendium demonstrates the depth, breadth and rigor of the scientific contributions into pathobiology, treatment, prevention and cures for childhood diseases. It really is a testimony of the spectacular quality of the research that goes on in the hallways of UC San Diego and Rady Children’s Hospital. This is an exciting time as we continue to grow and adapt to a changing world, remaining motivated and responsive to improving clinical care through research and discoveries. The reason for hope is related to both the superlative quality of clinical care at RCHSD and the outstanding basic and clinical research taking place in our research buildings into the late night, day after day.

We hope that this summary of faculty discoveries helps to facilitate potential collaborations between clinicians, clinician-investigators and scientists around the world. We trust that such collaborations and networking can bring investigators closer to curing diseases of childhood and hence building a better society for the future. This is so much the responsibility of pediatricians across the world.

Gabriel G. Haddad, MD

Gabriel G. Haddad, MD
Distinguished Professor of Pediatrics and Neuroscience
Chair, Department of Pediatrics
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BUGS: THE GOOD AND THE BAD

Revealing amazing host-microbe interactions, coordination, symbiosis and competition.
The recent and severe COVID-19 pandemic demonstrates the urgent need to understand not only factors that promote viral SARS-CoV-2 viability, but also mechanisms that encourage infectivity and establishment of lethal secondary bacterial infections, especially in enclosed, indoor environments where people spend the majority of time. Bacteria within our oral and respiratory cavities, and within our built environment may have an important influence on respiratory virus abundance and viability.

The laboratory of Dr. Gilbert has led the analysis of the microbiome of the built environment, which in the developed world consists predominantly of human skin, gut, oral and respiratory tract-associated bacteria. Previous studies including dense spatial and temporal sampling and microbiome analysis of the hospital environment has demonstrated that the hospital environment is selecting for a limited set of bacteria that can survive and persist on the floors and surfaces in the building – which primarily originate from the respiratory tract. Bacteria can alter the survival of viruses outside the host by enhancing stability of the capsid, and direct interaction between bacteria and viruses can impact viral transmission in mouse models. Considered together, these data suggest an intricate feedback loop in which viral-bacterial interactions, in hospital air and on surfaces, and within respiratory mucosa, are influenced by each other to impact viral persistence and potential virulence.

Recently, Dr Gilbert’s team, led by Dr Sarah Allard in collaboration with Dr Rob Knight’s team performed a survey to determine the mechanisms underlying the viral-bacterial interaction which has been validated by a group outside of our institution. The laboratory is now investigating how SARS-CoV-2 interacts with the oral and respiratory associated bacteria, Rothia denticarosa, both in the patient’s airway and within the patient’s room, especially the floors. This finding was independently validated by a group at another university. The team is now investigating how SARS-CoV-2 interacts with other bacteria including Rothia and found that the SARS SPIKE protein binds to the outside of many different types of bacteria (Fig. 1).

**IMPLICATION:** Broader sampling and experimentation are required to understand the extent to which Rothia influences the viability and infectivity of SARS-CoV-2. We are currently performing studies to identify whether Rothia influences viral persistence and viability on different surface materials and to determine the mechanisms underlying the viral-bacterial conjugate. The implication of this work is that definition of factors that impact SARS-CoV-2 abundance and viability and infectivity will enable the design of appropriate interventions and treatments to limit viral transmission and establishment of secondary bacterial infections.

**Reference:**

Staphylococcus aureus bacteremia (SaB) causes significant disease in humans, carrying mortality rates of ~25%. The ability to rapidly predict SaB patient responses and guide personalized treatment regimens could reduce mortality. Dr. Gonzalez and his team present a resource of SaB prognostic biomarkers. Integrating proteomic and metabolic techniques enabled the identification of ~10,000 features from >200 serum samples collected upon clinical presentation. They interrogated the complexity of serum using multiple computational strategies, which provided a comprehensive view of the early host response to infection. Their biomarkers exceed the predictive capabilities of those previously reported, particularly when used in combination. Last, the Gonzalez team validated the biological contribution of mortality-associated pathways using a murine model of SaB. Their findings represent a starting point for the development of a prognostic test for identifying high-risk patients at a time early enough to trigger intensive monitoring and interventions.

**IMPLICATION:** This finding is a leap forward toward a point-of-care predictive tool for SaB risk. Further refinement of SaB biomarkers will enable clinicians to identify patients who need intensified monitoring and therapy, rather than responding post hoc to failures in standard of care.

**Reference:**
Systematic characterization of the cancer microbiome provides the opportunity to develop techniques that exploit non-human, microorganism-derived molecules in the diagnosis of a major human disease. Following recent demonstrations that some types of cancer show substantial microbial contributions, the Knight lab re-examined whole-genome and whole-transcriptome sequencing studies in TCGA. Dr. Knight and his team could discriminate among samples discarded up to 92.3% of total sequence data. In addition, the use of very stringent decontamination analyses that commercial-grade cell-free tumor DNA platforms, despite lacking any genomic alterations currently measured on two mutations, provide the opportunity to develop techniques that are sensitive and specific for many cancers, providing both new diagnostic and therapeutic strategies based on the cancer microbiome.

Cancer Microbiome Diagnostics

The translational power of human microbiome studies is limited by high interindividual variation. Dr. Knight and his team describe a dimensionality reduction tool, compositional tensor factorization (CTF), that incorporates information from the same host across multiple samples to reveal patterns driving differences in microbial composition across phenotypes. CTF identifies robust patterns in sparse compositional datasets, allowing for the detection of microbial associations changes associated with specific phenotypes that are reproducible across datasets.

IMPLICATION: In this work and in follow-up studies (Song et al. 2021; Marotz et al. 2021; Martino et al. 2021), the Knight lab discovered that this improved method for revealing temporal signals in microbiome data can explain how early-life changes in the microbiome predict inflammatory bowel disease and possibly other conditions later in life, and how bacteria interact with SARS-CoV-2 in patients and hospital environments relevant to COVID. These results provide information about long-term impacts of early-life microbes.


Compositional Tensor Factorization

CTF utilizes feature abundance matrices for subjects over time. For each subject with a phenotype of interest, the data is represented as relative abundances of features (abundance gradient represented in grayscale) per time. (b) The matrices are concatenated, robust-centered log-ratio transformed (R-CLR) and structured into a tensor format with modes corresponding to subjects, features and time. (c) The resulting tensor is then factored based only on observed data into loading vectors for each dimension (i.e. subject, timepoint, and feature). (d) Simulated count data is plotted on the y-axis for three taxa with the mean counts in bold and missing values absent from the bold line. Standard deviation of distributions are shaded behind. Two phenotypes are compared; a control unchanging in time (left) and a dynamic phenotype with a perturbation at time point 2 (right). Taxon 1 (blue) is highly abundant and noisy, taxon 2 (red) is lowly abundant but growing exponentially in phenotype 2, and taxon 3 (orange) is oscillatory with increasing amplitude in phenotype 2. The first two principal component axes (i.e., loadings) from CTF (PC1 (top) and PC2 (bottom)) are plotted on the y-axis with the corresponding sample (x), time (f), and feature loadings (g). In PC1, phenotype 2 is linked to the unstable oscillatory waveform of highly loaded taxon 3 (orange, top). Similarly, in PC2, phenotype 2 is linked to the sigmoidal waveform of highly loaded taxon 2 (red, bottom).
Hyperosensitive sites sequencing (scTHS-seq) from human pediatric samples (full gestation, no known lung disease) collected at day 1 of life, 14 months, 3 years, chromatin accessibility at ACE2, TMPRSS, and CTSL loci across lung cells in early life. a. Schematic: single-cell chromatin accessibility by transposome and Erysipelotrichaceae. The most differentially abundant (p < 0.001) were Peptostreptococcaceae, Ruminococcaceae, were significantly different from each other in atherosclerotic mass spectrometry. In the aorta, IC-induced atherosclerosis of intermittent hypoxia (IH) or intermittent hypercapnia (IHC) increases atherosclerosis risk. However, the contribution to Air controls. Atherosclerotic lesions were examined, gut microbiome was profiled using 16S RNA gene amplicon sequencing and metabolome was assessed by untargeted mass spectrometry. In the aorta, IC-induced atherosclerosis was significantly greater than IH and Air controls (aorta, IC 11.1 ± 0.7% vs. IH 7.6 ± 0.4%, p < 0.05 vs. Air 8.1 ± 0.8%, p < 0.05). In the pulmonary artery (PA), however, IH, IC, and Air were significantly different from each other in atherosclerotic formation with the largest lesion observed under IH (PA, IH 40.9 ± 2.0% vs. IC 20.1 ± 2.8% vs. Air 12.2 ± 1.5%, p < 0.05).

The most differentially abundant microbial families (p < 0.001) were Peptostreptococcaceae, Ruminococcaceae, and Erysipelotrichaceae. The most differentially abundant metabolites (p < 0.001) were tauro-β-muricholic acid, ursodeoxycholic acid, and lysophosphoethanolamine (18:0). It was concluded that IH and IC (a) modulate atherosclerosis progression differently in distinct vascular beds with IC, unlike IH, facilitating atherosclerosis in both aorta and PA and (b) promote an atherosclerotic luminal gut environment that is more evident in IH than IC. We speculate that the resulting changes in the gut microbiome and metabolome interact differently with distinct vascular beds.

**IMPLICATION:** This work has major clinical implications since IH and IHC can change the gut microbiome, which can contribute to increased risk for atherosclerosis. Understanding which deleterious bacterial ecology is important and how the host interacts with gut bacteria to produce a certain gut metabolome will be important for devising innovation for better cardiovascular therapy of OSA.

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**Obstructive Sleep Apnea and Resulting Hypoxia and Hypercapnia**

**Alter the Gut Microbiome**

Hundreds of genes are implicated in autism spectrum disorder (ASD), but the mechanisms through which they contribute to ASD pathophysiology remain elusive. Dr. Lewis and his team analyzed leukocyte transcriptomics from 1- to 4-year-old male toddlers with ASD or typical development (TD) to identify transcriptional programs that are active in each diagnosis group. They found that the degree of dysregulation in this network correlated with the severity of ASD symptoms in the toddlers.

**IMPLICATION:** By finding core molecular pathways involved in the dysregulated brain development in autism, therapies can begin at a younger age when they may be more effective.

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**A Perturbed Gene Network Containing PI3K–AKT, RAS–ERK and WNT–ß-Catenin Pathways in Leukocytes is Linked to ASD Genetics and Symptom Severity**

Transcriptome analysis of 228 toddlers with ASD or TD identified 1,236 DE genes. A comprehensive static network of DE genes was built from high-confidence physical and regulatory interactions from interaction databases. To identify transcriptional programs that are active in each diagnosis group, pairs of interacting genes were retained in the static network that are highly coexpressed in each diagnosis group. This yielded networks that were specific to children with autism (DE-ASD) and typically developing children (DE-TD), allowing for a comparison of the activity of transcriptional programs between ASD and TD conditions. To connect the DE-ASD network to ASD genes, an XP-ASD network was built by connecting the DE-ASD network to autism risk gene (ASD) risk genes. The DE-ASD and XP-ASD networks were analyzed in the context of neural differentiation, ASD neuron models and ASD symptom severity. To ensure results were robust to variations in the interaction networks, the results were reproduced by replacing the high-confidence static network (the first step in pipeline) with a functional and a full coexpression network.

**IMPLICATION:** A perturbed gene network containing PI3K–AKT, RAS–ERK and WNT–ß-catenin pathways in leukocytes is linked to ASD genetics and symptom severity.

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**Perturbed Gene Network Containing PI3K–AKT, RAS–ERK and WNT–ß-Catenin Pathways in Leukocytes is Linked to ASD Genetics and Symptom Severity**

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**ASD children as young as 18 months old and predict the severity of their symptoms that may not present clearly for a few years. Thus, therapies can begin at a younger age when they may be more effective.**

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**GABRIEL G. HADDAD, MD**

Distinguished Professor of Pediatrics and Neuroscience

Respiratory Medicine

**RESEARCH INTERESTS:** Define and characterize the cellular and molecular alterations that can lead to survival of cells or to injury and cell death; obstructive sleep apnea and cardiovascular disease.

**NATHAN LEWIS, PHD**

Associate Professor of Pediatrics and Bioengineering

Host-Microbe Systems and Therapeutics

**RESEARCH INTERESTS:** Development of network-based diagnostics for childhood disorders using systems biology-based approaches that account for the regulation and activity of complex pathways such as metabolism, protein synthesis/secretion and glycosylation.
Advances in acne pathogenesis have been modest over the years because available animal models do not approximate human disease. The pathogen that causes acne vulgaris, C. acnes, is rapidly killed by laboratory animals upon subcutaneous introduction and, therefore, current models do not mimic what occurs in human disease.

Dr. Liu and his team applied a synthetic human sebum to the mouse skin at the time of C. acnes inoculation and noted dramatic and sustained survival of C. acnes along with induction of inflammatory lesions. Furthermore acne-associated C. acnes strains induced lesions that are consistently more inflammatory than lesions caused by health-associated C. acnes strains. Therefore, this new model represents a promising platform to study the pathogenesis and treatment of acne vulgaris.

**IMPLICATION:** Current therapeutics for acne vulgaris are modestly effective, non-specific, and require prolonged application. By generating an acne model that approximates human disease, the Liu lab is in a unique position to investigate human-relevant mechanisms of acne disease and treatment.


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Despite antibiotics and improvements in ICU care, Staphylococcus aureus (staph) bloodstream infection and sepsis still causes severe illness or death in 20% of patients. Dr. Nizet and his team discovered a battle that occurs between staph bacteria and platelets — blood cells known better for their role in clotting than in immune defense. In some sepsis cases, they found, the bacteria win out and platelet levels plummet, and those patients with fewer platelets were more likely to die of staph sepsis. Platelets secrete antimicrobial peptides that help the immune system destroy staph. At the same time, staph release a toxin that’s that pokes holes in platelets and causes them to produce an enzyme that trims off sugar molecules that decorate their own surfaces. The platelet’s new look is recognized by another molecule in the liver called the Ashwell-Morell receptor, which pulls “bald” platelets out of circulation.

To tip the balance of the “toxin-platelet-receptor” axis back in favor of the human patient, the Nizet lab performed studies in a mouse model of staph sepsis, testing several classes of drugs known to be safe in humans and known to act on platelets. Most drugs they tested had no effect, but two drugs made a big difference. Ticagrelor (Brilinta®) blocks staph’s toxin so it can’t injure platelets or stimulate its sugar-removing enzyme. Oseltamivir (Tamflu®) inhibits the platelet sugar-removing enzyme so the cells don’t go bald and aren’t cleared by the liver. Mice with staph sepsis and treated with either ticagrelor or oseltamivir maintained more platelets and had less bacteria in their blood. Ultimately, 60% percent of treated mice survived compared to 20% of untreated mice.

**IMPLICATION:** Discovering a new drug is tremendously expensive and takes many, many years. But by looking around at what is already available, what is already known to be safe, there may be many opportunities to improve patient outcomes. Rather than continue to throw more antibiotics at the problem, there is the option to boost the other side of the equation: the patient’s own immune system.

The Enterobacteriaceae are a diverse family of bacteria that inhabit the gastrointestinal tract. Members of this group include the enteric pathogens Salmonella enterica, as well as Escherichia coli, a species that comprises myriad commensals, pathobionts, and pathogens. Both Salmonella and E. coli can colonize the intestine of mammals and thrive in inflammatory conditions.

The laboratory of Dr. Raffatellu has previously discovered that, in response to zinc limitation, the gut pathogen Salmonella overcomes zinc sequestration by the host protein calprotectin, outcompetes the microbiota, and colonizes the gut to high levels. In addition to Salmonella, other Enterobacteriaceae can thrive in the inflamed intestine. One such example is the probiotic bacterium Escherichia coli Nissle 1917 (E. coli Nissle), a strain that was first isolated in WWII from the stool of a soldier who did not develop gastroenteritis during a Shigellosa outbreak. Since then, E. coli Nissle has proven to be effective in the treatment and prevention of inflammatory disorders including chronic constipation, ulcerative colitis, and inflammatory bowel disease.

Recently, Dr. Raffatellu’s team discovered the mechanisms by which E. coli Nissle acquires zinc in the inflamed gut and, in close collaboration with the laboratory of Dr. Pieter Dorrestein, identified the zinc-scavenging molecule. The team discovered that a small molecule secreted by E. coli Nissle (yersiniabactin) is capable of binding zinc and thus acts as a zincophore, delivering zinc to the bacterium. Moreover, the group demonstrated that E. coli Nissle utilizes yersiniabactin, in addition to other zinc transporters, to effectively acquire zinc in vitro, to resist the antimicrobial activity of calprotectin, and to colonize the inflamed gut.

**IMPLICATION:** Dr. Raffatellu’s studies elucidating how commensal and pathogenic Enterobacteriaceae thrive in the inflamed gut by acquiring essential metal nutrients have implication for developing new therapies to combat gastrointestinal infections while promoting the growth of beneficial microbes.

**REFERENCE:**


**Vibrio cholerae (V. cholerae),** the etiological agent of cholera, uses cholera toxin (CT) to cause severe diarrheal disease which is thought to promote transmission of the pathogen during outbreaks. Using animal models of disease and in vivo RNA-sequencing analysis, Dr. Rivera-Chavez and his team found that the catalytic activity of CT induces metabolic changes in the gut, leading to enhanced growth of V. cholerae during infection. However, the molecular mechanisms by which CT promotes transmission of the pathogen remain poorly understood.

The Rivera-Chavez lab continues to its research to further understand how CT-induced disease modulates host intestinal metabolism to enhance the transmission of V. cholerae. They are currently investigating whether CT may modulate the intestinal microbiota to promote fecal–oral transmission of V. cholerae. This research will continue the in-depth characterization of CT and other PAK-activating toxins in the modulation of host metabolism, the intestinal microbiota, pathogen growth, and transmission. These findings will have broad implications for how CT and other bacterial toxins modulate host metabolism and pathogen growth, which could lead to novel therapeutics and intervention strategies.

**IMPLICATION:** There are an estimated 100,000 to 120,000 deaths every year due to cholera, with about half of all cholera deaths occurring in children under five. Due to the lack of cost-effective vaccination and poor vaccine efficacy in children, there is a need for alternative preventative and therapeutic strategies. These findings represent a paradigm shift into our understanding of the function of cholera toxin and likely other bacterial toxins in the transmission of bacterial pathogens, which may point to both host and bacterial metabolism as new targets for the treatment or prevention of cholera and other infectious diseases.

**REFERENCE:**

The two major roadblocks to an HIV-1 cure are: 1. identification and elimination of HIV-1 from latent reservoirs that persist despite highly successful suppressive therapy; and 2. the identification and killing of all cells that express HIV-1 on the surface to prevent the new infection of neighboring cells.

To address the first challenge, Drs. Grant Campbell and Spector hypothesized that HIV-1 maintains the survival of latently infected CD4+ T cells through increased expression of cell survival factors including XIAP, BIRC2 and BECN1. They found that SMAC mimetics promote the degradation of XIAP and BIRC2 in latent HIV-infected resting memory CD4+ T cells (HIV-TCM) without activating viral transcription. Also in HIV-TCM, but not uninfected cells, degradation of XIAP and BIRC2 leads to induction of autophagy, and the formation of a cell death complex on phagophore membranes that results in killing of HIV-TCM while sparing uninfected cells (Fig 1).

To address the second obstacle, Dr. Spector’s laboratory in collaboration with the laboratory of Dr. Liangfang Zhang of bioengineering examined the potential of nanoengineered CD4+ T cell membrane-coated nanoparticles (TNP) to neutralize a broad range of HIV-1 strains. They found that TNP display outstanding neutralizing breadth and potency, and are able to neutralize all 125 HIV-1 viral strains from a global panel of HIV-1 subtypes and recombinant forms (Fig 2). TNP also selectively bind and kill HIV-1 infected CD4+ T cells and macrophages while having no effect on uninfected cells.

**IMPLICATION:** These findings show that certain SMAC mimetics can selectively kill HIV-1 latently infected cells. Moreover, TNP can be used to neutralize HIV-1 and to target cells expressing HIV-1 proteins on the surface. Thus, Dr. Spector and colleagues have developed a drug delivery platform that can be combined with small molecules including SMAC mimetics to target and preferentially kill HIV-infected replicating and latently infected cells.

**References:**


SMAC mimetics promote cell death of HIV-TCM via the autophagy dependent formation of a CASP8-activating platform on phagophore membranes. Treatment of a heterogeneous population of uninfected and latent HIV-infected TCM with SMAC mimetics induces the degradation of XIAP and BIRC2. This triggers the induction of autophagy and the formation of a cell death autophagy (FAAD, RIPK3, CASP9) and autophagy (ATG5, ATG7 and SQSTM1) proteins on unclosed autophagosomal/phagophor structures of HIV-TCM but not uninfected TCM resulting in the selective killing via apoptosis of the infected HIV-TCM while sparing the uninfected bystander cells in the absence of viral activation.

**FIGURE. 1**

**FIGURE. 2**

Neutralization profile of TNP against a panel of 125 HIV-1 viral strains from a global panel of HIV-1 subtypes and recombinant forms. Each bar represents the IC 80 (ng mL^-1) of TNP against a single virus. Viruses are ranked according to increasing IC80 values.
Dietary habits have been associated with alterations of the human gut resident microorganisms contributing to obesity, diabetes, and cancer. In Western diets, red meat is a frequently eaten food, but long-term consumption has been associated with increased risk of disease. Red meat is enriched in N-glycolyneuraminic acid (Neu5Gc) that cannot be synthesized by humans. However, consumption can cause Neu5Gc incorporation into cell surface glycans, especially in carcinomas. As a consequence, an inflammatory response is triggered when Neu5Gc-containing glycans encounter circulating anti-Neu5Gc antibodies. Although bacteria can use free sialic acids as a nutrient source, it was until recently unknown if gut microorganisms contribute to releasing Neu5Gc from food.

Dr. Zengler and his team found that a Neu5Gc-rich diet induces changes in the gut microbiota, and that these changes included an increased abundance of novel sialidases. They identified these bacterial sialidases with previously unobserved substrate preference for Neu5Gc-containing glycans. X-ray crystallography revealed key amino acids contributing to this substrate preference. Furthermore, they verified that these sialidases were able to release Neu5Gc from red meat. The release of Neu5Gc from red meat using bacterial sialidases could reduce the risk of inflammatory diseases associated with red meat consumption, including colorectal cancer and atherosclerosis.

**IMPLICATION:** This finding could provide a path forward to systematically reduce inflammation in the gut. Optimization of these new enzymes would increase release of Neu5Gc from the diet thus reducing its inflammatory properties.

**REFERENCES:**


CANCER PATHOGENESIS AND THERAPEUTICS

Discovering new cancer genes, pathways, therapies and survival.
Dr. Aristizabal and her research team established the Cross-Border Neuro-oncology Program (CBNP) between Rady Children’s Hospital San Diego and Hospital General Tijuana in Mexico to provide access to neuro-oncology care, including neurosurgical services, and improved survival for children with brain tumors diagnosed at Hospital General Tijuana. The CBNP facilitated access to complex neuro-oncology care for underserved children in Mexico through binational exchanges of resources and expertise.

Five-year overall survival dramatically improved from 0% before 2010 to 52% in 2017. The CBNP model offers an attractive alternative for children with brain tumors in low- and middle-income countries who require complex neuro-oncology care, particularly those in close proximity to institutions in high-income countries with extensive neuro-oncology expertise, such as Rady Children’s Hospital.

**IMPLICATION:** The Cross-Border Neuro-Oncology Program was established across the United States-Mexico border to facilitate access to neuro-oncology care for children with brain tumors in Tijuana, Mexico, through binational exchanges of resources and expertise. The cross-border model improved survival significantly and serves as a feasible model for other border regions across the world.

**Reference:**

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**REDUCING PEDIATRIC NEURO-ONCOLOGY SURVIVAL DISPARITIES ACROSS THE UNITED STATES-MEXICO BORDER**

**FIGURE 1**

**A**

**B**

Five-year overall survival improved from 0% before 2010 to 52% in 2017 (Fig. A). There was an incremental improvement in 3-year overall survival from 37% (2010-2013) to 53% (2014-2017) (P = 0.023) (Fig. B).
Cancer is the second leading cause of death in the United States and remains a formidable disease despite advances in medical science. The immune system plays a critical role in controlling the development of tumors and harnessing its innate ability to eliminate cancer has received much attention in recent times.

CD8+ cytotoxic T cells (CTLs) are vital components of anti-tumor immunity. Dr. Ganesan and her team discovered that a distinct subset of CD8+ CTLs, the tissue-resident memory (TRM) T cells, were most important in mediating robust anti-tumor immune responses and their presence within tumors correlated with improved survival in patients. However, they observed that only a subset of patients mounted a CD8+ TRM cell response. Therefore, it was imperative to understand what signals triggered TRM generation and persistence in these patients. To address this question, Dr. Ganesan and her team undertook a comprehensive evaluation of the key immune cell types in the tumor microenvironment (TME) to understand how they modulated TRM responses. They generated the transcriptional profiles of patient-matched, purified tumor-infiltrating immune cells from patients to define the molecular interactions between them. Dr. Ganesan found that a follicular helper T cell (TFH) program in CD4+ T cells was strongly associated with CD8+ TRM responses. She performed single-cell transcriptomic analysis to characterize this unique TFH-like cell subset and found that they possessed the ability to be cytotoxic and to provide 'help' to CD8+ TRM cells. Tumor-infiltrating TFH-like cells expressed PD-1 and were enriched in tumors following checkpoint blockade, suggesting that they may respond to anti-PD-1 therapy. Adoptive transfer or induction of TFH cells in mouse tumor models resulted in augmented CD8+ CTL responses and impairment of tumor growth in vivo, indicating an important role of TFH-like CD4+ T cells in supporting TRM responses and anti-tumor immunity.

**IMPLICATION:** From these studies, Dr. Ganesan and her team have discovered a number of novel molecular targets to bolster CD8+ TRM and CD4+ TFH-like responses which are likely to mediate tumor clearance. They are undertaking studies for functional validation of these targets to translate this knowledge for the design personalized immunotherapeutic strategies for cancer treatment.

**References:**


**FIGURE 1**
Transcriptomic profiling of patient tumors reveals that robust CD8+ TRM cell responses are associated with CD4+ follicular program and predict for improved patient survival. Novel immunotherapeutic strategies to bolster CD8+ TRM and CD4+ TFH responses will aid cancer treatment.
In 5-10% of pediatric cancer patients there are genetic variants which may have contributed to the cancer they have, which may put them at higher risk for cancer in the future, and which may affect other family members. In collaboration with Diane Masser-Frye and with the support of the St. Baldrick’s Foundation Grant, Dennis Kuo started the Cancer Genetics Clinic at Rady Children’s Hospital to provide cancer and surveillance for children, adolescents and young adults with genetic cancer predispositions. Two of the research initiatives that developed from this program explore the challenges in testing for cancer predispositions in the pediatric oncology population.

In their screening protocol where cancer predisposition testing was broadly offered to pediatric oncology patients and parents and parents were surveyed about their knowledge and attitudes, there was overwhelming interest. Parents felt very strongly that they wanted as much information as possible about such predispositions. Furthermore, the interest in testing was similarly highly held among both the patients and the participants.

However, while screening universally for cancer predispositions in the pediatric oncology population has some advantages and appeal, such an approach results in a rapid developing technologies, and optimal care for our patients and families.

**IMPLICATION:** Pediatric oncology patients and their families are highly interested in genetic cancer predispositions syndromes. Guidelines and algorithms can effectively identify the patients who would benefit from cancer predispositions screening.

Medulloblastoma is a highly malignant brain tumor that occurs predominantly in children. Recent studies have shown that medulloblastoma patients are very heterogeneous, but despite this, most patients receive the same therapies, and many end up dying of their disease.

Dr. Wechsler-Reya and colleagues hypothesized that tailoring therapy based on the characteristics of each patient’s tumor might improve outcomes. To test this, they established xenografts from 20 medulloblastoma patients and subjected them to DNA sequencing, gene expression profiling, and high-throughput drug screening, and then used the results to predict effective therapies. Importantly, they found that each patient’s tumor cells were sensitive to a distinct set of drugs, that there was significant variability in responsiveness to “standard-of-care” therapies, and that drug screening could help identify novel therapies for each patient.

**NOTABLY:** They discovered that Actinomycin D – a chemotherapy drug used for treatment of sarcoma but rarely for brain tumors – is active against Group 3 medulloblastoma, the most aggressive form of the disease. These studies suggested that incorporating functional analyses such as drug screening can enhance the predictive power of traditional, sequencing-based precision medicine.

**References:**

**Dr. Dennis John Kuo, MD**
Professor of Pediatrics
Hematology Oncology

**Dr. Robert J. Wechsler-Reya, PhD**
Professor of Pediatrics
Hematology and Oncology

**RESEARCH INTERESTS:** Understanding role of cancer predispositions syndromes in the treatment of pediatric oncology patients.

**RESEARCH INTERESTS:** Pediatric brain tumors and precision medicine.

**IMPLICATION:** Based on this work, Dr. Wechsler-Reya and Dr. John Crawford, Director of Neuro-Oncology at Rady Children’s Hospital, have begun to use a similar approach for patients undergoing treatment for brain tumors. Patient samples obtained from surgery are subjected to DNA and RNA sequencing, DNA methylation analysis and drug screening, and the results are discussed by a multi-disciplinary molecular tumor board, which is focused on identifying the most appropriate therapies for each patient. The goal of this analysis is to move away from a one-size-fits-all approach and begin to treat each patient with therapies that are effective against their specific tumor.
In collaboration with the UCSD Institute for Network Medicine, Dr. Zage and his team identified a pathway of 4 clusters of genes significantly associated with neuroblastoma differentiation. Preliminary analyses identified that genes from these gene clusters are involved in intracellular signaling and growth factor receptor intracellular trafficking pathways. The Zage lab has previously identified a key role for the ubiquitin ligase UBE4B in the regulation of growth factor receptor trafficking in neuroblastoma tumor cells, and the UBE4B gene was one of the genes identified in these clusters.

**IMPLICATION:** UBE4B gene expression is strongly associated with neuroblastoma patient outcomes, and UBE4B expression and activity are correlated with receptor trafficking, responses to therapy, and tumor differentiation, suggesting that UBE4B may function as a novel tumor suppressor, prognostic marker, and therapeutic target in neuroblastoma.

Reference:

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In collaboration with Tony Hunter, PhD at the Salk Institute for Biological Studies, the Zage lab has identified a novel role for NME1 in the growth and development of neuroblastoma. They have identified a role for NME1 as a histidine kinase and have shown that expression of NME1 is associated with neuroblastoma patient outcomes and that histidine phosphorylation is present in neuroblastoma cells and tumors. They have also determined that NME1 expression is involved in neuroblastoma cell migration and differentiation.

**IMPLICATION:** These results suggest novel roles for histidine kinases in neuroblastoma tumors and that NME1 represents a potential therapeutic target for the development of a completely new class of kinase inhibitors for neuroblastoma.

Reference:

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**UBE4B and Neuroblastoma Differentiation**

**NME1 Histidine Kinase Activity in Neuroblastoma**

**FIGURE 1**

Boolean network analysis identifies clusters of genes that are enriched in a continuum of cellular states. (Top left) Boolean Network Explorer (BoNE) software was used to analyze RNA expression profiles of neuroblastoma patient tumors. The path direction was derived from the connections established from high MYCN expression (representing undifferentiated neuroblastoma), with the arrow colors reflecting the implication relationships. (Top right) The Boolean relationships and candidate genes within each gene cluster in the identified cluster 3-4-5-6 pathway are shown. Representative Boolean gene expression relationships are shown. (Bottom) Parental and UBE4B-depleted (UBE4B KD) neuroblastoma cells were treated with 13-cis-retinoic acid and were monitored for cell confluence (bottom left) and for differentiation (bottom right).

**FIGURE 1**

NME1-depletion affects neuroblastoma cell differentiation and migration. (Left) Control SK-N-BE(2) cells and cells with depleted NME1 were treated with 13-cis-retinoic acid (CRA) or vehicle alone and analyzed using continuous live cell imaging. Imaging obtained of cells after 5 days of vehicle or CRA treatment, with cell bodies in yellow and neurite extensions mapped in blue. (Right) Control neuroblastoma cells and cells with depleted NME1 were plated and analyzed for migration into a scratch wound using continuous live cell imaging. Representative images are shown.

Control       NME1 Depletion
0hr                 24hr                48hr
CARDIAC DEVELOPMENT AND REPAIR

Exploring imaging of the heart for better diagnosis and treatment of congenital and acquired heart diseases.
Ductal-dependent cyanotic newborns require a secure source of pulmonary blood flow. There has been a recent migration to selective ductal (patent ductus arteriosus [PDA]) stenting for some of these children. Universal (nonselective) ductal stenting for all infants with ductal-dependent pulmonary blood flow is controversial. Dr. El-Said and her team examined the outcomes from a single center with this practice change. They compared outcomes from all ductal-dependent pulmonary blood flow infants (2013–2020 [January–June]) in the following treatment eras: Era 1 (selective PDA stenting; 2013–2017) or Era 2 (universal PDA stenting; 2018–2020 [January–June]).

Eighty-eight patients (Blalock-Taussig shunt, n=41; PDA stenting, n=47) met inclusion criteria. In Era 1, most received Blalock-Taussig shunt (62% [41/66]). In Era 2, all received PDA stents (100% [22/22]). There were more females in Era 2, but otherwise no demographic differences between eras.

There were less surgical revisions for PDA stent, n=47) met inclusion criteria. In Era 1, most received Blalock-Taussig shunt (62% [41/66]). In Era 2, all received PDA stents (100% [22/22]). There were more females in Era 2, but otherwise no demographic differences between eras. Post-procedure recovery days, P=0.02). There were no differences in mortality, treatment failures, or re-interventions between eras. Post-procedural length of stay was shorter in Era 2 (8 versus 22 days, P=0.001) length of stay and more symmetrical branch pulmonary arteries (0.9 versus 0.7, P=0.001) at subsequent surgery. It was concluded that PDA stenting for almost all ductal dependent cyanotic newborns can be safe and effective and may have lower morbidity than selective PDA stenting.

**IMPLICATION:** PDA stenting as an alternative to BT shunting can improve outcome for Neonates with CHD, particularity developmental outcomes and allows them to lead normal full lives.

**Reference:**

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**PDA Stenting for Neonates with Cyanotic Heart Disease and Ductal Dependent Pulmonary Blood Flow**

The focus of this study was to describe the early experience of using a new, commercially available Micro plug set for preterm neonate and infant transcatheter patent ductus arteriosus (PDA) occlusion. Transcatheter PDA occlusion in premature neonates and small infants is safe and effective. The procedure is early in its evolution.

Procedural and short-term outcomes of preterm neonates and infants undergoing transcatheter PDA occlusion with a new, commercially available device were reviewed. Eight preterm neonates and infants born at median 27 weeks gestation (23-36 weeks) underwent transcatheter PDA device closure with Micro Plug Set. The device is short (2.5 mm) with a range of diameters (3, 4, 5, 6 mm) and delivered through a microcatheter. Procedures were performed at median 41 days of age (12-88 days) and at 1690 grams (760-3310 grams). More than half of the patients required pre-procedure ventilator support. Transcatheter PDA device occlusion was performed with fluoroscopic and echocardiography guidance.

All procedures were successful with complete PDA occlusion. There were no procedural or short-term adverse events. It was concluded that Preterm neonate and infant transcatheter PDA device closure with a new, commercially available short and microcatheter delivered device (Micro Plug Set) was safe.

**IMPLICATION:** PDA device closure in premature infants can change their outcomes. The use of more user friendly occlusion techniques can minimize complications and improve overall outcomes.

**Reference:**

---

**Early Experience with the Micro Plug set for Preterm Patent Ductus Arteriosus Closure**

**Post-operative Length of Stay**

<table>
<thead>
<tr>
<th>Era 1</th>
<th>Era 2</th>
<th>Shunt</th>
<th>Stent</th>
</tr>
</thead>
<tbody>
<tr>
<td>22 days</td>
<td>20 days</td>
<td>8 days</td>
<td>10 days</td>
</tr>
</tbody>
</table>

Post-procedural length of stay for Era 1 (selective patent ductus arteriosus [PDA] stenting) vs Era 2 (universal PDA stenting) and surgical Blalock-Taussig shunt vs transcatheter PDA stent.
Pediatric infective endocarditis (IE) incurs significant morbidity and generally occurs among children with underlying heart disease. Identification of a pathogen is critical in determining appropriate therapy. However, standard diagnostic testing has limited sensitivity. In this case series of children with infective endocarditis, the use of plasma next-generation sequencing (Karius, Redwood, CA) identified an organism in 8 of 10 cases.

Cell-free plasma metagenomic next-generation sequencing (mNGS) utilizes a high-throughput approach that is culture-independent and noninvasive to detect microbial DNA. Dr. Gupta and her team identified 14 subjects with IE, in whom 10 had mNGS obtained as part of the diagnostic work up. Of these 10 subjects, 30% were female. Median age was 9.6 years [interquartile range (IQR) 7.7–15.0 years], median inpatient length of stay was 24.1 days (IQR 15.1–45.0 days), and median length of antibiotic treatment was 46 days (IQR 42.0–52.0 days). The average turnaround time (sample collection to results received) for mNGS was 3.2 days (SD 1.2 days). Seventy percent (7/10) of subjects had a prior history of underlying heart disease and 60% (6/10) had previously undergone cardiac surgery before diagnosis of IE. Five of 10 subjects had native valve IE. One subject was receiving chemotherapy for acute lymphoblastic leukemia. Two subjects did not have a predisposing condition. All subjects had an echogenic mass concerning for vegetation on echocardiography and 5 subjects experienced embolic complications associated with endocarditis. Nine subjects required surgical intervention for vegetation removal and/or valve replacement or repair. Six of 9 subjects who underwent surgery had negative tissue cultures. Tissue culture was not sent for 3 subjects.

**Use of Plasma Metagenomic Next-generation Sequencing for Pathogen Identification in Pediatric Endocarditis**

**IMPLICATION:** The potential value of mNGS in children with IE is consistent with studies that have evaluated clinical utility. Our case series is limited by small sample size and a lack of standardized criteria for ordering mNGS. However, the high positivity rate of mNGS in comparison to blood culture does support the potential utility of mNGS to help with clinical management of IE. Prospective trials are needed to clarify optimal use of this diagnostic tool in the evaluation of IE.

**References:**


**Use of Novel Nanocomposite Polymer Coating to Make Devices Safer under MRI**

**IMPLICATION:** Novel nanocomposite polymer coating to make devices safer under MRI does not heat-up significantly whereas bare guidewires heat-up.

**References:**


Anthracyclines are highly effective chemotherapeutic agents used in a majority of pediatric cancer patients, but they have cardiotoxic effects that result in a substantial risk of cardiac dysfunction and heart failure in childhood cancer survivors. Standard screening approaches to detect these cardiotoxic effects may identify cardiac disease too late, at a time when medical therapy has limited success in preventing disease progression. Earlier detection of cardiac disease is needed to allow for timely, targeted interventions to prevent the development of heart failure.

Dr. Narayan seeks to improve the detection and prognostication of anthracycline-related heart failure using novel cardiovascular imaging-based techniques to detect earlier changes in the heart that precede heart failure. In collaboration with investigators in the UC San Diego Department of Bioengineering, Dr. Narayan’s team generated three-dimensional computational models of left ventricular shape in a pilot study of childhood cancer survivors. Using a statistical atlas, left ventricular shape was compared between survivors and an asymptomatic reference population, and between survivors exposed to low- and high-dose anthracyclines. These shape comparisons demonstrated that survivors had significantly smaller hearts in comparison with the reference population. In addition, survivors treated with high-dose anthracyclines had specific differences in left ventricular shape in comparison with those treated with low doses.

**IMPLICATION:** These findings suggest that specific changes in left ventricular shape may be an important feature of early anthracycline-related heart failure progression. With further study, these results could lead to the development of new imaging-based disease markers to improve the detection and prognostication of heart failure.

**Reference:**

**Pediatric Anthracycline Exposure May Be Associated with Late Alterations in Heart Size and Shape During Survivorship**
Investigating repurposed drugs for different diseases or novel therapies for a variety of human diseases and conditions.
The deadly pediatric brain tumor, called medulloblastoma, is frequently associated with overexpressed and overactive Smoothened receptor. Existing mono-target treatments are not very efficient against this medulloblastoma subtype. Dr. Abagyan and his colleagues predicted computationally that an unrelated approved drug may also inhibit the Smoothened receptor. They found with a range of experiments and in vivo xenograft models that an existing treatment for chronic myeloid leukemia, Nilotinib, is suppressing Smoothened in addition to several other kinases essential for tumor growth. It results in reduction of tumor growth and desired effect on cancer stem cells.

**IMPLICATION:** A leukemia drug already approved for a different cancer may become a new therapy for a frequent subtype of childhood brain tumor called medulloblastoma. It is now a candidate to try it alone or in combination with surgery, radiation or chemotherapy.

**References:**

---

**TABLE 1**

<table>
<thead>
<tr>
<th>FRK (RTK)</th>
<th>EPHA8 (RTK)</th>
<th>LCK (RTK)</th>
<th>LYK (RTK)</th>
<th>Other Cancers</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABL1/2 (RTK)</td>
<td>DDR1/2 (RTK)</td>
<td>MLTK (RTK)</td>
<td>MK11 (RTK)</td>
<td>CSFR1 (RTK)</td>
</tr>
<tr>
<td>PDGFRA/β (RTK)</td>
<td>c-KIT (RTK)</td>
<td>SMO (TTM)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SMO-dependent Cancers (Hh-MB, Hh-GBM, BCC, ...)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Multi-pathway pharmacology of Nilotinib vs SMO-specific drugs for Hh-dependent cancers. Targets of Nilotinib include the Hh-pathway (identified in this study) and several other pathways that are either already known to be dysregulated in Hh-dependent cancers or may serve as escape pathways and are implicated in other cancers. The ability of Nilotinib to inhibit multiple targets simultaneously in Hh-dependent cancers makes it a suitable candidate for personalized medicine as compared to specific SMO inhibitors like Vismodegib. Target type is shown by color. RTK stands for receptor tyrosine kinase and rRTK stands for non-receptor tyrosine kinase.

**IMPLICATION:** Drug companies are not required to study drugs in pregnancy, so data on medication dosing and safety for most drugs is entirely absent, and for other drugs, is not available until years after the drug has already been on the market.

Dr. Best and her team conducted a multi-center phase-I/IV prospective study of darunavir and cobicistat pharmacokinetics in pregnant women with HIV and their infants in the U.S. Intensive steady-state 24 hour pharmacokinetic profiles were performed after administration of 800 mg of darunavir and 150 mg of cobicistat orally in fixed dose combination tablets once-daily during the second trimester, third trimester, and postpartum. Infant washout samples were collected after birth.

A total of 29 pregnant women receiving darunavir and cobicistat once-daily were enrolled in the study. Compared to paired postpartum data, darunavir overall exposure (AUC0-24) was 53% lower in the second trimester and 56% lower in the third trimester, while cobicistat AUC0-24 was 50% lower in the second trimester and 56% lower in the third trimester. Placental transfer of darunavir and cobicistat was limited.

**IMPLICATION:** Medications are often needed during pregnancy to protect the health of both the mother and the fetus/infant, but being pregnant may significantly alter the amount of medications in the bloodstream when standard non-pregnant adult doses are used. As an example, Dr. Best and her team showed that darunavir and cobicistat concentrations in many patients during pregnancy are too low - putting the mother at risk for disease progression and the infant at risk for viral transmission - so these commonly prescribed anti-HIV drugs should not be used during pregnancy.

**References:**
**Strategies for the Prevention and Treatment of HIV Infection in Infants**

While the rates of new HIV infection have fallen globally, more than 150,000 children get infected with HIV annually. Most of this occurs in infants during pregnancy, delivery, or breast feeding. Effective antiretroviral drug therapy given to mothers during pregnancy and post-partum is effective in mitigating transmission to infants. However, it is not always possible to identify and effectively treat HIV during pregnancy and post-partum during breast feeding. Infants from these mothers that are at risk for acquiring HIV infection can benefit from prophylactic treatment to prevent viral infection. As newborns can be sensitive to drug effects and have immature drug elimination, antiviral drug selection and age appropriate dosing are essential considerations both for preventing and treating infant HIV infection. Rapid, effective antiretroviral treatment in infants may also to limit the damage that HIV causes on development of their immune system.

The Capparelli lab designs and analyzes studies that measured drug concentrations (pharmacokinetics - PK) and safety of antiretroviral agents to determine optimal dosing of antivirals to prevent and treat HIV infections in newborn infants. These mathematical PK models include characterization of patient factors, such as age, weight, prematurity and genetics that can influence on drug elimination. These PK models can be used to develop dosing strategies for infants at birth and how to make dose adjustments to maintain optimal drug levels during the first few months of life. They recently demonstrated the independent influence of weight, therapy duration, genetic factors and prematurity on nevirapine (NVP) metabolism. NVP elimination doubles over the first 2 weeks of therapy with preterm infants and the CYP2B6 516TT genotype having slower elimination. This analysis now serves as the basis for US DHHS Pediatric Guidelines for HIV treatment of infants.

Dr. Capparelli and his team led the pharmaco-kinetic evaluation of the first monoclonal antibody (mAB) against HIV, VRC01, to be studied in infants through IMPAACT Study P1112. In infants following subcutaneous administration, VRC01 was absorbed faster but had a lower percent of the dose absorption than adults. They developed a population PK model and performed simulation of various dosing strategies to develop a regimen that maintains concentrations above the target concentration of 50 µg/mL in over 95% of infants.

**IMPLICATION:** This potential approach may improve prevention of mother to child HIV transmission via breast feeding and minimize the impact of HIV infection on infant health.

**References:**


**FIGURE 1**

- There is a large increase in nevirapine metabolism over the first 20 days of life. Infant nevirapine metabolism doubles and is also affected by genetic factors (CYP2B6 516) and prematurity. These factors need to be considered in dosing infants with nevirapine.

**FIGURE 2**

- Log10[µM] Pitavastatin

**Repurposing Statins for the Treatment of Primary Amoebic Meningoencephalitis**

Naegleria fowleri, commonly known as brain-eating amoeba, is a CNS-invasive pathogen that is responsible for severe primary amoebic meningoencephalitis (PAM). PAM mostly occurs in healthy children and young adults with recent recreational freshwater exposure. N. fowleri infection is particularly problematic due to the rapid onset and destructive nature of the disease as well as the lack of effective treatments. Despite the use of a cocktail of drugs including the standard of care amphotericin B, the mortality rate of PAM is more than 97% and only four patients survived so far in the US. The use of amphotericin B is also limited by severe dose-limiting side effects, thus development of more effective and safer therapies for PAM is a research priority.

Since ergosterol is one of the major sterols in the membrane of N. fowleri, disruption of isoprenoid and sterol biosynthesis by small-molecule inhibitors may be an effective, novel intervention strategy against PAM. HMGR is a rate-limiting enzyme in the pathway which catalyzes the conversion of HMG-CoA into mevalonate. HMGR inhibitors prevent the conversion of HMG-CoA to L-mevalonate resulting in the inhibition of the downstream sterol biosynthesis. The genome of N. fowleri contains a gene encoding HMGR, the catalytic domains of human and N. fowleri HMGR share ~60% sequence identity with only two amino acid substitutions in the active site of the enzyme.

Considering the similarity of human and N. fowleri HMGR, Dr. Debnath tested well-tolerated and widely used HMGR inhibitors or statins and identified blood-brain barrier permeable pitavastatin with nanomolar to low micromolar potency against five human N. fowleri strains of various genotypes originated from different geographic regions. Pitavastatin exhibited rapid killing effect and his cell biological study confirmed the on-target activity of HMGR inhibitors in N. fowleri. These results set the stage for future testing of pitavastatin in an animal model of infection.

**IMPLICATION:** Development of efficient new antimicrobials to primary amoebic meningoencephalitis (PAM) is a critical need to avert future deaths of children. Dr. Debnath and his team identified blood-brain barrier permeable FDA-approved pitavastatin as a fast-acting killing agent against multiple strains of N. fowleri originated from different continents. These results open up the possibility of repurposing pitavastatin for the treatment of PAM.

**References:**


**FIGURE 1**

<table>
<thead>
<tr>
<th>% Growth Inhibition</th>
<th>log[µM] Pitavastatin</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>3.16</td>
</tr>
<tr>
<td>1</td>
<td>3.16</td>
</tr>
<tr>
<td>5</td>
<td>3.16</td>
</tr>
<tr>
<td>10</td>
<td>3.16</td>
</tr>
<tr>
<td>20</td>
<td>3.16</td>
</tr>
</tbody>
</table>

**FIGURE 2**

- There is a significant increase in inhibition of N. fowleri at low micromolar concentration of pitavastatin against different strains of Naegleria fowleri. The data show a linear relationship between concentration and % inhibition of N. fowleri.
Dr. Dorrestein’s lab is a metabolomics lab that integrates the world’s metabolomics data for the betterment of understanding of biological aging, cognitive function and health and disease. His lab builds the computational infrastructure with and for the scientific community and the data analysis ecosystem in 2021 has grown to >450,000 accessions a month.

This year Dr. Dorrestein and his team introduced two new search engines and a synchronous collaborative analysis ecosystem allowing many people to analyze data simultaneously. This analysis ecosystem also uses machine learning technologies to link microbial metabolism, drug metabolism, diet, and how they influence health. For example, it is being used to understand how antibiotics given to the mother passed to the breastmilk alter the health of developing infants. In his lab, Dr. Dorrestein uses this platform to understand the molecules made by resident microbes (often referred to the microbiome).

In 2021, Dr. Dorrestein discovered that microbes make ~140 bile acids that were previously not yet been described. These molecules interact with receptors such as FXR. It is, therefore, revealing that resident microbes regulate metabolism and inflammatory bowel disease (IBD) and cystic fibrosis. These bile acids that were previously not yet been described. These findings are possible through the deep connection between our microbiota, our metabolites and our health.

**Global Chemical Effects of the Microbiome include New Bile-Acid Conjugations**

In 2020, Dr. Karen Klein led a pivotal study of a new agent for the treatment of children with precocious puberty. Her study findings demonstrated that Leuprolide acetate given in a single subcutaneous dose every 6 months is safe and effective in stopping the progression of early puberty in young children. The technology involves mixing a copolymer with the active peptide hormone to form a reservoir beneath the skin to release drug over 6 months. Hormone levels were suppressed to age-appropriate levels (Fig & Table below). Bone maturation slowed to appropriate rates with mean values for the differences between bone age and chronological age (BA-CA) decreasing from 3.0 ± 0.1 years at screening to 2.7 ± 0.1 years at week 48 (P < .001). Growth rate returned to prepubertal growth rate from 8.9 ± 1.7 cm/year prior to treatment to 6.0 ± 0.5 cm/year at week 48.

**New Treatment for Children with Precocious Puberty**

In 2020, Dr. Karen Klein led a pivotal study of a new agent for the treatment of children with precocious puberty. Her study findings demonstrated that Leuprolide acetate given in a single subcutaneous dose every 6 months is safe and effective in stopping the progression of early puberty in young children. The technology involves mixing a copolymer with the active peptide hormone to form a reservoir beneath the skin to release drug over 6 months. Hormone levels were suppressed to age-appropriate levels (Fig & Table below). Bone maturation slowed to appropriate rates with mean values for the differences between bone age and chronological age (BA-CA) decreasing from 3.0 ± 0.1 years at screening to 2.7 ± 0.1 years at week 48 (P < .001). Growth rate returned to prepubertal growth rate from 8.9 ± 1.7 cm/year prior to treatment to 6.0 ± 0.5 cm/year at week 48.

**IMPLICATION:** As the connections between humans and our microbial symbionts become increasingly appreciated, a combination of globally untargeted approaches and the development of tools that interlink these datasets (such as the Global Natural Products Social Molecular Networking and Mass Spectrometry Search Tool analysis infrastructure) will enable the more-efficient characterization of microbial molecules and efficient translation between model animals and human studies, leading to a better understanding of the deep connection between our microbiota, our metabolites and our health.

**Reference:**


**TABLE 2**

<table>
<thead>
<tr>
<th>Endpoint Target</th>
<th>Proportion of Children Achieving Endpoints, % (n/N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LH &lt;4 IU/L</td>
<td>Week 12</td>
</tr>
<tr>
<td></td>
<td>85 (51/60)</td>
</tr>
<tr>
<td>Estradiol &lt;20 pg/mL</td>
<td>98 (56/57)</td>
</tr>
<tr>
<td>Testosterone &lt;28.4 ng/dL</td>
<td>100 (2/2)</td>
</tr>
<tr>
<td>FSH &lt;2.5 IU/L</td>
<td>62 (37/60)</td>
</tr>
</tbody>
</table>

1 Post GnRH agonist stimulation 2 Primary efficacy endpoint

Distinct adipose gene signatures predict insulin resistance in young mice prior to high fat diet-induced obesity.
Therapeutic Drug Monitoring: New Way to Monitor an Old Drug

Despite more than 61 years of clinical use of vancomycin, knowledge gaps regarding the most appropriate approach for optimizing therapy and minimizing toxicity of vancomycin still exist. The revised consensus guidelines for dosing and monitoring vancomycin are an updated version of the 2009 guidelines developed by the American Society of Health-Systems Pharmacists, the Infectious Diseases Society of America, the Pediatric Infectious Diseases Society, and the Society of Infectious Diseases Pharmacists. Formerly adult-centric, this updated executive summary contains key recommendations that are specific for neonates and pediatrics. With numerous reports of increased nephrotoxicity in adults and pediatric patients when trough level monitoring was used, the area-under-the-curve (AUC) has been documented and now serves as the primary pharmacokinetic/pharmacodynamic target. The complete vancomycin guidelines can be cited and accessed at: https://academic.oup.com/ajhp/advance-article/doi/10.1093/ajhp/zxaa036/5810200

Recent pharmacologic and toxicodynamic studies have demonstrated a significant reduction in vancomycin exposure and nephrotoxicity rates when AUC/MIC monitoring was used vs. trough monitoring. New data from neonatal and pediatric pharmacologic studies were critically evaluated and, if robust, integrated to provide dosing recommendations in the effort to optimize exposure based on weight-based dosing.

**IMPLICATION:** While these guidelines originated from the United States, the widespread use of vancomycin worldwide makes these guidelines highly impactful and practice-changing in hospitalized premature infants and children. The complete vancomycin guidelines can be cited and accessed at: https://academic.oup.com/ajhp/advance-article/doi/10.1093/ajhp/zxaa036/5810200

**IMPLICATION:** In very-low birthweight neonates, ampicillin exposure remains therapeutic long after the last dose. Short-course ampicillin provided therapeutic exposures throughout the typical blood culture incubation period.

**Reference:**

**New Way to Improve Antimicrobial Stewardship: Post-Discontinuation Antibiotic Exposure in Early-Onset Sepsis**

Using Monte Carlo simulations (NONMEM 7.3), Dr. Le and colleagues analyzed antibiotic exposures in a retrospective cohort of 34,689 remature, very-low birthweight neonates (<1500 g, 22-27 weeks of gestation). Therapeutic exposure for ampicillin and gentamicin was evaluated relative to the minimum inhibitory concentration (MIC) for common pathogens (MIC 0.25-8 mcg/mL for group B streptococcus [GBS] and Escherichia coli). Post-discontinuation antibiotic exposure (PDAE) was defined as the time from the last dose to time when concentration decreased below MIC. Neonates had a median (range) gestational age of 26 (22-27) weeks and BW, 790 g (400-1497).

All ampicillin dosing regimens (50-100 mg/kg every 8-12 hours for 2-6 doses) achieved therapeutic exposures > MIC range. After the last dose, the PDAE mean (95% confidence interval [CI]) ranged from 34 to 50 hours (17-79) for E. coli (MIC 8) and 82 to 104 hours (95% CI: 39-122) for GBS (MIC 0.25); longer PDAE occurred with higher dose, shorter interval, and longer course. Short-course ampicillin (2 doses, 50 mg/kg every 12 hours) provided PDAE 34 hours for E. coli and 82 hours for GBS. Single-dose 5 mg/kg gentamicin provided PDAE > MIC 3 for 26 hours.

**IMPLICATION:** In very-low birthweight neonates, ampicillin exposure remains therapeutic long after the last dose. Short-course ampicillin provided therapeutic exposures throughout the typical blood culture incubation period.

**Reference:**
Chemotherapy-induced nausea and vomiting (CINV) is a common treatment-related adverse event that negatively impacts the quality of life of cancer patients. During pediatric drug development, extrapolation of efficacy from adult to pediatric populations is a pathway that can minimize the exposure of children to unnecessary clinical trials, thereby improving efficiency and increasing the likelihood of success in obtaining a pediatric indication. The acceptability of the use of extrapolation depends on a series of evidence-based assumptions regarding the similarity of disease, response to intervention, and exposure-response relationships between adult and pediatric patients.

This study conducted by Dr. Momper and colleagues evaluated data submitted to the US Food and Drug Administration for drugs approved for CINV to assess the feasibility of extrapolation for future development programs. For 5-hydroxytryptamine-3 and neurokinin-1 receptor antagonist antiemetic drugs, efficacy in adults was found to be predictive of efficacy in children, supporting the extrapolation of effectiveness of antiemetic products in children from adequate and well-controlled studies in adult patients with CINV.

**IMPLICATION:** Future development programs for antiemetic drugs to prevent chemotherapy-induced nausea and vomiting (CINV) in pediatric patients may be streamlined by avoiding lengthy and resource-intensive efficacy studies.

**Reference:**

**FIGURE 1**
Percentage of adult and pediatric patients achieving complete response in pivotal clinical trials of palonosetron for the prevention of nausea and vomiting associated with chemotherapy.
Primary amebic meningoencephalitis (PAM) due to a “brain-eating” amoeba Naegleria fowleri is a fulminating brain infection that can result in death within days. PAM has a worldwide distribution although it occurs most frequently in warm areas and during hot summer months in healthy children and young adults with recent recreational fresh water exposure. Based on the free-living amoeba registry maintained by the Centers for Disease Control and Prevention (CDC), the fatality rate of PAM is over 97%. Currently, there is no standard regimen for the treatment of Naegleria infections in humans. Identification of new therapeutic targets and development of the efficacious and safe drugs for the PAM treatment is unmet medical need.

Over the past few years, Dr. Podust and collaborators at UCSD and Texas Tech University explored the steroidogenic pathway in N. fowleri, validated several steroidogenic enzymes as drug targets and identified small molecule candidates for drug repurposing strategies and for development of novel therapeutics. It was discovered that the FDA-approved CYP51 inhibitors, posaconazole and itraconazole, are superior in their anti- Naegleria activity to Amphotericin B, a cornerstone of the posaconazole and itraconazole, are superior in their anti-

Sterol Biosynthesis in “Brain-Eating” Ameoba is a Drug Target for Anti-Fungal Drugs

An antifungal drug posaconazole tightly binds to the active site of N. fowleri sterol 14-demethylase (CYP51), as detected by X-ray structure analysis, and inhibits production of the essential to amoebae sterol, ergosterol. Instead, accumulation of the 21-norsterol substrate is detected by GC-MS analysis in treated N. fowleri cells.

Despite years of attention, hospitals continue to struggle to implement successful medication reconciliation. Aiming to increase the percentage of hospital admission medication reconciliation (AdmMedRec) completion to ≥95% in 12 months at a large academic children’s hospital, Dr. Rungvivajaranus along with an interdisciplinary team of physicians, nurses, pharmacists, and analysts, co-led by a pediatric hospitalist and chief medical information officer, initiated a quality improvement (QI) project in April 2017. Interventions were implemented through sequential Plan-Do-Study-Act cycles. Process maps, fishbone diagrams, and failure mode and effects analysis were used to identify AdmMedRec failures. Baseline data from 12,481 admission encounters July 2016–April 2017 were analyzed. Interventions included electronic health record (EHR) workflow redesign, clarification of clinicians’ responsibilities, targeted training, Best Practice Advisory alert, and weekly reporting of specialty- and physician-specific performance data. Data from 13,082 postintervention period admission encounters were examined. Reconciliation by therapeutic drug classes was calculated as a proxy for quality. Study results showed AdmMedRec completion rate increased from a baseline of 73% to 95% within 7 months from the start of this project and was sustained at 94% during the post intervention period. Psychiatry and hospital medicine demonstrated the largest improvements, with rates increasing from 17% to 88% and 76% to 98%, respectively. Percentages of reconciled medications in all 13 therapeutic classes, including high-risk drugs, improved significantly (p < 0.05). It was concluded that this QI initiative using an interdisciplinary team and interventions focused on process and culture changes was successful at increasing AdmMedRec rates and reducing omission errors across all therapeutic drug classes.

References:

TIRANUN (JANE) RUNGVIVATJARUS, MD
Assistant Professor of Pediatrics
Hospital Medicine
RESEARCH INTERESTS: Improving medication reconciliation and pediatric medication safety.

Pediatric Medication Reconciliation

References:
Malaria remains a devastating disease, affecting 216 million people annually, with 445,000 deaths occurring primarily in children under 5 years old. Malaria treatment relies primarily on drugs that target the disease-causing asexual blood stages (ABS) of Plasmodium parasites, the organisms responsible for human malaria. Whereas travelers may rely on short-term chemoprotective drugs, those living in endemic regions require long-term malaria protection such as insecticide-treated nets (ITNs) and vector control. However, ITNs do not fully shield individuals from malaria, may lose potency with time, and can be bulky and difficult to use. Another concern is that mosquitoes may become resistant to the active insecticides that are used in ITNs and vector control. An alternative strategy to insecticide treated bednets is chemoprevention. Although millions of compounds have been screened for activity against parasite ABS, and some have been subsequently tested for potential prophylactic activity, large-scale searches that begin with prophylactic activity have not been performed because of the complexity of the assay. This assay requires the production of infected laboratory-reared mosquitoes and hand-dissection of the sporozoite-infected salivary glands from mosquito thoraces. To discover leads for next-generation chemoprotective antimalarial drugs, the Winzeler lab used luciferase-expressing Plasmodium spp. parasites, dissected from more than a million mosquitoes over a 2-year period, to test more than 500,000 compounds for their ability to inhibit liver-stage development of malaria (681 compounds showed a half-maximal inhibitory concentration of <1 μM). These leads were further tested through multiple phenotypic assays that predict stage-specific and multispecies antimalarial activity. Results revealed compound classes that are likely to provide symptomatic relief from blood-stage parasitemia in addition to providing protection. These findings substantially expand the set of compounds with demonstrated activity against two known targets of chemoprotective drugs, cytochrome bC1 and dihydroorotate dehydrogenase. These present a rich collection of chemical diversity that may be used to accelerate malaria elimination. Work is currently underway to translate these discoveries into next generation drugs.

**IMPLICATION:** The development of new, more effective methods for preventing malaria could save the lives of millions of children over the next decade.


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**ADRIANA TREMOULET, MD**
Professor of Pediatrics
Host-Microbe Systems and Therapeutics

**RESEARCH INTERESTS:**
Pediatric infectious diseases, Kawasaki disease (KD), multisytem inflammatory syndrome in children (MIS-C), and pediatric clinical pharmacology.

**FIGURE 1**

As compared to serum from KD patients that blocks the transcription factor KLF4, promoting endothelial mesenchymal transition (EndoMT) and formation of coronary artery aneurysms, incubation of human umbilical vein endothelial cells with KD serum from patients treated with atorvastatin increases KLF4, blocking EndoMT and formation of myofibroblasts.

**Discovery of New Starting Points for Chemopreventative Antimalarials**

Elisa A. Bertsch & Elizabeth Winzeler


**IMPLICATION:**
The malaria parasite lifecycle. Infection begins with the bite of infected mosquito which releases Plasmodium sporozoites which migrate to the liver and establish a silent infection. To identify drug candidate molecules we obtain mosquitoes that have fed on malaria-infected mice and dissect malaria sporozoites from these mosquitoes. The sporozoites are used in an assay that can detect whether they develop productivity in cultured liver cells. Subsequent dose response curves determine whether compounds have enough potency to protect patients from malaria.

**References:**

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**ELIZABETH WINZELER, PHD**
Professor of Pediatrics
Host-Microbe Systems and Therapeutics

**RESEARCH INTERESTS:**
Drug discovery, genomics, host pathogen interactions, malaria, eukaryotic pathogenesis, and infectious diseases.
Discovering the importance of nurture and interaction with nature in disease predisposition.
Patterns of Prenatal Alcohol Exposure that Affect Infant Growth and Development

Prenatal alcohol exposure (PAE) is often categorized into crude categories of use that remove information on timing, amount and duration of exposure. Dr. Bandoli and her team characterized PAE into longitudinal trajectories and found that low-to-moderate sustained alcohol use was more strongly associated with most negative infant outcomes than moderate-to-high PAE with early reduction.

**IMPLICATION:** These findings reinforced the important public health message that no matter the initial amount of consumption, alcohol intake cessation increases the chances for improved offspring outcomes, and demonstrated a new tool to better classify PAE.

**Reference:**
Patterns of Prenatal Alcohol Use That Predict Infant Growth and Development

Patterns of Prenatal Antidepressant Use and Adverse Neonatal Outcomes

Using longitudinal trajectory methods, Dr. Bandoli classified patterns of antidepressant use from a cohort of 15,000 women with antidepressant use during pregnancy. There was an increased risk of preterm birth among the highest-use groups, and all patterns of use, even those with early pregnancy discontinuation of antidepressants, were associated with greater risk for neonatal respiratory distress.

**IMPLICATION:** This methodological approach can help clinicians caring for pregnant women to identify groups whose infants may be at higher risk for preterm birth or neonatal respiratory distress.

**Reference:**
Prenatal Antidepressant Use and Risk of Adverse Neonatal Outcomes
The COVID-19 pandemic has exacerbated an ongoing epidemic of burnout among healthcare workers (HCWs). Most literature on pandemic-related stress has focused rightly on clinicians, but other HCWs have also been affected. This study investigates burnout and sources of COVID-related stress among a group of healthcare quality and safety practitioners.

Members of the Southern California Association for Healthcare Risk Management (SCAHRM) were asked to complete an online survey including a validated burnout instrument, the Oldenburg Burnout Inventory (OBI), and one open-ended question: “Since the start of the COVID-19 pandemic, what work or non-work-related issues have been causing you the most stress?”

31 participants (~17% of the organization’s 187 members) completed the OBI and 27 responded to the open-ended question. Over 70% of participants were experiencing burnout. Stressors were organized into several key themes, including: The impacts of social distancing, changing duties and workload, the real and potential impacts of the virus (e.g., fear of infection for self or others), and financial concerns (both personal and organizational). Less common themes included untrustworthy and constantly changing guidance, feeling abused by persons in power, and positive comments about the experience of working during the pandemic.

Although a larger sample will be needed to estimate the true prevalence of burnout among quality and safety professionals, these findings suggest that burnout and pandemic-related stress may be very common in this population. This high level of burnout has implications beyond the direct human cost to the workers involved. Among clinicians and other professionals, burnout is associated with impaired work performance. If this holds true among members of the healthcare quality and safety workforce, high levels of burnout represent a threat to patient safety and the delivery of high-quality care. Turnover related to occupational stress may also lead to poorer outcomes, given the dearth of experienced quality & safety professionals prepared to step into these roles.

**IMPLICATION:** Shortfalls in healthcare safety and quality are a major cause of illness and death in the United States and around the world. Burnout among healthcare quality and safety professionals may reduce the ability of healthcare organizations to effectively address the causes of quality and safety problems. This study demonstrates that these professionals may be a high-risk group for burnout and related outcomes, and identifies key pandemic-related stressors that could be targeted to reduce those risks.

**Reference:**
Since the UC San Diego Mommy’s Milk Human Milk Research Biorepository was established in 2014 in the Department of Pediatrics, Dr. Chambers and her team have been able to leverage this nationwide platform to make novel contributions to the health of breast/chest-fed infants worldwide. In 2018, they demonstrated for the first time that currently available, more potent cannabis products when used by lactating individuals result in measurable levels of THC in human milk that persist up to 6 days following last use.

**IMPLICATION:** This discovery influenced recommendations made by American Academy of Pediatrics and others regarding cannabis use and lactation.

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Reassuring Findings for Lactating Parents with COVID-19 Infection

In early 2020, at the onset of the SARS-CoV-2 pandemic, Dr. Chambers and her team were able to leverage the Mommy’s Milk platform to enroll lactating individuals who had documented COVID-19 infection. At the time, there was concern that human milk itself could be a source of infection for the infant. They demonstrated for the first time that although viral RNA could be detected in some samples, replication-competent virus could not.

**IMPLICATION:** This discovery helped support recommendations by the World Health Organization and to support the safety of the donor milk supply.

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References:
Marijuana Use by Breastfeeding Mothers and Cannabinoid Concentrations in Breast Milk.

Evaluation for SARS-CoV-2 in Breast Milk From 18 Infected Women.
The fetal alcohol spectrum disorders (FASD) are among the most prevalent causes of neurodevelopmental disorders. Prompt diagnoses are necessary to provide early intervention services in a timely manner; however, the availability of diagnostic expertise is limited. Based on their research finding, Dr. Del Campo and his team have concluded that telemedicine is a valid and reliable method for the examination of the physical features of FAS.

This study was performed at two different sites at the University of California, San Diego that were physically sequestered to assure isolation of the two examiners from each other. Two different Telemedicine (TM) systems were compared. The first utilized hand-held devices, an iPhone and an iPad, and secure Zoom conferencing. The second utilized a Transportable Examination Station (TES) which included a fully mobile telemedicine platform equipped with an integrated High Definition video camera, and its own secure transmission, with an approximate cost of $20,000 (Figure 1).

All children in the study were examined face to face (F2F) on two separate occasions by each examiner. These physical examinations were viewed in real time by the other examiner at the receiver end, one using Zoom, and the second using TES. All data for the 4 physical examinations were compared, agreement using Cohen’s K was calculated for the two exams F2F, and the agreement between the F2F exams and the two exams using the two different TM methods.

The collected data that were compared between the 4 were the measurements of the head circumference (HC) and the evaluation of the three key facial features, including palpebral fissure length (PF), smooth philtrum and thin and smooth vermillion of the upper lip. For the two examiners combined, the agreement observed between the two F2F examinations (K=0.89) for the diagnosis of FAS was in the almost perfect range. The agreement between F2F exams and ZOOM (K=0.85) and TES (K=0.89) for the individual features contributing to the diagnosis of FAS were all in the almost perfect range.

In conclusion, the finding of high agreement between F2F and TM exams demonstrates the validity and reliability of telemedicine for the identification of the physical features of FASD through remotely guided dysmorphology examinations, using specialized equipment or widely available, affordable hand-held devices with cameras.

**IMPLICATION:** Proving the validity of an accurate telemedicine recognition of dysmorphic features will impact the diagnosis of FAS without a need for expert examiners in place.

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**Reference:**

The Safer at School Early Alert (SASEA) project is sponsored by San Diego County Health and Human Services Agency (SDHHSA). SASEA was developed to ensure that all students, staff, and their families feel safe at school regardless of their neighborhood, race/ethnicity, or economic status.

Dr. Fielding-Miller and her team developed and pilot tested SASEA in partnership with 15 schools serving low-income and racial/ethnic minority communities across San Diego County throughout the 2020-2021 academic year. Together with these partner schools and SDHHSA they have developed an environmental monitoring and diagnostic testing system that consists of: (1) Daily environmental sampling and testing for SARS-CoV-2 using (a) wastewater from the whole site and (b) surface swabs from specific classrooms (typically collected from the center of a classroom floor) with genomic sequencing of samples that test polymerase chain reaction (PCR) positive; (2) Rapid reporting to site administrators when environmental samples test positive (current turnaround time approx. 36 hours); (3A) Responsive on-site diagnostic testing using FDA authorized PCR tests via the CLIA-certified UCSD EXCITE Lab led by Drs. Knight and Laurent (co-Is) to diagnose asymptomatic positive cases when environmental monitoring suggests there is a potential case on site; (3B) Mitigating risk via environmental modification (e.g., moving classes outdoors, increasing ventilation in classrooms with a potential case) and health communication messaging (e.g., encouraging double masking, recommending wider testing among household members) until a case is identified and/or until the wastewater or surface samples remain negative for at least 2 days; and (4) Identify resources and recommendations for safe isolation of cases and quarantine of their contacts. SASEA’s goal is to transform schools and childcare sites from places of perceived risk to trusted community assets for historically marginalized communities.

IMPLICATION: Wastewater and surface monitoring in schools has the potential to provide ongoing sentinel surveillance data for the community served by a school, including genomic surveillance to monitor the introduction and prevalence of SARS-CoV-2 variants of concern with neighborhood-level resolution.

Reference:
Dr. Fielding-Miller and her team are currently developing their primary outcome manuscripts. They have submitted 8 abstracts to the American Public Health Association. Public facing information, including an open-access manual for any school site interested in implementing SASEA is available on their website: www.saseasystem.org
Although Fetal Alcohol Spectrum Disorders (FASD) was thought to be the most common cause of developmental disability in the U.S., prior to 2018, no national study of the actual prevalence of the disorder in the U.S. had been conducted. Dr. Jones and his team demonstrated in 4 communities in different regions of the U.S. that FASD prevalence is at least 1-5% in first grade children, and it is at least as common as autism spectrum disorders, but largely unrecognized.

Active case ascertainment methods using a cross-sectional design were used to assess children for fetal alcohol spectrum disorders between 2010 and 2016. Children were systematically assessed in the 4 domains that contribute to the fetal alcohol spectrum disorder continuum: dysmorphic features, physical growth, neurobehavioral development, and prenatal alcohol exposure. The settings were 4 communities in the Rocky Mountain, Midwestern, Southeastern, and Pacific Southwestern regions of the United States. First-grade children and their parents or guardians were enrolled.

Prevalence of fetal alcohol spectrum disorders in the 4 communities was the main outcome. Conservative estimates for the prevalence of the disorder and 95% CIs were calculated using the eligible first-grade population as the denominator. Weighted prevalences and 95% CIs were also estimated, accounting for the sampling schemes and using data restricted to children who received a full evaluation. A total of 6639 children were selected for participation from a population of 13,146 first-graders (boys, 51.9%; mean age, 6.7 years [SD, 0.41] and white maternal race, 79.3%). A total of 222 cases of fetal alcohol spectrum disorders were identified. The conservative prevalence estimates for fetal alcohol spectrum disorders ranged from 11.3 (95% CI, 7.8-15.8) to 50.0 (95% CI, 39.9-61.7) per 1000 children. The weighted prevalence estimates for fetal alcohol spectrum disorders ranged from 31.1 (95% CI, 16.1-54.0) to 98.5 (95% CI, 57.5-139.5) per 1000 children.

**IMPLICATION:** This discovery increased global recognition of the public health impact of FASD, and has led to a number of initiatives to improve access to diagnosis, treatment, and prevention.

**References:**

**FIGURE 1**

- First-graders (boys, 51.9%; mean age, 6.7 years and white maternal race, 79.3%) in 4 communities in different regions of the U.S. (n=12,146)
- Selected for participation (n=6,639)
- Assessed in the four domains that contribute to FASD
- Demonstrated in 4 communities in different regions of the U.S. that FASD prevalence is at least 1-5% in first grade children

### The Prevalence of Fetal Alcohol Spectrum Disorders in an American Indian Community

The prevalence of fetal alcohol spectrum disorders (FASD) differs among populations and is largely unknown among minority populations. Prevalence and characterization of FASD is necessary for prevention efforts and allocation of resources for treatment and support. However, prevalence data are lacking, including among many minority populations.

The aim of this study was to obtain an FASD prevalence estimate in a Southern California American Indian community employing active case-ascertainment. In 2016, American Indian children aged 5-7 years and their caregivers were recruited in collaboration with Southern California Tribal Health Clinic. Children were assessed using physical examinations and neurobehavioral testing. Parent or guardian interviews assessed child behavior and prenatal exposures including alcohol. Of 488 children identified as eligible to participate, 119 families consented and 94 completed assessments to allow a classification for FASD. Participating children (n=94) were an average of 6.61 ± 0.91 years old and half were female. Most interviews were conducted with biological mothers (85.1%). Less than one third (29.8%) of mothers reported consuming any alcohol in pregnancy and 19.1% met study criteria for risky alcohol exposure prior to pregnancy. Overall 20 children met criteria for FASD, resulting in an estimated minimum prevalence of 410 per 1000 (4.1%). No cases of fetal alcohol syndrome (FAS) were identified; 14 (70.0%) met criteria for alcohol related neuro-developmental disorder (ARND).

**Minimum prevalence estimates found in this sample are consistent with those noted in the general population.**

Dr. Jones and his team concluded that the prevalence estimate in this reservation-based community was not different from the estimate obtained in the national study and that diagnoses were similarly primarily based on behavioral variables. It’s important because, even though a smaller proportion of Native women consume alcohol compared to their non-Native counterparts, FASDs must still be prevented, diagnosed, and treated among indigenous communities.

**IMPLICATION:** These study findings contribute to the understanding of FASD among minority populations, specifically AIAN populations. In addition to providing information helpful for clinical resource allocation, this study raises questions about community resilience and the effect of culture. Future research into community-specific risk and protective factors is warranted.

**Reference:**
Cigarette advertising contributes to initiation of cigarette smoking among young people which has led to restrictions on use of cigarette advertising. However, little is known about other tobacco advertising and progression to tobacco use in youth and young adults.

Dr. Pierce and colleagues investigated whether receptivity to tobacco advertising among youth and young adults is associated with progression (being a susceptible never user or over user) to use of the product advertised, as well as conventional cigarette smoking. The Population Assessment of Tobacco and Health (PATH) Study at wave 1 (2013-2014) and 1-year follow-up at wave 2 (2014-2015) was conducted in a US population-based sample of never tobacco users aged 12 to 24 years from wave 1 of the PATH Study (N = 10,989). Household interviews were conducted using audio computer-assisted self-interviews.

Of the 10,989 participants (5410 male [weighted percentage, 48.3%]; 5579 female [weighted percentage, 51.7%]), receptivity to any tobacco advertising at wave 1 was high for those aged 12 to 14 years (44.0%; 95% confidence limit [CL], 42.6%-45.4%) but highest for those aged 18 to 21 years (68.7%; 95% CL, 64.9%-72.2%). Cigarette advertising had the highest receptivity among all age groups. For those aged 12 to 17 years, susceptibility to use at wave 1 was significantly associated with product use at wave 2 for conventional cigarettes, e-cigarettes, cigars, and smokeless tobacco products.

Among committed never users aged 12 to 17 years at wave 1, any receptivity was associated with progression toward use of the product at wave 2 (conventional cigarettes: adjusted odds ratio [AOR], 1.43; 95% CL, 1.23-1.65; e-cigarettes: AOR, 1.62; 95% CL, 1.41-1.85; cigars: AOR, 2.07; 95% CL, 1.62-2.49; and smokeless [mala only]: AOR, 1.42; 95% CL, 1.07-1.89) and with use of the product (conventional cigarettes: AOR, 1.54; 95% CL, 1.03-2.32; e-cigarettes: AOR, 1.45; 95% CL, 1.19-1.75; cigars: AOR, 2.07; 95% CL, 1.6-3.40). Compared with those not receptive to any product advertising, receptivity to e-cigarette advertising, but not to cigarette advertising, was independently associated with those aged 12 to 21 years having used a cigarette at wave 2 (AOR, 1.60; 95% CL, 1.08-2.38). Receptivity to tobacco advertising among adolescent never users was significantly associated with experimentation progression with cigarettes, e-cigarettes as well as cigars. Moreover, there was evidence that receptivity to e-cigarette advertising was also associated with trying a cigarette.

**IMPLICATION:** Just as for cigarettes, marketing of e-cigarettes and other tobacco products is associated with increases in initiation of tobacco use. E-cigarette advertising, which is currently not restricted, not only encourages adolescents to try an e-cigarette but it was also associated with trying cigarettes.

Experimentation with e-cigarettes and other forms of tobacco can lead to young people becoming daily cigarette smokers. Dr. Pierce and his team identified 12- to 24-year-olds at wave 1 of the US Population Assessment of Tobacco and Health Study and determined ever used, age at first use, and daily use through wave 4 for 12 tobacco products. Their findings showed sixty-two percent of 12- to 24-year-olds (95% confidence interval [CI]: 60.1% to 63.2%) tried tobacco, and 30.2% (95% CI: 28.7% to 31.6%) tried 2 or more tobacco products by wave 4. At wave 4, 12% were daily tobacco users, of whom 70% were daily cigarette smokers (95% CI: 67.4% to 73.0%); daily cigarette smoking was 20.8% in 25- to 28-year-olds (95% CI: 18.9% to 22.9%), whereas daily electronic cigarette (e-cigarette) vaping was 3.3% (95% CI: 2.4% to 4.4%).

Compared with single product triers, the risk of progressing to daily cigarette smoking was 15 percentage points higher (adjusted risk difference [aRD] 15%; 95% CI: 12% to 18%) among those who tried ≤5 products. In particular, e-cigarette use increased the risk of later daily cigarette smoking by threefold (3% vs 10%; aRD 7%; 95% CI: 6% to 9%). Daily smoking was 6 percentage points lower (aRD -6%; 95% CI: 8% to 4%) for those who experimented after age 18 years.

**Results:**
"The recent large increase in e-cigarette use will likely reverse the decline in cigarette smoking among US young adults.**

**IMPLICATION:** Young people becoming addicted smokers is a public health concern because it is so hard to quit. This study demonstrates that trying an e-cigarette or multiple other tobacco products before age 18 years of age is associated with young people becoming addicted cigarette smokers within 4 years. It emphasizes the need for public health action to apply the same restrictions on marketing for e-cigarettes as exist for marketing cigarettes.

**Reference:**

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**Figure 1:**
Panel B compares experimentation with e-cigarettes vs other tobacco products.

**Figure 2:**
Adjusted Risk Difference (RD) from Multivariable Logistic Regression on Progression To Daily Cigarette Smoking in the PATH Nationally Representative Longitudinal Study. Panel A compares number of Tobacco Products Tried.
Children and adolescents gain more weight in the summer than during the school year, with African American and Latino youth gaining the most. Some studies found youth are less physically active in the summer, but it is unknown whether this seasonal difference varies across race, ethnic, and sex subgroups.

The aim of this study was to examine race/ethnic and sex differences in adolescent physical activity, sedentary behavior, and related variables, comparing the school year and summer. In a study of over 200 highly-diverse adolescents, daily physical activity was 14 minutes per day lower in the summer than the school day. This decline was seen in all race/ethnic groups and both sexes. American Indians, Latinos, and girls were the least active groups in the summer. All racial and ethnic groups were sedentary between 8 and 9 hours per day, which did not differ from the school year to the summer, even though youth were not forced to sit in school for hours each day in the summer. All groups of adolescents reported more screen time in the summer, except for American Indians, and African Americans increased the most. Walking was the most preferred activity for virtually all subgroups, and around the home was the most preferred place to be active. Declines in physical activity and increases in screen time from the school year to the summer were documented in virtually all subgroups of low-income adolescents.

Thus, population-wide interventions are needed to increase physical activity in the summer.

**IMPLICATION:** Reduced physical activity and more screen time in the summer among low-income adolescents could contribute to excess weight gain and diminished mental health, making health disparities worse. Pediatricians should consider providing guidance and resources to help adolescents stay active and minimize screen time during the summer. Study findings suggest that interventions providing safe conditions and support for walking in neighborhoods are promising for all race, ethnic, and sex subgroups of adolescents.

**Reference:**

**FIGURE 1**

**Average MVPA** (minutes per valid day)

<table>
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<td>Interaction Sex * Time</td>
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</table>

**Moderate-to-Vigorous Physical Activity (MVPA, accelerometer measured). School-year vs summer differences by race/ethnicity and sex. *Note that physical activity was lower in summer than in the school year for all subgroups.**
In a large sample of >22,000 women, Dr. Shadyab found that women whose mothers and fathers survived to age 90 years relative to women whose parents did not achieve longevity were 38% more likely to achieve healthy aging, defined as survival to age 90 without major chronic diseases (coronary heart disease, stroke, diabetes, cancer, or hip fracture) or physical limitations (e.g., difficulties with walking several blocks, bathing or dressing, climbing one flight of stairs, or lifting or carrying groceries).

IMPLICATION: (and relevance to childhood health): Parental longevity represents the combined effects of genetic, behavioral, socioeconomic, and environmental factors transmitted from parents to children that, throughout the life course, influence future aging outcomes among children. Study findings suggest that children who inherit genes for longevity from their parents, grow up in better socioeconomic environments (i.e., with more wealth), and learn more positive lifestyle behaviors from their parents (e.g., eating well and exercising) are predisposed to achieving longevity and healthy aging themselves. Overall, Dr. Shadyab’s data suggest that early life factors in childhood have long-term impacts on adult health and aging.

Reference:

Children with Long-Lived Parents are More Likely to Live a Long and Healthy Life

Children born to older fathers tend to have greater access to economic resources. Therefore, these study findings suggest that childhood socioeconomic status strongly predicts longevity and healthy aging. Children born to older fathers also tend to have longer leukocyte telomere length, a biomarker of aging that is associated with longevity and healthy aging.

Reference:

Children Born to Older Fathers are More Likely to Live a Long and Healthy Life
Children living in agricultural settings have an increased risk of pesticide exposures agricultural sources. Insecticides, such as organophosphates, carbamates or pyrethroids, are designed to hijack the nervous system of insects, but there is growing evidence that exposures to these insecticides may affect the brain development of children.

The study of Secondary Pesticide Exposures among Children and Adolescents (ESPINA) is a study that has examined 623 children and adolescents living in agricultural settings in the Ecuadorian Andes since 2008. The 14th year of follow-up of study participants is currently planned for 2022. In analyses from 2008 and 2016, Dr. Suarez and his team observed that residential proximity to greenhouse crops was associated with greater biomarkers of pesticide exposure among children living within 275m of crops. Furthermore, performance on neurobehavioral tasks including Memory and executive tasks. The findings of this study can inform the planning of agricultrual land use and residential zoning policy considerations. Added precautions to reduce the off-target drift of pesticides from greenhouse crops onto nearby homes are recommended.

**IMPLICATION:** These findings reflect the upstream effects of the global demand for flowers, which result in increased chemical contaminants, including pesticides and persistent pollutants.

**References:**


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**Children Living Near Flower Crops Have Greater Insecticide Exposures and Lower Performance on Mental Health Processes**

Fetal alcohol spectrum disorders (FASD) are thought to be the leading cause of developmental disabilities worldwide. Accurate estimates of the prevalence of FASD have been lacking. An improved estimate of FASD prevalence in the U.S. was recently reported in Journal of the American Medical Association (2018) where Dr. Xu led the Data Analysis and Coordination Core. The study used multistage methods of active case ascertainment of first-grade children with FASD in four regions in the U.S. Each method relied on parental consent and therefore had potential non-response bias.

In a follow-up paper Dr. Xu and her colleagues considered weighted approaches, where the weights were formed using the distribution of observed variables in the population from which the samples were drawn. The researchers further described sensitivity analyses using methodology developed for causal inference, to account for other unobserved variables that are likely to affect both non-response and FASD outcome.

**IMPLICATION:** Fetal alcohol spectrum disorders (FASD) are thought to be the leading cause of developmental disabilities worldwide. Accurate estimates of the prevalence of FASD have been lacking. This work provided new data as well as methodology to address the challenges in the estimation of FASD prevalence.

**References:**


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**Estimating FASD Prevalence Accounting for Non-Response Bias**

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**IMPLICATION:** Accurate estimates of the prevalence of FASD have been lacking. This work provided new data as well as methodology to address the challenges in the estimation of FASD prevalence.
GENOMIC METHODS AND DISEASE

Using novel methods to interrogate genes, genetics, epigenetics and genomes.
Wolfram syndrome is a rare, autosomal recessive syndrome characterized by juvenile-onset diabetes and optic atrophy and is caused by bi-allelic mutations in the WFS1 gene. A range of phenotypes, including diabetes insipidus, retinal abnormalities and sensorineural deafness, are observed in individuals with Wolfram syndrome. In the absence of optic atrophy and other non-diabetic phenotypes, Wolfram syndrome can be clinically misdiagnosed as non-autoimmune type 1 diabetes.

Dr. Bansal and his team identified a novel, mild-form of Wolfram syndrome - characterized by later onset of diabetes – that is caused by a population-specific missense variant in the WFS1 gene. This variant (p.R558C) that affects a conserved amino acid residue has an allele frequency of 1.4% in individuals with Ashkenazi Jewish ancestry and represents a founder mutation in Ashkenazi Jewish individuals. Bansal V, Boehm BO, Darvasi A. Diabetologia. Identification of a missense variant in the WFS1 gene that causes a mild form of Wolfram syndrome and is associated with risk for type 2 diabetes in Ashkenazi Jewish individuals. Bansal V, Boehm BO, Darvasi A. Diabetologia. 

The mutation (p.R558C) affects a conserved residue in the transmembrane protein Wolframin. Individuals homozygous for this variant have a later age at diabetes diagnosis (yrs), compared to Wolfram syndrome patients.

The cardiac transcription factor (TF) gene NKX2-5 has been associated with electrocardiographic (EKG) traits through genome-wide association studies (GWASs), but the extent to which differential binding of NKX2-5 at common regulatory variants contributes to these traits has not yet been studied. Dr. Frazer and her team analyzed transcriptomic and epigenetic data from induced pluripotent stem cell–derived cardiomyocytes from seven related individuals, and identified ~2,000 single-nucleotide variants associated with allele-specific effects (ASE-SNVs) on NKX2-5 binding. NKX2-5 ASE-SNVs were enriched for altered TF motifs, for heart-specific expression quantitative trait loci and for EKG GWAS signals. Using fine-mapping combined with epigenomic data from induced pluripotent stem cell–derived cardiomyocytes, the Frazer lab prioritized candidate causal variants for EKG traits, many of which were NKX2-5 ASE-SNVs. Experimentally characterizing two NKX2-5 ASE-SNVs (rs1807969 and rs6900443) showed that they modulate the expression of target genes via differential protein binding in cardiac cells, indicating that they are functional variants underlying EKG GWAS signals. Results show that differential NKX2-5 binding at numerous regulatory variants across the genome contributes to EKG phenotypes.

**New variant for Wolfram syndrome**

**Figure 1**

The mutation (p.R558C) affects a conserved residue in the transmembrane protein Wolframin. Individuals homozygous for the mutation are diagnosed with diabetes at an average of 17.8 years compared to 5 years for Wolfram syndrome.

**Figure 1**

Prioritization of candidate causal variants at heart rate loci. Functional annotation of a SNP (rs6801957) associated with heart rate. From top to bottom: regional plot of association P values, with SNPs color coded based on linkage disequilibrium (r2; squared Pearson correlation) values from the 1000 Genome Project CEU population and the altered TF motif. These analyses show SNP rs6801957 is associated with heart rate through disrupting NKX2-5 binding.
Type 1 diabetes (T1D) is a complex autoimmune disease that affects over 1 million individuals in the US yet the etiology remains poorly understood. Genome-wide studies have revealed many genetic loci contributing to T1D risk and understanding how these loci function can reveal molecular mechanisms underlying disease, yet most loci are non-coding and have no obvious function.

In their recent study Dr. Gaulton and his team combined the largest to-date genome-wide association study (GWAS) of T1D with single cell epigenome maps to interpret the function of genetic variants influencing T1D risk. 136 signals associated with T1D risk were identified, including rare variants with large effects on T1D, almost half of which were novel. Using single cell ATAC-seq of peripheral blood and pancreas, Dr. Gaulton derived accessible chromatin profiles for 28 different cell types and defined candidate cis-regulatory elements (cCREs) in each cell type. T1D-associated variants were highly enriched in cCREs specific to pancreatic acinar and ductal (exocrine) cells, and multiple T1D variants mapped in exocrine-specific cCREs linked to genes with exocrine-specific expression. At one locus T1D variants mapped in a ductal cell-specific cCRE near the CFTR gene, and the Gaulton lab validated effects on CFTR using CRISPRi in ductal cells. T1D variants at exocrine signals were also associated with pancreatitis, suggesting a link between T1D and pancreatic disease. Together these findings provide evidence that the exocrine pancreas plays a causal role in the development of T1D and implicate specific genes such as CFTR in mediating T1D risk within exocrine cells.

**IMPLICATION:** The discovery of genetic factors for T1D can help predict disease risk, including in individuals carrying rare, large effect variants. Furthermore, the exocrine pancreas and genes affecting exocrine cell function may represent new areas for therapeutic targeting to prevent disease.

**Reference:**

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**JANE J. KIM, MD**
Professor of Pediatrics
Endocrinology

**RESEARCH INTERESTS:** Profiling the metabolome, gut microbiome, and genome in children with type 1 or type 2 diabetes; working with industry to innovate the use of continuous glucose monitoring devices in infants, currently FDA-approved for individuals over 2 years of age.

**Distinct Genetic and Metabolomic Signatures Associated with Insulin Resistance and Type 2 Diabetes in Youth**

Dr. Kim and her team have identified novel gene and metabolite signatures associated with childhood insulin resistance and type 2 diabetes through studies in both mice and human subjects. In the first study, they exploited the wide phenotypic variation among highly inbred male C57BL/6 mice in response to diet-induced obesity to identify distinct transcriptomic profiles in adipose tissue in early life that predicted the later development of insulin resistance in adulthood. In the second, they discovered a signature consisting of 22 urine metabolites that was uniquely associated with type 2 diabetes in adolescents at Rady Children’s Hospital when compared to obese children without diabetes, and healthy, age- and gender-matched normal weight controls.

**IMPLICATION:** These studies provide insight into the pathogenesis of type 2 diabetes in children, which is increasing more rapidly in adolescents than in any other age group. Dr. Kim hopes to utilize the metabolomic data to develop a non-invasive test to identify those youth at highest risk for diabetes onset.

**References:**
Extracting common gene signatures from diverse heterogeneous datasets are challenging. Technologies in this space has the potential to deliver robust translational impact in understanding both infectious and immune-mediated diseases. To achieve the full potential of a shared gene signature, Dr. Sahoo and his team use a mathematical concept of “invariant” that describes formula in Boolean logic that are true in every conditions. To showcase the powerful and superior nature of this approach over other conventional methods, their preliminary work has molded into two distinct discoveries:

(i) the development of a drug-discovery platform to solve an unmet and urgent grand challenge in inflammatory bowel disease (IBD). The Sahoo lab demonstrated how to compute invariant disease gene signatures and developed machine learning approaches to extract spectrum of disease continuum states from healthy to IBD.

(ii) A computational framework (disease map) to navigate the uncharted territory of COVID-19. An unbiased AI-guided approach was used to identify and validate a set of gene expression signatures that is an indicator of disease severity in respiratory viral pandemics (ViP and severe (s)ViP signatures). The signatures: (a) defined the nature (IL15-centric), extent and source of the cytokine storm in COVID-19; (b) set objective therapeutic goals; (c) assess the efficacy of a given drug/intervention on the disease.

**IMPLICATION:** The computational approach helps model a disease using a map of successive changes in gene expression at the onset and during the progression of the disease. What sets this mapping apart from other existing models is the use of mathematical precision to recognize and extract all possible fundamental rules of gene expression patterns, many of which are overlooked by current methodologies. The approach could help determine whether new treatments and vaccines are working in patients.

**References:**


**Drug Discovery at Your Fingertips Using Boolean Logic**

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**References:**

While exposed to very similar environmental stressors, premature infants show a marked diversity in their inflammatory response and BPD severity. Dr. Sajti in collaboration with Dr. Glass, applied genomic and epigenomic techniques to identify molecular mechanisms underlying the individual variation in lung inflammation. As a model they used inbred mouse strains that differ in their susceptibility to lung injury. By comparing gene expression and gene regulation in lung immune cells isolated from mice that are susceptible and resistant to lung injury, Dr. Sajti identified the transcription factors and signaling molecules likely regulating cell-specific functional potential and response to inflammatory stimuli.

To understand cell heterogeneity in the human developing lung, the Sajti lab collaborated with Dr. Xin Sun, the Center for Genomic and Epigenomic Analysis and the NHLBI LungMap Consortium. The analysis of non-diseased lung samples obtained from donors of 30 weeks gestation, 3 years and 30 years at the single cell level provided molecular insight into lung development at an unprecedented stage. This offers clues why young children account for only a small percentage of COVID-19 infections.

**IMPLICATION:** Determining the specific role of immune cell subsets in lung inflammation and identifying candidate genes and pathways that contribute to or protect against disease will allow targeted pharmacological therapy in a selective and developmentally appropriate manner.

**References:**


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**NATHALY M. SWEENEY, MD, MPH**

Assistant Professor of Pediatrics

**RESEARCH INTERESTS:** The application of precision medicine in the care of neonates with congenital heart disease and suspected genetic disease.

**Rapid Whole Genome Sequencing Impacts Care and Resource Utilization in Infants with Congenital Heart Disease**

Congenital heart disease (CHD) is the most common congenital anomaly and a major cause of infant morbidity and mortality. While morbidity and mortality are highest in infants with underlying genetic conditions, molecular ascertainment remains low. Furthermore, cost of care for children and adults with CHD are high. Rapid whole genome sequencing (rWGS) of newborns in intensive care units with suspected genetic diseases has been associated with increased rate of diagnosis and a net reduction in cost of care.

To determine whether the clinical utility of rWGS extended to critically ill infants with structural CHD, a retrospective review of rWGS study data obtained from inpatient infants ≤1 year with structural CHD was performed. rWGS diagnosed genetic disease in 46% of the enrolled infants. Moreover, genetic disease was identified five times more frequently with rWGS than microarray+/- gene panel testing in twenty-one of these infants. rWGS diagnosed 43% with microarray±Y gene panels, p=0.02. Molecular diagnoses ranged from syndromes affecting multiple organ systems to disorders limited to the cardiovascular system. The average daily hospitalization period was lower in the time period post blood collection for rWGS compared to prior (p=0.003) and further decreased after rWGS results (p=0.000). The cost was not prohibitive to rWGS implementation in the care of this cohort of infants. rWGS provided timely actionable information that impacted care and there was evidence of decreased hospital spending around rWGS implementation.

**IMPLICATION:** rWGS can be effectively implemented in the care of critically ill children and aids in more effective healthcare delivery. Furthermore, the database of variants of unknown significance suspicious for implication in structural CHD curated during sequencing will serve as a powerful resource for further research in the etiology of structural CHD. Investigation and validation of these variants as pathogenic will revolutionize the care of children with structural CHD by accelerating the application of precision medicine in this patient population and drastically improve outcomes.

**References:**

GUT, LIVER AND NUTRITION

Investigating a variety of factors important for proper growth and good nutrition.
Breastfeeding and human milk exert remarkable influence on infant survival and health, including reduced risk from infections and promoting various aspects of postnatal development. The many maternal benefits include protection from breast and ovarian cancer and cardiometabolic disorders. Although the mechanisms underlying some of these benefits have been elucidated, the origins of others that have been reported, such as influence on adult IQ and later protection against obesity and diabetes remain more obscure.

In March 2020, Dr. Bode authored a Perspective article in Science introducing a novel concept that human milk is more than just nutrition for babies, but an integral part of the mother–milk–infant ‘triad’, where maternal physiology, milk composition, and infant physiology form a co-adapting system that is embedded in socioeconomic, cultural, behavioral, and environmental contexts. The article calls for timely investments in research designed to clarify the operations and biological effects of the mother–milk–infant triad and their translation into public health. – One week after the article was published, the WHO declared COVID-19 a pandemic and it became strikingly evident how urgently investments in human milk research were needed. Parents and healthcare providers struggled with the complete lack of knowledge related to the transmission of SARS-CoV-2 through breastfeeding and human milk as well as safety and efficacy of COVID-19 vaccines for breastfeeding women. While the teams at UC San Diego were able to respond to these crises fairly quickly [4], it highlighted the urgent need for substantial and sustainable investments in human milk research during crises and beyond.

**IMPLICATION:** Elevating human milk research from a mere “food for babies” perspective to the center of maternal and infant health and fully understanding the mechanistic underpinnings of the mother–milk–infant triad holds massive opportunities for innovative approaches to better predict health outcomes, improve recommendations for preventing diseases, and develop new therapeutic targets as well as diagnostics to improve the health and development of infants, mothers and people of all ages.

### References:
- How does human milk affect maternal and infant health? Mechanistic insights hold the promise of providing more informative definitions of health status, better predictions of health outcomes, improved recommendations for preventing diseases, and new therapeutic targets and diagnostics.

### Human Milk at the Center of Maternal and Infant Health – A Systems Biology Approach Tested During the Pandemic

Breastfeeding and human milk, Human Milk Oligosaccharides (HMOs) and how they can serve as natural templates for the development of preventative, therapeutics, and diagnostics for people of all ages.

#### Maternal Diet and Exercise Alter Human Milk Composition with Consequences for Infant Health and Development

Not all human milk is created equal; in fact, human milk composition varies widely between women and changes over the course of lactation with potential immediate as well as long-term consequences for infant health and development. In a series of manuscripts the Bode lab and collaborators from around the world have shown that both fixed factors like maternal genetics as well as modifiable factors like maternal nutrition, exercise, and health medications influence human milk composition. Human milk oligosaccharides (HMOs) are complex glycan, sugars that represent the third most abundant component of human milk after lactose and lipids, and are the prime research focus of the Bode lab at UC San Diego. In a landmark article published in Nature Metabolism, Dr. Bode and collaborators at Ohio State University Wexner Medical Center and the Arkansas Children’s Nutrition Center have shown both in mice as well as in humans that consumption of a high-fat diet leads to a reduction of a specific milk oligosaccharide called 3’-sialyllactose. The group further identified this specific oligosaccharide as a critical mediator to improve metabolic health and cardia function in the breastfed infant receiving the milk. The Bode lab together with collaborators at the Baylor College of Medicine in Texas later confirmed in an independent study published in Scientific Reports that maternal diet affects human milk oligosaccharide composition.

**IMPLICATION:** These discoveries have several implications as it shows that (i) human milk composition is not the same in all women, (ii) can be changed by nutrition and exercise interventions, (iii) and has significant effects on infant health and development.

### References:
Changes in BMIz by appetitive trajectory group.

Over 1/3 of children are overweight or obese, which is associated with numerous health and psychological comorbidities. The gold standard treatment for children with overweight or obesity is Family-Based Treatment (FBT), which includes nutrition and physical activity education, behavior therapy skills and parenting skills. Although FBT results in weight reduction, there is wide variability in treatment response, and 2/3 of children continue to struggle with overweight or obesity into adulthood. Dr. Boutelle’s goal is to identify individual-level factors underlying treatment response that can be targeted to develop novel treatments.

Children are born with specific appetitive traits, such as how full they feel when eating and how much they feel driven to eat when physically full. These traits interact with the current food environment and put certain vulnerable children at risk for weight gain. Dr. Boutelle and her team examined the impact of appetitive traits on weight loss success among 150 children with overweight and obesity who participated in a 6-month FBT program with their parent.

Children were characterized into three groups: highly responsive to internal cues for satiety (HighSR), highly responsive to external food cues (High FR), or frequently engaged in emotional eating (HighEE). Children in each of these appetitive trait categories lost weight at a similar rate during the 6-month intervention. However, those who were highly responsive to external food cues or engaged in emotional eating regained their weight at the 12-month follow-up assessment, while children who were high on satiety responsiveness maintained their weight (see Figure 1).

IMPLICATION: These study findings highlight the need for additional tailoring of current behavioral weight-loss interventions to increase long-term success for children. The Boutelle lab is currently testing a treatment, called Regulation of Cues that targets both food responsiveness and satiety responsiveness and is expected to be more effective for children who do not respond to traditional FBT.

References:

TABLE 1

<table>
<thead>
<tr>
<th>Variables (unit)</th>
<th>Estimate</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Δ home food index – high fat (1 sd)</td>
<td>1.101</td>
<td>0.168 to 2.033</td>
</tr>
<tr>
<td>Δ home food index – low fat (1 sd)</td>
<td>–3.733</td>
<td>–5.67 to –1.791</td>
</tr>
<tr>
<td>Δ CFQ – restriction (1 sd)</td>
<td>–0.132</td>
<td>–1.532 to –1.267</td>
</tr>
<tr>
<td>Δ CFQ – monitoring (1 sd)</td>
<td>–1.107</td>
<td>–1.708 to –0.508</td>
</tr>
<tr>
<td>Δ CFQ – perceived responsiveness (1 sd)</td>
<td>0.313</td>
<td>–0.566 to –1.192</td>
</tr>
<tr>
<td>Δ kcal (1 sd; adult)</td>
<td>1.482</td>
<td>–0.627 to 3.591</td>
</tr>
<tr>
<td>Δ kcal (1 sd; child)</td>
<td>–1.407</td>
<td>–3.376 to 0.563</td>
</tr>
<tr>
<td>Δ parent knowledge questionnaire (1 sd)</td>
<td>–0.292</td>
<td>–7.244 to 6.661</td>
</tr>
<tr>
<td>Δ average MVPA day (1 sd)</td>
<td>–0.267</td>
<td>–2.140 to 1.605</td>
</tr>
</tbody>
</table>

Conditional estimation weighted predictors of child’s intra-individual weight changes. Abbreviations: BMI, body mass index (unit kilograms/meters squared); CFQ, Birch child feeding questionnaire subscales; MVPA, moderate and vigorous intensity physical activity. All models adjust for time, sex, randomization, time* randomization, and corresponding baseline demographics. P-value using Benjamini-Hochberg adjustment procedure. Negative estimates is better in the context of evaluating the weight-loss treatment. Weighted predictors include balancing time-varying covariates through inverse probability weighting of adult BMI and attendance.

IMPLICATION: These results are being used to develop an FBT program that is less burdensome and focuses on these mechanisms of change.

References:
The Role of Executive Function in Child Weight-Loss Treatment

Executive functions (EFs) are higher level cognitive functions that guide behavioral responses during complex tasks or socio-emotional situations to achieve a goal. The three main EF domains include: inhibitory control (the ability to control and regulate impulsive behaviors), working memory (the ability to hold memories in order to complete a task), and cognitive flexibility (the ability to shift thoughts in order to respond to a situation). Current treatment recommendations require significant EF; thus, it was hypothesized that executive function may impact treatment outcomes. Dr. Eichen and her team examined the impact of EF tasks on weight loss success among 150 children with overweight and obesity who participated in a 6-month weight loss program with their parent. Children who had poorer cognitive flexibility or set-shifting ability had greater weight regain at the follow-up assessment (see Figure 1).

**IMPLICATION:** The results of this study suggest that current programs need to target EF to improve long-term maintenance of weight loss obtained during treatment. Currently, Dr. Eichen is developing programs that incorporate EF training to improve outcomes for obesity and binge eating disorder treatment. A program has been developed that targets compensatory strategies to manage EF risk factors. An evaluation of this treatment delivered to parents of children with Attention Deficit Hyperactivity Disorder (ADHD) is in progress. ADHD is associated with lower EF so combining EF training with weight loss treatment for this population has the potential to improve weight loss maintenance in children and parents participating in family-based treatment for childhood obesity.

**Reference:**

**FIGURE 1**

Chronic liver disease represents a major cause of morbidity and mortality worldwide. Fibrosis is an intrinsic response to chronic persistent liver injury that results in a wound-healing process to mitigate the damage but also can lead to scar formation. Unfortunately, in many instances liver fibrosis progresses over time and results in the development of cirrhosis where the normal liver parenchyma is replaced by scar tissue resulting in severe disruption of the liver architecture and vascular distortion, and is associated with feared complications including portal hypertension, liver failure, and hepatocellular carcinoma with liver transplantation being the only curative therapeutic option. Cell death and inflammation are two central elements in the development of liver fibrosis.

Investigators in Dr. Feldstein lab uncovered hepatocyte pyroptosis, a novel form of cell demise and release of inflammasome components as important mechanisms to preempt liver injury and liver fibrosis development. Experiments in cell cultures, mice, and human samples show that specific form of cell death, called pyroptosis, leads to the release of complex inflammatory particles, the NLRP3 inflammasome, from inside hepatocytes into the extracellular space. From there they are taken up by other cells and thereby mediate inflammatory and pro-fibrogenic stress signals. The discovery of this mechanism may lead to novel treatments for chronic liver diseases in the future.

**IMPLICATION:** Chronic inflammation has created a global health burden and is a common driver of some of the most common diseases limiting people’s health and longevity. Dissecting the molecular mechanisms driving chronic inflammation may help identify novel precision medicine approaches to treat these conditions.

**References:**
Health Literacy, Transition, and Informatics

According to the Centers for Disease Control and Prevention, health literacy is “the degree to which individuals have the ability to find, understand, and use information and services to inform health-related decisions and actions for themselves and others.” In pediatrics, health literacy is essential for a smooth transfer from pediatric-centered to adult-centered care, the preparation for which is a process known as transition. Urgency to address transition readiness has been well-recognized by national health agencies because of demonstrated poor psychosocial and health outcomes in youth with poor transition experiences. Delivery of transition services needs to be integrated into clinical workflows, but this historically has been difficult to achieve because of lack of time and systematic supports.

Dr. Huang and her lab, along with a multidisciplinary Transition Task Force at Rady Children’s Hospital in San Diego, have been evaluating the role of the electronic health record (EHR) to essentially support distribution of transition services in a manner friendly to clinical workflow. Currently, EHR-supported transition service delivery occurs annually to youth with chronic disease needs to be integrated into clinical workflow, but this historically has been difficult to achieve because of lack of time and systematic supports.

The Transition Activity Protocol

This figure demonstrates the transition activity protocol where (going left to right) assessment occurs at an annual visit at age 12y to inform goal setting and resource delivery regarding transition readiness and skills to be gained prior to the next visit. In addition, a patient’s medical summary is reviewed annually to familiarize the patient with his/her own medical history. At age 18y+, after this period of preparation, the patient then identifies an adult provider and direct pediatric provider-to-adult provider communication occurs to ensure sharing of relevant information for continuous care delivery and appropriate transfer of care.

Prevalence of Nonalcoholic Fatty Liver Disease in Children with Obesity

Nonalcoholic fatty liver disease (NAFLD) is the most common cause of liver disease in children with a prevalence in the U.S. of 5 to 8 million. NAFLD is a metabolic form of liver disease that places children at future risk for end-stage liver disease, diabetes and coronary heart disease.

In April 2019, working in Dr. Jeffrey Schwimmer’s lab, Dr. Yu and her team evaluated the prevalence of NAFLD in children with obesity. This study utilized magnetic-resonance imaging – proton density fat fraction (MRI-PDFF) as a non-invasive diagnostic tool for pediatric NAFLD and identified evidence-based diagnostic thresholds for alanine transaminase (ALT), which validates the clinical utility of ALT as a screen. Prevalence of NAFLD in children with obesity was 26%, thus demonstrating that NAFLD is indeed more common in children with obesity but NAFLD and obesity are not concomitant.

References:

Prevalence of Nonalcoholic Fatty Liver Disease in Children with Obesity

<table>
<thead>
<tr>
<th>PDFF thresholds</th>
<th>Prevalence of fatty liver</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥5.0%</td>
<td>All: 26.0%</td>
<td>24.2%-27.7%</td>
</tr>
<tr>
<td></td>
<td>Boys: 29.4%</td>
<td>26.1%-32.7%</td>
</tr>
<tr>
<td></td>
<td>Girls: 22.6%</td>
<td>16.0%-29.1%</td>
</tr>
<tr>
<td>≥3.5%</td>
<td>All: 49.3%</td>
<td>47.9%-50.7%</td>
</tr>
<tr>
<td></td>
<td>Boys: 50.0%</td>
<td>49.0%-51.9%</td>
</tr>
<tr>
<td></td>
<td>Girls: 48.5%</td>
<td>48.5%-52.8%</td>
</tr>
<tr>
<td>≥6.4%</td>
<td>All: 19.9%</td>
<td>16.7%-21.4%</td>
</tr>
<tr>
<td></td>
<td>Boys: 23.5%</td>
<td>19.1%-27.9%</td>
</tr>
<tr>
<td></td>
<td>Girls: 14.7%</td>
<td>5.5%-23.8%</td>
</tr>
<tr>
<td>≥9.0%</td>
<td>All: 11.5%</td>
<td>8.5%-14.4%</td>
</tr>
<tr>
<td></td>
<td>Boys: 15.2%</td>
<td>9.2%-21.3%</td>
</tr>
<tr>
<td></td>
<td>Girl: 7.7%</td>
<td>0.2%-19.6%</td>
</tr>
</tbody>
</table>

Prevalence of NAFLD by MRI/PDFF threshold value in children with obesity.
Vancomycin is a medication with potential for significant harm with both overdosing and underdosing. Obesity may affect vancomycin pharmacokinetics and is increasingly common among children. Dr. Khare and her research team aimed to determine if children with overweight or obesity have increased vancomycin trough concentrations with total body weight (TBW) dosing compared with children with normal weight. They conducted a search of Medline and Medline In-Process & Other Non-Indexed Citations from 1952 (the year vancomycin was discovered) to November 2017. Search terms included vancomycin, body weight, and body composition terms and were limited to children. Studies were reviewed and screened by ≥2 reviewers. The primary outcome was vancomycin level. Data were extracted by 2 reviewers. Quality assessment was performed using the Newcastle-Ottawa quality assessment scale. 271 records were identified. After abstract and full-text screening, Dr. Khare and her team identified 7 studies for full review. Six of the 7 studies used a matched case-control design, although there was significant variation in study methodology. Four of the 7 studies were included in a meta-analysis, which revealed a small but significant difference in vancomycin trough levels between children with normal weight and children with overweight or obesity when dosed by using TBW (N = 521; mean difference 2.2 U [95% confidence interval: 1.0–3.4]). High-quality data to guide vancomycin dosing in children with obesity are lacking. More studies evaluating dosing strategies in children with obesity are warranted because using TBW to dose vancomycin may lead to higher vancomycin concentrations and potential toxicity.

**IMPLICATION:** This study describes the variation in vancomycin dosing by weight and examines the association between weight measures and pharmacokinetic outcomes in large, multicenter studies. Providers should be aware of the variation in pharmacokinetics by weight status to guide their dosing practice.


### TABLE 1

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>Overweight or Obese</th>
<th>NW</th>
<th>Mean (ug/mL)</th>
<th>SD (ug/mL)</th>
<th>Mean (ug/mL)</th>
<th>SD (ug/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heble 2013</td>
<td>14.35</td>
<td>5.12</td>
<td>42</td>
<td>10.77</td>
<td>4.27</td>
<td></td>
</tr>
<tr>
<td>Madigan 2013a</td>
<td>7.7</td>
<td>2.1</td>
<td>11</td>
<td>7.1</td>
<td>2.4</td>
<td></td>
</tr>
<tr>
<td>Madigan 2013b</td>
<td>13.8</td>
<td>7.1</td>
<td>25</td>
<td>10.4</td>
<td>4.6</td>
<td></td>
</tr>
<tr>
<td>Miller 2011</td>
<td>9.6</td>
<td>8.9</td>
<td>70</td>
<td>7.4</td>
<td>5.7</td>
<td></td>
</tr>
<tr>
<td>Moffett 2011</td>
<td>6.9</td>
<td>4.3</td>
<td>24</td>
<td>4.8</td>
<td>3.1</td>
<td></td>
</tr>
<tr>
<td>Total (95% CI)</td>
<td></td>
<td></td>
<td>172</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heterogeneity: x² = 6.58, df = 4 (P = .16); I² = 39%</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Test for overall effect: Z = 4.63 (P &lt; .00001)</td>
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</tbody>
</table>
Overweight and obesity affect one-third of children in the US. Treatment programs have traditionally involved family-based behavioral therapy which promotes a range of behavioral strategies to help increase physical exercise and decrease caloric intake. However, only a third of families are able to maintain these changes over a ten-year period.

In an effort to identify new targets for treatment, Dr. Rhee used the dual-process model to examine the interplay between food responsiveness (an appetitive trait) and executive functioning (cognitive traits) on obesity risk. In a study with preschool-age children, she found that those who were highly responsive to food cues (appetitive traits) had higher BMI percentiles if they had poor emotional control skills (an executive function) (Figure 1). However, children who were not highly responsive to food cues did not demonstrate this variability in weight status as executive functions differed. Dr. Rhee and her team have begun to test an intervention to promote self-regulation in preschool children and decrease consumption of energy-dense snack foods, and has obtained promising results.

**IMPLICATION:** Focusing on executive functioning skills may enhance current behavioral weight loss programs, especially for children who are sensitive to environmental food cues.

**References:**

**Guided Self-Help Pediatric Obesity Treatment in the Primary Care Setting**

In addition to work on novel targets for intervention, Dr. Rhee is working to disseminate current programs to make them more accessible to families who have a hard time attending treatment at a tertiary care academic center. She and Dr. Kerri Boutelle have developed a Guided Self-Help (GSH) model of obesity treatment that provides multi-component treatment recommended by the AAP and several other national organizations, but delivers it in individually scheduled 20-minute visits with a health coach. This program includes the same behavioral strategies that are covered in their traditional family-based behavioral program, but allows for increased flexibility in scheduling and can potentially be delivered in the primary care setting. Their recent clinical trial demonstrated similar changes in weight status as the traditional program, but had a significantly higher rate of attendance (Figure 1).

**IMPLICATION:** Greater attendance at obesity treatment programs is the first step towards obtaining weight loss.

**References:**
Nearly all pediatric gastroenterologists recommend dietary intervention for Nonalcoholic Fatty Liver Disease (NAFLD), but there are limited data to guide this intervention. Dietary sugar is thought to be a major contributor to liver fat through de novo lipogenesis. A challenge for nutrition research has been how to test a change in a single nutrient of a free living individual’s diet.

Dr. Schwimmer designed a multi-center, randomized controlled trial in which the research team evaluated the entire family diet. They provided the meal planning, grocery shopping, and cooking in order to replicate the family diet, with the only change being to lower the free sugar in the diet to < 3% of total calories. To measure the change in liver fat, this study also used the approach validated in the MRI Rosetta Stone Project. It was demonstrated that 8 weeks of a diet low in free sugar content, compared to a usual diet, substantially improved hepatic steatosis and liver chemistry. The improvements observed were superior to the effects observed in medication trials to date.

**IMPLICATION:** These findings set the stage for sustainable dietary interventions to make a difference in the outcomes of children with NAFLD.

**Reference:**

Disturbances of the Intestinal Microbiome associated with Pediatric NAFLD Presence and Severity

A growing body of literature shows the potential role of the intestinal microbiome in health and disease. Dr. Schwimmer and his team performed the most carefully phenotype case-control study of Nonalcoholic Fatty Liver Disease (NAFLD) to date to evaluate the association of the intestinal microbiome with the risk for NAFLD and the severity of NAFLD. The development of the control group was done using advanced MRI technology developed at UC San Diego and validated in collaboration with the Schwimmer Lab (MRI Rosetta Stone Project). It was discovered that the fecal microbiomes of children with NAFLD were disrupted (dysbiosis) compared to children without NAFLD. When evaluating the relationship with disease severity, among children with NAFLD, Lactobacillus and Oribacterium were higher in patients with steatohepatitis. With respect to liver scar tissue, children with severe fibrosis were more likely to have high abundance of Prevotella. In addition, increased levels of bacterial genes that regulate synthesis of lipopolysaccharide and assembly of flagella were associated with disease severity.

**IMPLICATION:** Through this work, Dr. Schwimmer and his team demonstrated the potential to use these biological relationships to develop clinically relevant, non-invasive biomarkers based upon the intestinal microbiome.

**Reference:**
Congenital tufting enteropathy (CTE) is one of several intractable diarrheal diseases of infancy that typically presents in the neonatal period with chronic watery diarrhea and impaired growth. Patients suffer from intestinal failure necessitating parenteral nutrition and, in some cases, intestinal transplant. The diagnosis of CTE is made with the recognition of structural changes in the small intestinal epithelium. Typical findings include villous atrophy and focal epithelial tufts in the small intestine.

Dr. Sivagnanam has identified mutations in the gene, EpCAM (epithelial cell adhesion molecule), contributing to the cause of this disease. In her lab, Dr. Sivagnanam is team is growing 3D “mini guts” to further understand how EpCAM contributes to the health of the intestine. These mini-guts, known as enteroids, possess all the cell types and architecture of the intestines. The enteroids mimic features of disease and allow for intricate study of the crypt villus axis. Thus, they serve as a powerful tool for understanding the dynamics diseases such as CTE, where structure is altered.

Using this model of disease, investigators in the Sivagnanam lab have gone on to understand that EpCAM plays a role in the delicate balance of intestinal epithelial cell differentiation, with decreases in goblet, Paneth and enteroendocrine cells. They discovered that these changes can be reversed with Notch inhibitor DAPT. Absorptive enterocytes in this disease are also dysfunctional, potentially contributing to nutrient malabsorption and impaired weight gain, which are hallmarks of CTE.

**IMPLICATION:** The knowledge acquired from these studies will allow a paradigm shift in the understanding of EpCAM, CTE, diarrheal diseases, and intestinal failure. Patients with CTE don’t have treatment options currently and these new findings set the framework for new therapies to treat this devastating disease.

**FIGURE 1**

**A Fine Balance Between Health and Disease**

Alterations in intestinal epithelial cell differentiation and enterocyte dysfunction lead to the features of Congenital Tufting Enteropathy.

**Lack of Weight Gain**

**Decreased Glucose Absorption**

**Secretory & Osmotic Diarrhea**

**Malabsorption**

**CTE Disease Manifestation**

<table>
<thead>
<tr>
<th>Decrease</th>
<th>Increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absorptive Enterocytes</td>
<td></td>
</tr>
<tr>
<td>Enteroendocrine Cells</td>
<td></td>
</tr>
<tr>
<td>Goblet Cells</td>
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<td>Paneth Cells</td>
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<td>Non-secretory Enterocytes</td>
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Absorptive enterocytes in this disease are also dysfunctional, potentially contributing to nutrient malabsorption and impaired weight gain, which are hallmarks of CTE.

**References:**


INFLAMMATION AND IMMUNITY

Investigating basic mechanisms to understand allergic and immunologic diseases.
Eosinophilic esophagitis (EoE) is a chronic allergic food and aero-allergen driven disease of increasing prevalence in children and adults. Complications include food impactions and poor growth and esophageal rigidity and luminal narrowing. Loss of esophagus luminal patency occurs via tissue fibrosis and remodeling, a pathogenesis in which esophageal fibroblasts play a pivotal role. New discoveries in the Aceves lab, which is focused on understanding the mechanisms and clinical implications of tissue remodeling, have begun to unravel the phenotypes of EoE fibroblasts and the differences in the extracellular matrix that they create.

The ability of T cell derived TNF superfamily member LIGHT/TNFSF14 to create a transcriptional and functional inflammatory fibroblast phenotype were reported for the first time in the November 2020 issue of Gastroenterology (cover article). Human esophageal fibroblasts treated with LIGHT had increased transcription of pro-inflammatory cytokines (CXCL-5, -6, CSF1), interleukins (IL32, -33, -34), TNF superfamily members (TNFSF15, TNFSF13B, TNFSF4), and adhesion molecules (ICAM-1, VCAM-1). The pro-fibrotic factor, TGFß1, enhanced a subset of LIGHT effects. LIGHT treated fibroblasts tethered human eosinophils to their surface in an ICAM-1 dependent manner.

**IMPLICATION:** These studies place the fibroblast as potential central mediator of both inflammation and fibrosis in EoE.

In other studies, the Aceves lab reported the novel experimental approach of culturing a normal esophageal fibroblast on the extracellular matrix (ECM) derived from an EoE esophageal fibroblast. The EoE ECM altered the phenotype of the normal fibroblast to a higher collagen-producing myofibroblast (Journal of Allergy and Clinical Immunology, 2021, Editor’s Choice article). Using an unbiased proteomic approach, Aceves and colleagues found that thrombospondin-1 (TSP-1) was increased in the fibroblast-derived and in vivo esophageal ECM and that TSP-1 could induce collagen production from esophageal fibroblasts.

**IMPLICATION:** This novel approach demonstrates that the EoE fibroblast ECM is sufficient to change fibroblast phenotype and function and could provide a new way to gauge disease severity in EoE.

**References:**

B cell development is a highly regulated process involving multiple differentiation steps, yet many details regarding this pathway remain unknown. Sequencing of patients with a B cell–restricted immunodeficiency (Hoffman syndrome), revealed autosomal dominant mutations in TOP2B. TOP2B encodes a type II topoisomerase, an essential gene required to alleviate topological stress during DNA replication and gene transcription, with no previously known role in B cell development.

Dr. Broderick and her colleagues used a multi-species model approach, from Saccharomyces cerevisiae to patient cells, to demonstrate that the patient mutations in TOP2B have a dominant negative effect on enzyme function, resulting in defective proliferation and survival of B cells causing a block in B cell development, and impaired humoral function in response to immunization.

**Mutations in TOP2B Underlie Syndrome B Cell Immunodeficiency**

**IMPLICATION:** Topoisomerases are significant in the cancer literature; however, their involvement in primary immunodeficiency and in hematopoietic cell development has not been previously described. Thus, this discovery defined an entirely new class of B cell immunodeficiency and the first Mendelian disease due to mutations in a topoisomerase.

**Reference:**

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**Local Tonsillar Inflammation Drives PFAPA Syndrome**

Periodic Fever, Aphthous stomatitis, Pharyngitis and Adenitis (PFAPA) syndrome is an immune disorder of childhood classically characterized by recurrent attacks of fever, pharyngitis, stomatitis, cervical adenitis and leukocytosis. Despite these unique clinical features, little is known about the underlyingpathogenesis, though tonsillitis has been suggested to be a therapeutic option. Given the location of the tonsils at the junction of the respiratory and gastrointestinal tracts and their inherent exposure to environmental agents, as well as recent data suggesting a role in immune tolerance, resolution of PFAPA after tonsillectomy suggests that the syndrome may represent dysregulation of the immune system.

Dr. Broderick and her team reported on the clinical features of large cohort of PFAPA patients seen in a tertiary care center, their response to tonsillectomy, following this cohort for 8 years, and demonstrated a unique pro-inflammatory signature present even during the afebrile intervals, which distinguish these tissues from those from pediatric patients with recurrent pharyngitis. Furthermore, they provided the first in depth microbiome evaluation of PFAPA tonsils.

**IMPLICATION:** This work provides clinical and translational data supporting the success of tonsillectomy as an effective surgical treatment option. The differential expression of several genes and microbial signatures suggests the potential for a diagnostic biomarker for PFAPA syndrome, and brings new insights into this often overlooked pediatric syndrome.

**Reference:**

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**FIGURE. 1**

Mutations in TOP2B cause peripheral B cell immunodeficiency and dysmorphic features. Top, 3D modeling of the identified variants, with green and blue helices representing two domains of the homodimer, and black and white spheres representing the DNA helix in the binding site. Mutations are displayed in red. Bottom, B cells with heterozygous mutations (right) fail to proliferate resulting in an early arrest compared to wildtype (left).

**FIGURE. 1**

PFAPA tonsils have an inflammatory signature. A, Gene expression analysis from whole tonsillar tissue from PFAPA (n=12) and control tonsils (n=6), demonstrates significant increases in ILIRN and TNF gene expression in PFAPA tonsils compared to controls. Each circle represents a different patient, average of technical triplicates, shown as the mean ± SEM. *, p<0.05, **, p<0.01 by Student’s two-tailed t-test.
Microglia are the resident immune cell of the brain, they enter the brain early in fetal development (gestational week 6) and contribute not only to inflammation in cases of infection or insult, but also perform many crucial neurodevelopmental tasks such as pruning synapses and modulating neural progenitor pools.

Dr. Coufal and her team have described the epigenetic and transcriptomic landscape of human microglia, the adequacy of current modeling techniques, and utilized bioinformatics to predict the key signaling mediators in microglial development and maturation (Figure). They are now defining how microglia in the context of genetic disease contribute to neurodevelopmental disease. To address this challenge, they have utilized patient derived induced pluripotent stem cells to generate microglia in vitro, and have developed novel methods for understanding how human microglia contribute not only to brain development but also to pediatric neuroinflammatory disease-risk association. Science. 2019 Nov 29;366(6469):1134-1139.

The laboratory identified a molecule called interleukin-1 (IL-1) immune cells can release the molecule to amplify and orchestrate excessive inflammation and organ damage. During infection, pathogens because they are filled with anti-microbial compounds are defective in the PTPN6 protein also develop inflammatory dermatoses are skin lesions driven by white blood cells called neutrophils, and patients often report fever and malaise, and in some cases present with severe non-resolving skin wounds that expose underlying muscles and tendons. PTPN6 is a molecular brake that was reported to have coding and transcript variants in blood cells of patients with neutrophilic dermatoses. Mice that are defective in the PTPN6 protein also develop inflammatory skin lesions and autoimmune disease, enabling researchers to study the cellular and genetic causes of the disease.

In a study published in Nature Immunology, the Croker laboratory has now revealed the molecular and cellular basis of disease, opening the door to off-the-shelf treatments for these patients. The laboratory identified a molecule called interleukin-1 (IL-1) as the key to disease but the source of IL-1 was not known. IL-1 is kept under lock-and-key by cells to prevent it from causing excessive inflammation and organ damage. During infection, immune cells can release the molecule to amplify and orchestrate immune responses. PTPN6 is one of the locks that prevent IL-1 from being released by neutrophils.

The research team recognized that neutrophils can control release of IL-1 by co-opting the molecular machinery normally reserved for triggering the death of the cell. By studying mice with neutrophils that were genetically deficient in these cell death proteins, they could block the inflammatory skin disease in mice. IL-1 is still produced by neutrophils but is no longer released to drive disease. Neutrophils in this disease appear to function normally but their lifespan is very short, and they die in the wrong place at the wrong time. Neutrophils are highly toxic to pathogens because they are filled with anti-microbial compounds and inflammatory molecules including IL-1.

References:

Nicole G. Coufal, MD, PhD
Assistant Professor of Pediatrics
Allergy, Immunology & Rheumatology

Research Interests: The contribution of microglia to neurodevelopment, aging, and neuroinflammatory and neurodegenerative disease.

Mapping the Role of Microglia in Brain Development and Neuro Inflammatory

Implication: Both rare and common neurodevelopmental and neurodegenerative disorders have an inflammatory component. As the primary immune cell of the brain, microglia mediate many of these inflammatory interactions and have become as a primary treatment target in neurodevelopmental disease. Dr. Coufal’s studies focus on the contribution of microglia to neurodevelopment and pediatric neurodevelopmental disease with a goal of discovering novel pathways amenable to drug screening.

References:

Ben Croker, PhD
Associate Professor of Pediatrics
Allergy, Immunology & Rheumatology

Research Interests: Obstructive sleep apnea, endothelial dysfunction, and pediatric sleep.

Negative Regulation of Inflammatory Cell Death in Neutrophilic Dermatoses

Chronic inflammatory skin lesions can be disfiguring and painful, and in some cases associated with inflammatory bowel disease, rheumatoid arthritis, autoimmunity and leukemia. Neutrophilic dermatoses are skin lesions driven by white blood cells called neutrophils, and patients often report fever and malaise, and in some cases present with severe non-resolving skin wounds that expose underlying muscles and tendons. PTPN6 is a molecular brake that was reported to have coding and transcript variants in blood cells of patients with neutrophilic dermatoses. Mice that are defective in the PTPN6 protein also develop inflammatory skin lesions and autoimmune disease, enabling researchers to study the cellular and genetic causes of the disease.

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Reference:
Dr. Franco and her team have defined the fine specificity and immunodominance of the peptides that activate a Treg population that recognizes the heavy constant region of the IgG (Fc). Fc-specific Treg can be activated by IgG+ B cells by processing their surface IgG and presenting the Fc peptides, as well as by dendritic cells through the uptake of exogenous IgG.

In Kawasaki disease, their data suggest that the expansion of these cells is one of the mechanisms of success for intravenous immunoglobulin therapy (IVIG). Functionally, this Treg population showed a capability in inhibiting the polarization of naive T cells toward a proinflammatory phenotype. Healthy adult donors have always Fc-specific Treg in circulation, which underlines their importance in the immune homeostasis, and the immunodominant Fc peptides have been found comparable in subacute KD children after IVIG and RA subjects. In acute KD this Treg population is missing before IVIG but expand because of the high concentration of IgG that leads in Treg priming. The Fc is processed and presented by Treg by tolerogenic myeloid dendritic cells (tmDC), namely CD14+CD16+CD8+CD3+DMHC. Four of the immunodominant Fc peptides (Fc 51-65, 61-75, 181-195, and 186-200) can bind multiple HLA class II alleles therefore applicable as therapeutic in the general population.

The success of identifying these immune modulatory pan-HLA peptides would serve as a novel therapeutic approach to treat inflammatory conditions as Rheumatoid Arthritis and Kawasaki disease (the models that have been explored). Lack of the Treg responses may have a profound impact in the pathogenesis of autoimmune and inflammatory disorders as a defect of the “central” immune regulation that the peptide-based therapy could restore.

**IMPLICATION:** This discovery may be an alternative to chronic administration of immune suppressive medications such as steroids by instead boosting the immune regulation. Studies are in progress for the filing of an investigator new drug (IND) for a Phase 1 in Rheumatoid Arthritis.

**References:**

**FIGURE 1**
A model for mature IgG-B cells in controlling inflammation by expanding natural, thymic-derived regulatory T cell (Treg) via a unique antigen processing that shapes adaptive regulation through the presentation of peptides derived from the heavy constant region (Fc) of IgG. IgG-B cells present Fc peptides via processing of the surface IgG and activate a human Treg population that recognizes the Fc underlying a model for B-cell-Treg cooperation in the human immune regulation.
Autoinflammatory diseases are monogenic and polygenic disorders due to dysregulation of the innate immune system. The inherited conditions have been clustered with primary immunodeficiencies in the latest practice parameters, however, these diseases have unique clinical presentations, genetics and available therapies. While there has been attention in the literature to diagnosis and treatment of rare, genetically-defined autoinflammatory disorders, physicians are challenged by increasing numbers of patients with intermittent or periodic fevers who face unnecessary morbidities due to a lack of a diagnosis. The broad differential of diseases presenting with fever includes autoinflammatory syndromes, infections associated with immunodeficiency and/or allergies complicated by infection, and less commonly, autoimmune disorders or malignancy.

To address this challenge, Dr. Hoffman and his team review the history of the medical approach to fever, current diagnostic paradigms, and controversies in management. They describe the spectrum of disorders referred to a recurrent fever disorders clinic established in an Allergy/Immunology division at a tertiary pediatric care center.

**IMPLICATION:** The Hoffman team provides practical recommendations including historical features and initial laboratory investigations that can help clinicians appropriately manage these patients, to reduce delays in diagnosis and encourage use of targeted therapies.

**Reference:**

**Alternative Splicing as a Mechanism for Immune Regulation**

Dr. Hoffman and his team characterized the phenotype of familial cold autoinflammatory syndrome and positionally cloned a novel gene using DNA from affected individuals and controls to identify gain of function missense mutations in the gene NLRP3 as the cause of this disease. NLRP3 is expressed in peripheral blood leukocytes and encodes a protein we termed cryopyrin, a crucial component of the inflammasome and regulator of immune mediated inflammation that has been referred to the “Rosetta Stone of Innate Immunity.” How varied triggers activate the NLRP3 inflammasome leading to differential immune responses has long been studied. They recently reported how different alternatively spliced NLRP3 isoforms affect inflammasome activation.

**IMPLICATION:** Alternative splicing may contribute to regulation of the human innate immune response and development of immune dysregulatory disorders.

**Reference:**
Food allergy prevalence continues to increase and there is no cure. The current standard of care is limited to avoidance of the allergenic food and treatment of allergic reactions from accidental ingestion with injectable epinephrine. Peanut is among the most prevalent of the food allergies, affecting approximately 1 in 70 children and 1 in 160 adults in the United States. Peanut allergy typically begins in early childhood, but unlike many food allergies, it persists into adulthood in 80% of patients. Reactions to peanut can be triggered by milligram exposures, are frequently severe, and account for most food allergy-related deaths. Researchers have been studying oral immunotherapy (OIT) as a method to gradually desensitize patients to their food allergen with the goal of reducing reactivity.

In a phase 3 peanut OIT clinical trial, peanut-allergic participants aged 4 to 55 years who reacted to ≤100 mg of peanut protein (approximately 1/3 of a peanut kernel) were enrolled. Participants were randomly assigned, in a 3:1 ratio, to receive AR101 (a peanut-derived biologic OIT drug) or placebo. Subjects received escalating doses of peanut protein up to 300 mg per day and stayed at this maintenance dose for approximately 24 weeks. Of the 496 subjects aged 4 to 17 years on active treatment, 77% tolerated 300 mg peanut protein at the end-of-study food challenge vs. 8% on placebo; 67.2% tolerated 600 mg vs. 4% on placebo; and 50% tolerated 1000 mg vs. 2% on placebo. During the food challenge, the maximum severity of symptoms was moderate in 25% of the participants on active treatment vs. 59% of those on placebo, and severe in 5% and 11%, respectively.

This trial showed that peanut OIT treatment with AR101 in children and adolescents who were highly allergic to peanut resulted in higher tolerated doses of peanut protein and in lower symptom severity at the end-of-study food challenge compared to placebo.

**IMPLICATION:** In 2020, the first ever food allergy treatment, Palforzia®, was approved by the FDA based on the peanut OIT Phase 3 trial done here by Dr. Leonard and at other centers around the world. This year the new Food Allergy Immunotherapy Clinic opened at Rady Children’s Hospital and this state-of-the-art food allergy treatment is now available to help protect patients and give families peace of mind. To assist other allergy centers and practices, Dr. Leonard authored a publication in the Journal of Allergy & Clinical Immunology on integrating OIT into clinical practice that will provide guidance for this new therapy for years to come.

**References:**


KIDNEY DEVELOPMENT AND DISEASE

Exploring acute, developmental and chronic mechanisms of kidney disease.
Treatments for Cachexia in Chronic Kidney Disease (CKD)

Cachexia/wasting is present in up to 50% of children with mild to moderate chronic kidney disease (CKD). The cachexia syndrome in CKD children consists of anorexia, reduced body and muscle mass, poor nutrition and short stature, the latter two features of which have been clearly associated with increased morbidity (hospitalization) and mortality risk.

Inflammammasomes are intracellular multi-protein complexes nucleated by innate immune signaling receptors such as NLRP3 (discovered by Dr. Hoffman, collaborator). NLRP3 inflammasome activation results in caspase-1 activation leading to production of IL-1β and IL-18 cytokines. The Mak lab hypothesized that the primary source of these cytokines is myeloid derived circulating cells via a NLRP3 dependent mechanism.

Using specific cytokine deficient mice and anakinra cells via a NLRP3 dependent mechanism.

IMPLICATION: There are a number of novel NLRP3 inhibitors available or becoming available. The Mak lab investigates whether these novel molecules will provide a novel unmet need for treatment of cachexia in CKD which is associated with high mortality and morbidity in CKD patients.

References:

Congenital anomalies of the kidney and urinary tract (CALKUT) are the most common cause of chronic kidney failure in children, and they can increase an individual’s risk for high blood pressure or urinary tract infections. The relatively high degree of familial aggregation of CALKUT suggests a significant genetic component, and disease-causing mutations have already been identified in at least 20 genes. Many of these genes have been implicated in early aspects of kidney development. Therefore, a comprehensive understanding of the genetic regulation of kidney development should assist in improving the diagnostic, prognostic and therapeutic options available for individuals with CALKUT. In addition, elucidation of the genes that regulate kidney development will be valuable for targeting stem cell and regenerative technologies to therapeutic uses for kidney disease.

Genetic Regulation of Early Kidney Development

Congenital anomalies of the kidney and urinary tract (CALKUT) are the most common cause of chronic kidney failure in children, and they can increase an individual’s risk for high blood pressure or urinary tract infections. The relatively high degree of familial aggregation of CALKUT suggests a significant genetic component, and disease-causing mutations have already been identified in at least 20 genes. Many of these genes have been implicated in early aspects of kidney development. Therefore, a comprehensive understanding of the genetic regulation of kidney development should assist in improving the diagnostic, prognostic and therapeutic options available for individuals with CALKUT. In addition, elucidation of the genes that regulate kidney development will be valuable for targeting stem cell and regenerative technologies to therapeutic uses for kidney disease.

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References:
About 3-5 percent of human proteins participate in handling drugs (e.g., ibuprofen, penicillin, antivirals, diuretics). Some years ago, Dr. Nigam and his team discovered the main kidney drug transporter, now called OAT1, as well as other members of a large family of transporters called SLC22. These are among the most important transporters of drugs in the kidney, liver, and other organs.

Drug transporters are abundant in flies, worms, fish and other organisms. Thus, it is likely that their main function is something other than transporting man-made pharmaceuticals. Dr. Nigam’s studies in knockout mice have begun to define “what drug transporters really do.” This has led to the formulation of the Remote Sensing and Signaling Theory, which proposes a central role for drug transporters in inter-organ (gut-liver-kidney) and inter-organismal (gut microbes-host) communication by small molecules. The Remote Sensing and Signaling Network consisting of over 500 genes, can be viewed as a homeostatic system working in parallel with the endocrine system.

One of the surprises from these omics studies of knockout mice is that OAT1 and OAT3 in the kidney are responsible for the systemic regulation of many gut microbe-derived metabolites and uremic toxins. The latter are thought to be responsible for the progression of chronic kidney disease (CKD). OAT1 and OAT3 also appear to play a key role in handling lipids, uric acid, and other molecules associated with metabolic syndrome.

**IMPLICATION:** Defining the pathways by which metabolites are transported during inter-organ communication can lead to the design of “metabolite-like drugs” that can target particular organs. Metabolic side effects of drugs can be diminished by considering whether drugs and metabolites compete with each other in the Remote Sensing and Signaling Network. Strategies to alter the expression or function of “drug” transporters may help slow down the progression of kidney disease and improve metabolic profiles in diabetes and gout.

**References:**
MEDICAL AND SURGICAL CARE RESEARCH

Implementing novel medical and surgical in-patient care research as well as quality improvement projects.
Severe bronchomalacia in infants is a complex and potentially life-threatening condition. Traditional approaches to treating the condition include prolonged mechanical ventilation and placement of a tracheostomy. Endobronchial stents have demonstrated a poor track record secondary inflammatory response, erosion into adjacent structures and occlusion. Through a well-developed collaboration between otolaryngology and cardiology, Dr. Brigger and Dr. Howaida El-Said have developed the novel application of principles of interventional cardiology into the pediatric airway. By identifying the shortcomings of traditional stents used in the airway, the team identified that the properties of specific vascular stents were better suited to the needs of the pediatric airway. In a subset of infants with severe bronchomalacia, soft corona stents provide the physical properties that overcome many of the shortcomings of traditional stents. In particular, the radial strength combined with flexibility overcomes the limitations that previously resulted in exuberant granulation tissue and erosion into adjacent structures. Furthermore, the team’s experience demonstrates the safety and ability to remove the stents up to 16 months after placement while maintaining a successful airway outcome secondary to airway remodeling a stiffening.

IMPLICATION: The ability to offer airway stenting to infants with severe bronchomalacia is an important adjunct to the management of critically ill children. The ability to provide relief of bronchomalacia allows such infants to sustain lower weight bearing and mobility. A number of common pediatric conditions including developmental dysplasia of the hip, Legg-Calvé-Perthes disease, slipped capital femoral epiphysis, and femoroacetabular impingement affect normal hip development and can lead to limited childhood function. Understanding the three-dimensional morphology of the acetabulum is critical for surgeons treating orthopedic hip conditions.

Dr. Upasani and his team recently developed a technique to quantify the shape and volume of the acetabulum. They published the technique in the Journal of Hip Preservation Surgery in 2020. The technique gives a clear and reproducible measurement of acetabular morphology that can be directly compared with a cohort of age- and sex-matched typically developing children. This analysis has been used at Rady Children’s Hospital – San Diego for the past 5 years when treating patients with pediatric hip disease and has allowed surgeons to better understand the unique pathology present in each child.

This technique also allows these findings to be shared with patients and their parents to demonstrate the magnitude and location of the clinical abnormality and guide surgical corrections that can best optimize the shape and function of each individual child’s hip joint. Dr. Upasani and his team have utilized this technique in a number of pediatric hip conditions.

IMPLICATION: The next step in advancing the surgical management of pediatric hip diseases will be to integrate this 3D understanding of hip deformity into the operating room. Technologies that allow navigation and robotic guidance may soon become the standard of care to improve the safety and accuracy of our surgical corrections. In the meantime, Dr. Upasani and his team will continue to work to better understand the development and mechanics of the hip joint.
Cerebral Palsy is the most common motor disability in children with a prevalence of 3–4 children per 1000 in the United States. While it is appreciated that this disorder is secondary to an insult to the developing brain, either in-utero or in the first 2 years of life, little was known about the end-organ: muscle. With a brain injury, there is often a lack of inhibitory neurotransmitters such as GABA to modulate the reflex action of the skeletal muscles of the body. This leads to spasticity with painful spasms and eventual muscle contractures.

Most treatments for cerebral palsy are not focused on the brain but on treating the muscles. This can be in the form of physical therapy with muscle stretching and strengthening, oral or intrathecal GABA agonists such as baclofen or diazepam, botulinum toxin injections or selective dorsal rhizotomies to diminish spasticity and orthopedic surgery to lengthen or transfer spastic and contracted muscles. However, little was known about muscle in children with cerebral palsy.

Working with one of the world’s leading muscle researchers, Richard Lieber, PhD, who until recently was the Vice-Chair of Research of the Orthopedic Department of UCSD and currently the Chief Scientific Officer at the Shirley Ryan Ability Center, Dr. Chambers and Dr. Lieber’s team conducted a series of studies to characterize muscle in children and young adults with cerebral palsy. Since he performs many surgeries on spastic and contracted muscle at Rady Children’s Hospital they were able to obtain muscle samples. One of the challenges of studying muscle in children and adolescents is that there is little access to control samples and it is difficult to obtain these samples. Fortunately, there is a very active sports medicine practice at Rady and Dr. Chambers was able to easily obtain muscle from adolescents in whom they were performing ACL reconstructions, especially from their hamstring muscles.

The findings from these studies were surprising in that the muscle shouldn’t have been that different from typically developing children as there is no “disease” of the muscle. However, a series of investigations demonstrated that there was a direct relationship between the severity of the cerebral palsy (ambulators vs nonambulators, for example) and a multitude of muscle properties. The muscle fibers were actually longer in those children with cerebral palsy instead of shorter making them less efficient and powerful, the stiffness of the muscle was found to be secondary to increased production of collagen surrounding the muscle bundles, there were fewer satellite cells (muscle stem cells) as the severity of the disorder increased, there were changes in the enzyme activity and, there were many genes which were up- or down-regulated in all areas of the cell.

**IMPLICATION:** This characterization of muscle and understanding of some of the pathways that spasticity affects muscle is leading to further discovery; investigating potential agents to increase satellite cell production or stem cell treatment, increase various components of muscular activity, modulate the regulation of cellular activity and small molecule research to perhaps decrease the influence the direct effect of spasticity on the muscle cell. The goal of this research is to minimize the effect of brain injury on our prime movers: our muscles.

**References:**


**Diagram:** Demonstrating the genes which were either up- or down-regulated in the muscle cell in a child with severe cerebral palsy. (Courtesy of Lucas Smith, PhD.)
Dr. Edmonds and his team sought to determine if the torsional growth could be modulated based on the angles of the tension band plating and whether or not oblique plating affected overall longitudinal growth. New Zealand White rabbits either underwent sham surgery that included screw placement only, or experimental surgery with obliquely placed unlocked plates, angled from 0 to 75 degrees, fixed to the bone with screws and spanning the physis. Radiographs were taken at biweekly intervals.

After 6 weeks of growth, hind limbs were harvested and microCT scans performed. Femoral length, distances between screw heads and angle between the plates were measured on radiographs. Femoral length differences were compared between groups. Femoral version was measured from 3D microCT. Plate angle changes were correlated to the difference in femoral version between limbs using Pearson correlation (significance was set to $P<0.05$ for all comparisons).

Femur length difference between the contralateral and the operative side was significantly greater in the plate group compared with the sham group over time ($P=0.049$). Medial and lateral screw distances changed significantly more in the sham group than the plate group on both sides ($P<0.001$). A greater initial angle between plates resulted in a greater change in the angle between plates ($P=0.001$). Significant correlations were found between right-left side femoral version differences and initial plate angle ($P=0.003$) and plate angle change ($P=0.014$).

The torsional effect of oblique plating seems to correlate with the amount of initial plate angle, with an additional, not negligible, longitudinal growth effect. Therefore, placing plates at given angles across open physes may result in predictable changes in bone torsion allowing for a safer and less invasive option when treating childhood rotational deformities, but the resulting shortening of the operative femur must be considered.

**Implication:** This study demonstrated that not only was this method feasible, but it showed that it could be done in a less invasive manner when treating rotational deformities in children. However, it was concluded that further study on an animal model was imperative before any human trials are attempted, because not only did shortening of the hind limb occur, but there were histologic changes to the orientation of the cartilage cells in the physis. Therefore, further study is warranted to examine the cellular effects on the physis and true 3D changes within the structure of the physis itself.

**Reference:**

**Using the Power of the Physis to Correct Pathologic Rotation in Long Bones of Children Via a Rabbit Model**

**Physis and Pathologic Rotation in Long Bones**

**ERIC EDMONDS, MD**
Professor
Orthopedic Surgery

**RESEARCH INTERESTS:** Youth sports medicine and musculoskeletal trauma.
High flow nasal cannula (HFNC) use for bronchiolitis is increasing on general wards. Little is known about outcomes or adverse events at different flow rates. The objective of this study was to examine the impact of increasing maximum flow rates on treatment failure, ICU transfer, and adverse events. Dr. Levy and his team conducted a retrospective chart review of patients age <24 months admitted to the general ward from March 2017-May 2020 with a first diagnosis of bronchiolitis treated with HFNC.

During the study period, the maximum allowable HFNC flow rate increased from 6 liters per minute to 12 liters per minute. Primary outcomes were incidence of treatment failure and hospital length of stay. Secondary outcomes were incidence of pressure ulcer, aspiration pneumonia, pneumothorax, and seven-day readmission. Findings showed that treatment failure decreased as maximum allowable flow rates increased. The effect was most pronounced in infants ≤6 kg for whom failure rates decreased from 41% at the beginning of the study period to 10% at the end. There was no increase in length of stay or incidence of adverse events.

**IMPLICATION:** Bronchiolitis is one of the most common reasons for pediatric hospital admission. This study shows that for young infants with bronchiolitis who weigh ≤6 kg, high flow nasal cannula is a safe treatment that can prevent ICU admission without increasing hospital length of stay or adverse events.

**Reference:**
It has been the observation of Dr. Scott Mubarak that a bony fullness or “double medial malleolus” over the middle facet is a consistent finding with most talocalcaneal coalitions. To document this observation, the Mubarak team reviewed records and radiographs in 3 patient groups. They found that talocalcaneal coalitions have a bony prominence below the medial malleolus on clinical exam and not present in flatfeet or other coalitions. This abnormal middle facet is almost twice the size of the normal middle facet. If a palpable bony prominence is noted just below the medial malleolus during examination of a painful foot with a decrease in subtalar motion, the likely diagnosis is talocalcaneal coalitions based on clinical examination. With this added clinical finding, appropriate images can be ordered to confirm the diagnosis of the latter. CT scans with 3D images for surgical planning is advised. The primary finding for tarsal coalitions in textbooks is decreased subtalar motion and this is an added finding.

**IMPLICATION:** This new finding of a palpable enlarged medial prominence just below the medial malleolus is highly associated with talocalcaneal coalitions and allows for more specific X-ray studies.

**Reference:**

**FIGURE 1**
Bony fullness or “double medial malleolus” over the middle facet is a consistent finding with most talocalcaneal coalitions.

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Prompt evaluation of a younger child (age <5) with gait disturbance or refusal to walk is necessary to distinguish orthopedic urgency (trauma or infection) from relatively benign processes, hip synovitis, and chronic problems such as arthritis. Physical examination should include evaluation of gait, supine and simulated prone hip examination (with the parent holding the child for comfort), and the crawl test.

In conjunction with the detailed history, a thorough physical exam and radiographs from the orthopedic provider can determine the need for laboratory tests and other imaging. Physical examination of the limping child can be optimized by utilizing the parent holding the child chest-to-chest while the examiner moves the extremities, especially hip internal rotation, and checks the back. Asymmetric hip internal rotation likely is indicative of hip joint pathology.

**IMPLICATION:** These new physical examination tips, prone hip examination and the crawl test, will focus the provider’s X-rays orders to the appropriate region of the lower extremities.

**Reference:**
This series of radiographs demonstrates the initial preoperative scoliosis of the thoracic spine (Pre) and subsequent correction following anterior spinal growth modulation with a tether. After anterior spinal tether placement (a radiolucent cord is connected between the vertebral body screws) there is modest correction seen in the "Post" image. The 1 and 2 year post-surgery images demonstrate the continued reduction of the scoliosis that has been driven by growth of the spine. The correction has been largely maintained at 3 and 5 years after surgery once growth was finished.

**TABLE 1**

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<th>Pre</th>
<th>Post</th>
<th>5 years</th>
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**FIGURE 1**

This series of clinical anteroposterior radiographs of medial epicondyle fractures demonstrates the three methods of fixation: cannulated screw (i), suture bridge (ii) and divergent Kirschner-wires (iii).

Adolescent idiopathic scoliosis (abnormal curvature of the spine) is common in teenagers and when severe may require surgical treatment. The gold standard method for surgically correcting scoliosis remains a spinal fusion procedure in which rods and screws are used to realign the spine. The spinal fusion is accomplished by adding bone graft thus yield the region of the spine treated immobile. An option that avoids spinal fusion thus allowing some motion through the corrected region of the spine has now reached a point of routine clinical application in patients.

A technique known as anterior spinal growth modulation, utilized a flexible cord attached to the spine that limits growth on the convex side of the curve while allowing growth on the concave side such that the curve resolves as the patient grows. This technique was first studied in Dr. Newton’s lab over 20 years ago in several animal models. In 2 recent publications, the clinical assessment was completed by retrospective and prospective multicenter research is underway through the Setting Scoliosis Straight - Harms Study Group trials. The best clinical paper award was given to this group and Dr. Newton at the 2021 Scoliosis Research Society’s International Meeting on Advanced Spine Techniques.

**IMPLICATION:** This method has the potential to reduce the need to perform spinal fusion in patients treated for scoliosis.

**References:**


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**KATHLEEN D. RICKERT, MD**

Assistant Professor Orthopedic Surgery

**RESEARCH INTERESTS:** Limb deficiency and deformity, trauma.

**TABLE 1**

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**FIGURE 1**

This series of clinical anteroposterior radiographs of medial epicondyle fractures demonstrates the three methods of fixation: cannulated screw (i), suture bridge (ii) and divergent Kirschner-wires (iii).

Medical epicondyle fractures are common fractures that occur about the elbow in children. This fracture is typically an avulsion of the medial epicondyle with the attachment of the common flexors of the forearm and through its apophysis from the medial distal humerus. The most common method of fixation for displaced medial epicondyle fractures is with the use of a screw. However, younger patients have a smaller fracture fragment that may not be able to accommodate a screw due to their size and minimal ossification. Younger patients with displaced medial epicondyle fractures are treated with Kirschner wires (K-wires) or suture anchors as alternative options.

The three fixation methods were evaluated for their biomechanical properties, clinical outcomes, cost and complications for patients 10 years of age or younger. The biomechanical assessment was performed using immature pig forelimbs. An osteotomy was created through the medial epicondyle apophysis to simulate a fracture that were fixed with each of the fixation methods: screw, K-wires or suture anchors. The biomechanical properties were tested using an MTS machine evaluating for: cyclic elongation (mm), displacement (mm), load to failure (N), and stiffness (N/mm). Biomechanically, screw fixation proved to be stronger (P=0.047) and stiffer (P<0.01) than the K-wire or suture anchor constructs. Screw, K-wire and suture anchor fixation were all found to be acceptable methods for displaced medial epicondyle fractures as the forces used to test the constructs exceeded the forces observed across the elbow joint with typical range of motion.

The clinical assessment was completed by retrospective review of patients 10 years of age or younger with a medial epicondyle fracture fixed with these three strategies. The complications, radiographic and postoperative outcomes were compared and were to complete a cost-analysis of each fixation method. All (100%) of the 51 fractures reviewed healed independent of the fixation method. K-wires were used as fixation for younger patients (p<0.05). Screw fixation was found to have the shortest duration of casting (3 weeks, p<0.02). All three groups had similar clinical outcomes and complications although a second surgery for implant removal was less likely in the suture anchor group (p<0.05). The decreased incidence of reoperation rate translated to a 10% cost-savings for the suture anchor group.

**IMPLICATION:** All three methods of fixation are biomechanically and clinically appropriate for fixation of displaced medial epicondyle fractures in children. Screw fixation provides the strongest, stiffest construct with the shortest duration of immobilization but the added cost of frequent reoperation due to symptomatic implants. K-wires and suture anchors are a suitable alternative clinically and biomechanically to screws for small or comminuted displaced medial epicondyle fractures in children.

**References:**


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An increasing number of surgical strabismus (eye misalignment) patients are taking oral anticoagulant and antiplatelet agents, with more diverse mechanisms of action than those used in the past. The decision as to whether to continue these drugs throughout the perioperative period is difficult and must be based on the balance between hemorrhagic and thrombotic risk.

To help guide strabismus surgeons with clinical management in these cases, Dr. Robbins and her team reviewed the literature for potential hemorrhagic complications of strabismus surgery and examine the use of anticoagulant and antiplatelet drugs during the perioperative period. (See Fig 1). Surgical strategies that might help minimize intraoperative hemorrhage in patients on anticoagulant therapy are also discussed.

**IMPLICATION:** Bleeding is an issue all surgeons face while trying to cure disease in the operating room. This research takes a deep dive into how to reduce bleeding and thus decrease scarring in delicate eye muscle surgery, especially when patients are on anti-coagulants for other systemic disease.


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Retinopathy of Prematurity Guidelines for Diagnostic Examination

Retinopathy of Prematurity (ROP) is a pathologic process that occurs in immature retinal tissue and can progress to a tractional retinal detachment, which may then result in visual loss or blindness. For more than 3 decades, treatment of severe ROP that markedly decreases the incidence of this poor visual outcome has been available. However, severe, treatment-requiring ROP must be diagnosed in a timely fashion to be treated effectively. The sequential nature of ROP requires that infants who are at-risk and preterm be examined at proper times and intervals to detect the changes of ROP before they become destructive. This statement presents the attributes of an effective program to detect and treat ROP, including the timing of initial and follow-up examinations.

**IMPLICATION:** This major policy statement provides screening guidelines for diagnosis representing ALL the major medical organizations dealing with the potentially blinding premature infant eye disease of the retina. Most countries worldwide utilize this research as a basis for their own country’s guidelines.


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**FIGURE 1**

Dr. Shira Robbins examining a patient after eye re-alignment surgery using an ocular microscope.

**FIGURE 1**

An infant in the UC San Diego neonatal intensive care unit who required eye examinations to screen for the blinding disease of Retinopathy of Prematurity (Photography credit: Peter Durdaller).
Perforated appendicitis is a well-documented child health disparity. Geographic patterns in perforated appendicitis exist in several United States regions, but such patterns had not yet been described in California.

Drs. Schwartz and Nguyen aimed to analyze spatial-temporal patterns of pediatric perforated appendicitis and identify population characteristics contributing to these cluster patterns. Risk-adjusted perforated appendicitis rates per 1000 appendicitis cases in patients 1-17 years from 2005-2015 in California were geocoded and a space-time cube analysis was performed to identify hot spot trends. Drs. Schwartz and Nguyen performed logistic regression to estimate rural classification associated with spatial-temporal hot spots and multivariate analysis to assess effects of socioeconomic factors.

In 2005-2015, 43,888 cases of pediatric perforated appendicitis occurred in California. The median risk-adjusted perforated appendicitis rate was 312 per 1000 appendicitis cases. Eleven spatial-temporal hot spots of perforated appendicitis were identified (see Figure 1). Rural micropolitan counties had 14 times higher odds of being classified as a hot spot ($p<0.05$, 95% CI 1-185). Poverty was a significant predictor of high perforated appendicitis median risk-adjusted rate ($p<0.004$).

**IMPLICATION:** This work highlights the importance of geographic location in pediatric health. Using space-time analysis, we gained insight on how rural living and poverty might impact pediatric appendicitis outcomes. Incorporating the role of geography alongside an understanding of socioeconomic factors is a critical step in addressing this important child health disparity.

**Reference:**
Novel Electronic Glove to Enhance Muscle Hypertonicity Assessments

Spasticity is a motor disorder characterized by an increase in muscle tone during movement. A variety of debilitating clinical conditions, including stroke, multiple sclerosis, head trauma, spinal cord trauma, and cerebral palsy can cause hypertonicity. Untreated symptoms can become permanently disabling, causing severe bone/joint deformities and reduction in joint biomechanical range of motion. The evaluation of spasticity severity level is a critical step in selecting appropriate treatments for patients. The lack of an objective measurement limits understanding and advancements in this area.

Dr. Skalsky and a team of engineers at the UCSD Jacobs School of Engineering designed an instrumented glove system to measure the severity of hypertonicity in patient’s spastic limbs. This device introduces the use of frequency modulation (FM) and frequency division multiplexing (FDM) to improve multi-sensor signal acquisition with collocated sensors. As illustrated below, the spastic muscles were measured when the patient was i) fully awake, ii) being induced under anesthesia, iii) under full anesthesia. Anesthesia greatly reduces spasticity. The electronic glove system measured a significant increase in the force applied while awake versus under anesthesia indicating increased resistance in the patient’s muscle. This demonstrates that the glove system can differentiate measurements of physical attributes related to normal and spastic states of the muscle groups involved.

**IMPLICATION:** This novel approach by using a sensor-filled glove enables a high interrater reliability and removes the observer bias that can stem from subjective assessments. This should result in more sensitive and objective spasticity assessments that can be done by clinicians, caregivers or researchers with high-caliber reproducible data. Such a discovery of an objective measurement can dramatically improve the accuracy in tracking patient’s response to therapy to likely reveal fine-level changes that are not currently observable and lessen the healthcare burden cost by imprecise medication adjustments based on subjective models. Dr. Skalsky’s team envisions this revolutionary quantitative tool to help in enhancing feasibility of studies that can bring more robust and valuable advancements to the field and most importantly to improve patient’s quality of life with an objective outcome measures in clinical research looking at spasticity and its treatment.

**References:**


**Children Undergoing Outpatient Complex Penile Surgery, Hypospadias Repair May Not Require Opioid Analgesics**

Pain control is important after penile surgery. Non-opioid pain management is a common postoperative strategy after penile surgery, but literature describing this is sparse. Dr. Swords and her team sought to describe their experience performing complex penile surgery with vs without post-operative opioids.

A retrospective review was performed of the institution’s penile surgeries performed by four current attending surgeons, including 3998 surgeries between 2009-2019. Patients identified were <8 years of age who underwent outpatient penile surgery requiring either penile degloving for correction of an anomaly or any variation of a hypospadias repair. One provider was compared to the others to associate the practice of prescribing no opioids vs routine prescription. Patients were matched 1:1 by age and type of penile surgery. Baseline features and post-operative outcomes were examined. The primary outcome of interest was post-op encounters related to pain, delayed opioid prescription, and predictors of pain complaint on multivariate analysis.

200 children were identified, 100 per group, matched with both groups’ mean age 1.3±0.8 years. 48% of each group were penile degloving procedures, 31% hypospadias repairs with tubulization/catheter placement, and the remaining 21% were hypospadias repairs without catheter placement. Patients receiving opioids received a mean 12.5 doses. OR time, concomitant surgery, caudal use, and 30-day complications were comparable between groups (P>0.05). 59%(39%) of patients without opioids had an impromptu post-operative encounter vs 41% of those prescribed opioids, and 20% had an associated pain complaint vs 9% (p=0.006). 2 patients in both the non-opioid and opioid groups received a delayed opioid prescription(n=1,00), as most pain complaints were managed with non-opioid strategies. Assessing all baseline and perioperative features, it was found that the presence of a catheter (OR 2.9) and no opioid prescription (OR 2.6) were independent predictors for post-operative pain complaint.

Patients discharged without an opioid were more likely to contact a provider postoperatively, and were more likely to endorse a concern of pain, but rarely required a delayed opioid prescription as most patients found relief with non-opioid measures.

**IMPLICATION:** As the opioid crisis continues to progress, it is vital to scientifically examine the need for post-operative opioid analgesics and reduce or discontinue their use when able. This study demonstrates the decreased need for opioids in a complex penile surgery, which was previously thought to require regular opioid prescription. Reducing exposure in our children has been shown to reduce later in life opioid use.

**References:**
Optimizing Anticoagulation for Children with Blood Clots

Blood clots in children, including deep vein thrombosis and pulmonary embolism result in significant morbidity and mortality and anticoagulants that have been used to date require frequent injections and/or lab monitoring. Direct oral anticoagulants (DOACs) do not require injections or lab monitoring.

Dr. Thornburg participated in a think tank to consider the optimal use in children and is evaluating the DOACs in children with blood clots. She was the principal investigator at Rady Children’s Hospital San Diego for an international randomized open label phase three clinical trial of rivaroxaban versus standard of care anticoagulation in children, 0-17 years of age, with venous thromboembolism, Einstein-Jr. This was the largest study of a blood thinner in children (N= ~500). Three hundred twenty-nine received at least one dose of dose-adjusted rivaroxaban, in tablets or suspension, and one hundred sixty-two received at least one dose of standard of care anticoagulation. Symptomatic recurrence of venous thromboembolism occurred in 1% of those who received rivaroxaban and 3% who received standard anticoagulation. On repeat imaging of the thrombus, rivaroxaban was associated with a decrease in thrombus burden compared to standard of care anticoagulation. Major bleeding was rare.

Overall, the results of the study show that the medication is safe and effective in children, and the results will likely lead to FDA approval of the medication for children and adolescents. The new drug application for this indication was submitted in June 2021. Dr. Thornburg and pediatric colleagues in the Venus Thromboembolism Network US (VENUS) are collecting real-world safety and effectiveness data on use of DOACs in children.

IMPLICATION: This work should lead to more effective and less burdensome treatment for children with blood clots.

References:

Towards a Better Understanding of Intraventricular Hemorrhage in Premature Infants

Dr. Thornburg collaborated with investigators in the Eunice Kennedy Shriver National Institute of Children Health and Human Development Neonatal Research Network to evaluate genetic predictors of intraventricular hemorrhage (IVH) in extremely low birthweight infants.

The multicenter study included 899 infants. Of these, 139 had grade III-IV IVH, 73 had grade II IVH, and 687 had grade 0 or I IVH. Candidate gene analysis, including 1279 single nucleotide polymorphisms involved with coagulation, angiogenesis, inflammation, and organ development, found that genetic variants for central nervous system neuronal and neurovascular development may be associated with severe intraventricular hemorrhage in premature infants. Genes of interest with SNPs with significant associations included NAA15, IGF1R, and NOS2. Further validation studies are indicated.

IMPLICATION: Intraventricular hemorrhage is associated with significant morbidity and mortality in this vulnerable population and better understanding of the causes may lead to preventive strategies.

Reference:
ORGANOIDs, MUTATIONS AND EARLY NEUROLOGICAL HUMAN DISEASE

Utilizing novel technologies to understand early brain and lung development and disease pathogenesis.
Genetic forms of pediatric brain disease represent many individually rare conditions. Investigators in Dr. Gleeson’s lab identified a new pleiotropic human multisystem condition caused by deficient Wnt secretion. Wnts are secreted proteins involved in cell fate decisions. Patients missing the WLS gene showed microcephaly, defects in heart, kidney and hair. Patient cells showed global Wnt attenuation. Defects observed in mouse models could be prevented with a drug administered during pregnancy to boost Wnt levels. This exciting work suggests some structural birth defects might be preventable if caught early.

Dr. Gleeson and his team have also been studying ways to prevent pediatric brain disease by preventing mutations in the first place. In previous work, Dr. Gleeson reported that these mutations likely arise when the father was an embryo.

**IMPLICATION:** These clonal sperm mosaic mutations are incredibly stable over time, and predict that carrier screening could reduce the burden of disease in children.

References:


Microtubules are integral to the normal function of healthy cells. In the nervous system, neurons rely on the microtubule cytoskeleton to form the synaptic connections where information is transferred from a neuron to its target. Microtubule growth and stability must be carefully balanced for proper synaptic development.

In their studies, Dr. Wildonger and her team uncovered a new mechanism that regulates microtubule growth. They found that an enzyme called α-tubulin acetyltransferase (αTAT) has a surprising enzyme-independent role in restraining microtubule dynamics in neurons. Loss of αTAT results in the overgrowth of synaptic connections during development. The new model generated from this work is that non-enzymatic αTAT activity regulates the remodeling of microtubule networks to generate proper synaptic connections.

**IMPLICATION:** Neurological disease impacts the health of nearly 100 million Americans yearly, making it essential to increase our understanding of how neurons achieve their proper shape and function. Many neurodevelopmental and degenerative diseases arise from perturbing the microtubule cytoskeleton. Through these studies, Dr. Wildonger and her team have uncovered a new mechanism that regulates the balance of stable and dynamic microtubules at synapses; in the future, this knowledge may be leveraged to advance innovative treatments of diseases associated with microtubule dysfunction.

References:
Prenatal opioids exposure can lead to both neonatal abstinence syndrome in newborns and neurological deficits later in life. Although opioids have been well studied in general, the cellular and molecular mechanisms by which opioids affect human fetal brain development has not been well understood.

In this work, we have taken advantage of a human 3D-brain cortical organoid (hCO) that facilitated enormously the investigation of early human brain development. Using imaging, immunofluorescence, multi-electrode array (MEA) and patch clamp recording techniques, they have investigated the effect of methadone, a frequently used opioid during pregnancy, on early neural development, including neuronal growth, neural network activity and synaptic transmission in hCOs.

Their results demonstrated that methadone dose-dependently halted the growth of hCOs and induced organoid disintegration after a prolonged exposure. In addition, methadone dose-dependently suppressed the firing of spontaneous action potentials in hCOs and this suppression could be reversed upon methadone withdrawal in hCOs treated with lower dosages. Further investigation using patch clamp whole cell configuration revealed that, at clinically relevant concentrations, methadone decreased the frequency and amplitude of excitatory postsynaptic currents in neurons, indicating a critical role of methadone in weakening synaptic transmission in neural networks in hCOs. In addition, methadone significantly attenuated the voltage-dependent Na+ current in hCOs. They concluded that methadone interrupts neural growth and function in early brain development.

**IMPLICATION:** Understanding the deleterious effect of opioids on fetal brain development will be critical for better treatment of mother and fetus when pregnant women are exposed (whether for treatment or abuse) to methadone or other opioid forms.

**Reference:**
**“Archealization” of Human Brain Organoids**

Ancient DNA sequencing from Neanderthals and Denisovan’s bones has opened the door for comparative paleogenomics, providing the genetic information of our closest relatives. Most of the comparative genetics between archaic and modern humans has been on introgressed sites, genome regions where modern humans have adopted archaic elements via ancestral interbreeding and admixture.

Dr. Muotri and his team performed a comprehensive analysis of genetic variation available from diverse modern human populations, revealing that only 61 non-synonymous, derived coding variants are fixed or nearly fixed in extant humans and human-specific. Of these candidates, the Neuro-Oncological Ventral Antigen 1 (NOVA1) is a disease-related RNA binding protein that contains a nearly fixed, derived non-synonymous change in humans, the modern human NOVA1 was replaced with the ancestral allele in pluripotent stem cells. The stem cells were then differentiated into functional cortical organoids. The "archealization" of NOVA1 in brain organoids led to early exoctic synaptic maturation. Neurodevelopmental differences between species are essential components in evolutionary studies, as small changes in the timing of development can often turn into functional implications.

**IMPLICATION:** The systematic understanding of human brain evolution using this novel "archealization" strategy might change our ability to understand and treat human-specific neurological disorders. Our unique cognitive capabilities likely came as evolutionary trade-offs. Thus, just as understanding the evolutionary history of bipedalism has contextualized the hernias, hemorroids, varicose veins, spine disorders, osteoarthritis, uterine prolapse, and difficult childbirth experienced by humans today, so too will understanding the evolutionary history of brain development illuminate conditions, such as autism, schizophrenia, speech and language disorders, learning disabilities among others. These findings could provide a leap in our further understanding of human brain evolution and the treatments of modern neurological conditions.

**References:**


**FIGURE 1**

Gene editing technology was used to replace the NOVA1 gene in human cells by the archaic genetic variant. Brain cortical organoids carrying the Neanderthal version of the gene was created and used to compare.

**COVID-19, the disease caused by the pandemic coronavirus SARS-CoV-2, is primarily regarded as a respiratory infection. Yet the virus has also become known for affecting other parts of the body in ways not as well understood, sometimes with longer-term consequences such as heart arrhythmia, fatigue and "brain fog."**

The Rana lab uses stem cell-derived organoids — small balls of human cells that look and act like mini-organs in a laboratory dish — to study how the virus interacts with different organ systems and develop therapies to block infection. They have found that SARS-CoV-2 doesn’t infect the entire body in the same way because in different cell types, the virus triggers the expression of different genes, leading to different outcomes. Like many organs, lung and brain organoids produce the molecules ACE2 and TMPRSS2, which sit like doorknobs on the outer surfaces of cells. SARS-CoV-2 grabs these doorknobs with its spike protein, as a means to enter cells and establish infection.

**IMPLICATION:** These findings may help explain the wide variety in COVID-19 symptoms and aid the search for therapies.

**References:**


**Human Lung and Brain Organoids Respond Differently to SARS-CoV-2 Infection in Lab Tests**

Dr. Rana and his team developed a pseudovirus — a noninfectious version of SARS-CoV-2 — and labeled it with green fluorescent protein, or GFP, and bright molecule derived from jellyfish that helps researchers visualize the inner workings of cells. The fluorescent label allowed them to quantify the binding of the virus’ spike protein to ACE2 receptors in human lung and brain organoids, and evaluate the cells’ responses. They generated human iPSCs-derived lung organoids (LORGs), cerebral organoids (CORGs), neural progenitor cells (NPCs), neurons and astrocytes. LORGs containing epithelial cells, alveolar type I and type 2, highly express ACE2 and TMPRSS2 and are permissive to SARS-CoV-2 infection. Surprisingly, they observed 10-fold more ACE2 and TMPRSS2 receptors and correspondingly much higher viral infection in lung organoids, as compared to brain organoids. SARS-CoV-2 infection induces IFNs, cytokines and chemokines and activates critical inflammasome pathway genes. Infection in neuronal cells activates TLR3, TLR7, OAS2, complement system and apoptotic genes. Further, treatment with viral spike protein or TMPRSS2 inhibitors reduced infection levels in both.

**IMPLICATION:** These findings may help explain the wide variety in COVID-19 symptoms and aid the search for therapies.
Sleep Apnea, Lungs and Oxygen Biology

Investigating the various components of the respiratory system.
Sleep disorders are highly prevalent amongst children. Although the precise function of sleep in humans remains unclear, there are several studies that have associated sleep deprivation and/or disorders sleep to abnormal physical and mental health as well as child development. Sleep disordered breathing, including obstructive sleep apnea (OSA), is a highly prevalent condition affecting 2-10% of all children. OSA manifests as recurrent upper airway obstruction during sleep which culminates in cortical arousals and/or aberrations in gas exchange including episodic oxygen desaturations. In children, OSA is associated with neurocognitive dysfunction, inattentiveness, hyperactivity, school problems, depressed mood and reduced quality of life.

Recent evidence has also emerged that implicates OSA and other complications related to pediatric OSA. Other diseases are among the leading causes of global death and disability. Surprisingly, a great deal about the cellular heterogeneity of the lungs and the function of different cell types remain unknown. Recent review papers have characterized OSA, or other conditions, in children, or adult patients using single cell transcriptomic and epigenomic datasets to identify pathways amenable to therapeutic intervention. In the lung, single cell epigenomics and computational biology have demonstrated that endothelial specific microRNA (miR92a) is associated with OSA severity in both children and adults, and may represent a novel biomarker of OSA mediated CVD.

**References:**


**RESEARCH INTERESTS:** Obstructive sleep apnea, endothelial dysfunction, and pediatric sleep.

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**Organizations:**

- UC San Diego & Rady Children’s Hospital
- Translating Science into Cures for Children
In the US, asthma affects 8% adults and 7% children. Despite advancement in treatment options such as biologics, effective therapy remains elusive for many patients. Contributing to this clinical limitation is incomplete knowledge of asthma etiology, especially on how allergen is sensed by the lung, leading to immune and lung physiological responses.

Investigators in the Sun lab identified a rare cell airway cell type called pulmonary neuroendocrine cells (PNECs). These are unusual airway surface cells that had a dually characteristics of epithelium and neural cells. They are strategically enriched at airway branchpoints where inhaled particles carrying signals such as allergen are enriched. In response to allergen, PNECs are activated to secrete neuropeptides and neurotransmitters. These potent bioactive molecules signal to underlying resident immune cells such as innate lymphoid group 2 cells (ILC2s) to trigger their production of Th2 cytokines such as IL5. This leads to a cascade of recruitment of Th2 cells and eosinophils, key immune cells of the allergen-triggered immune response. The Sun lab team found that blocking PNECs or their secretion of neuropeptides dampened allergen response, with the highest effectiveness in early postnatal stage.

**IMPLICATION:** These landmark findings suggest PNECs and the neuropeptides that they produce as novel therapeutic targets for the treatment of asthma.

**Reference:**

Inhaled corticosteroids (ICS) are key treatments for controlling asthma and preventing asthma attacks. However, the responsiveness to ICS varies greatly among individuals. MicroRNAs (miRNAs) have been lauded for their prognostic utility. We hypothesized that circulating miRNAs obtained at baseline/prerandomization in the Childhood Asthma Management Program (CAMP) could serve as biomarkers and biologic mediators of ICS clinical response over the 4-year clinical trial period.

Dr. Tantisira and his team selected baseline serum samples from 462 CAMP subjects subsequently randomized to either ICS (budesonide) or placebo. Samples underwent small RNA sequencing, and read counts were normalized and filtered by depth and coverage. Linear regression was used to associate miRNAs with change in FEV1% (prebronchodilator FEV1 as a percent predicted) over the 4-year treatment period in both main effects and interaction models. They validated the function of the top associated miRNAs by luciferase reporter assays of glucocorticoid-mediated transrepression and predicted response to ICS through logistic regression models.

Seven miRNAs were identified significantly associated with FEV1% change (P ≤ 0.05) and 15 miRNAs with significant interaction (P ≤ 0.05) to ICS versus placebo treatments. Three miRNAs were designated for functional validation, of which hsa-miR-155-5p and hsa-miR-532-5p were significantly associated with changes in dexamethasone-induced transrepression of NF-κB. Combined, these two miRNAs were predictive of ICS response over the course of the clinical trial, with an area under the receiver operating characteristic curve of 0.86.

**IMPLICATION:** Two functional circulating miRNAs were identified as predictive of asthma ICS treatment response over time. Since these miRNAs were also associated with cellular response of the glucocorticoid receptor, they may also represent therapeutic targets. Dr. Tantisira’s lab is pursuing similar prognostic models using miRNAs and other genomic platforms, and has recently published a long term prognostic model related to which children will undergo asthma remissions by adulthood (PMID: 32888944).

**Reference:**

**FIGURE 1**
ROC Curve for Logistic Regression

Two miRNA prediction model. Baseline miR-155-5p and miR-532-5p can predict subsequent 4 year asthma response to ICS medications with an AUC of 0.86 (good to excellent prediction).

**FIGURE 2**
Luciferase activity assay

miRNA effects on glucocorticoid signaling. Compared with controls, miR-155-5p and miR-532-5p mimics decrease and increase, respectively, corticosteroid mediated transrepression.
STEM CELL DIFFERENTIATION AND DISEASE

Exploring the use of gene therapy to treat diseases of childhood.
Cystinosis is a lysosomal storage disorder characterized by the accumulation of cystine within the lysosomes of all organs, and caused by mutations in the CTNS gene encoding the transmembrane lysosomal cystine transporter, cystinosin. Major complications of cystinosis include early renal Fanconi syndrome, kidney failure, photophobia, hypothyroidism, myopathy and neurological defects. Cysteamine, the FDA-approved drug to treat cystinosis, only delay the progression of the disease.

Dr Cherqui and her team developed an autologous transplantation approach for cystinosis of CD34+ HSPCs modified ex vivo using a Self-Inactivated-lentiviral vector to introduce a functional version of the CTNS cDNA (drug product name: CTNS-RD-04). Starting with the preclinical studies [1], they translated this project to the clinic by performing the work necessary for an investigational new drug application (IND) and received FDA-approval for a phase I/II clinical trial in December 19, 2018. They started the clinical trial at UC San Diego in July 2019 to evaluate the safety and efficacy of CTNS-RD-04. Three patients have been infused so far. Preliminary data are encouraging as white blood cell cystine and tissue cystine crystals have been shown to be decreased in the treated patients.

The extent of efficacy of HSPCs to rescue cystinosis was surprising especially considering that cystinosin is a transmembrane lysosomal protein. Dr. Cherqui and her team demonstrated that the mechanism of action involved the differentiation of HSPCs into macrophages that transferred cystinosin-bearing lysosomes via tunneling nanotubes [2]. Their study is the first demonstration of cross-correction in the context of a lysosomal transmembrane protein after HSPC transplantation suggesting that HSPC gene therapy strategy could be applied to other lysosomal disorders. They confirmed this hypothesis in Danon disease, a lysosomal disorder due to the dysfunction of the Lysosomal Associated Membrane Protein type-2 (LAMP-2), leading to heart failure.

**IMPLICATION:** This project demonstrates that hematopoietic stem and progenitor cell (HSPC) transplantation could reverse progressive tissue injury in multiple compartments, even in the context of an intracellular membrane protein as opposed to secreted enzymes. This led to the first-in-human HSPC gene therapy clinical trial for cystinosis, and the underlying mechanism of action is expected to have implications beyond cystinosis, with potential applications to other genetic diseases with systemic defects and transmembrane organelle proteins.

**References:**
Friedreich’s ataxia (FRDA) is an autosomal recessive disease caused by the expansion of GAA repeats in the intron 1 of the frataxin gene (FXN). Frataxin is a mitochondrial iron-binding protein. FRDA is a progressive, neurodegenerative disorder, often associated with cardiomyopathy and diabetes mellitus. FRDA’s pathogenesis is primarily caused by degeneration of the sensory neurons within dorsal root ganglia (DRG). Ultimately patients require the use of a wheelchair for mobility and often die from the cardiomyopathy. There is no effective treatment for FRDA.

Because Dr. Cherqui and her team showed that HSPC transplantation could rescue multi-organ failure in cystinosis via the differentiation of HSPC into macrophages that could transfer organelles such as lysosomes and mitochondria to the diseased cells via tunneling nanotubes, they tested HSPC transplantation FRDA in the mouse model. It was found for the first time that transplantation of wildtype HSPC led to the complete rescue of the neurologic and muscular complications of FRDA [1]. Degeneration of large sensory neurons in the DRGs was prevented as well as the mitochondrial dysfunction in brain and muscle.

For future clinical application, the goal is to develop an ex vivo gene-corrected autologous HSPC transplantation for FRDA. The Cherqui lab thus optimized a targeted genome editing approach using CRISPR/Cas9 in patients’ CD34+ HSPCs to remove the hyper-expansion mutation leading to restoration of frataxin expression and mitochondrial function [2]. This represents the manufacturing feasibility of the human product, which is the first step towards a clinical translation of this strategy for FRDA.

The Cherqui lab also demonstrated that the mechanism underlying the rescue of FRDA by HSPCs involved the differentiation of HSPCs into microglial cells in the brain and spinal cord, and macrophages in DRGs, heart and skeletal muscle leading to the transfer of frataxin mitochondrial protein to the diseased neurons and myocytes. They are now investigating in vitro and in vivo if the entire the mitochondria are transferred in this context. These studies will be particularly interesting for the nervous system for which intercellular communication and mitochondrial exchange in the complex neuronal/microglia network might be demonstrated.

**IMPLICATION:** These results represent the first demonstration of complete phenotypic rescue of Friedreich’s ataxia following HSPC transplantation, and the development of a gene-corrected HSPC approach using CRISPR/Cas9 technology for future clinical application. These results also suggest that mitochondrial cross-correction from HSPC-derived phagocytic cells may be the mechanism of rescue, opening new perspectives in the treatment of Friedreich’s ataxia and other mitochondrial diseases.

**Refereces:**


**CRISPR/Cas9 Gene Edited-Hematopoietic Stem Cell transplantation for Friedreich’s Ataxia**

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**RESEARCH INTERESTS:** Development of hematopoietic stem cell and gene therapy-based therapeutic approaches for multi-systemic genetic disorders, and understanding of the mechanism of action for hematopoietic stem cell-mediated tissue repair.
The Use of Gene Therapy to Reverse a Fatal Newborn Lung Disease in Mini Lungs

Surfactant protein B deficiency results in respiratory distress syndrome in the newborn. The lungs are unable to exchange gas and the newborns are ventilator dependent and die unless they get a lung transplantation. This fatal disease is a result of a simple frameshift mutation which can be altered through gene therapy.

The objective of Dr. Leibel’s study was to use induced pluripotent stem cells (iPSC) that were reprogrammed from fibroblasts of a patient with surfactant protein B (SFTPB) deficiency and study the gene and protein expression changes throughout the stages of endoderm and early lung development using a novel 3D organoid differentiation protocols. Data analysis demonstrated that this mutation impacted the expression of key proximal and distal lung epithelial cells compared to the wild type iPSC derived lung organoids. Dr. Leibel then transduced the mutant iPSCs with a lentivirus carrying the wild type SFTPB gene and showed wild type expression of proximal and distal surfactant associated genes, proteins and lamellar bodies and rescued functional surfactant secretion.

Dr Leibel was one of the first to show the phenomenon of SFTPB surfactant secretion. Despite robust SFTPB gene expression protein expression only in the 3D lung organoid, and not in the newborns are ventilator dependent and die unless they get a lung transplantation. This fatal disease is a result of a simple frameshift mutation which can be altered through gene therapy.

The focus for Dr. Polk and his team for three decades has been to better understand intestinal development and repair processes that could translate to new therapies and prevention strategies for ulcerative colitis and Crohn’s disease, the two most common inflammatory bowel diseases (IBD). These are chronic diseases characterized by intestinal inflammation, ulceration and the progression of intestinal injury from incomplete or abnormal repair. Mouse models have provided important clues about the genetics, immunology, intestinal microbiome and the environment in the causes and potential prevention and treatment approaches. However, very little is known about the cells contributing to ulcer repair in these diseases.

In a recent publication, featured by the journal, Dr. Polk and his team compared a variety of IBD models for their stem cells involved in repair. Their findings advance the field in several ways. First, this is the first comparison of stem cell lineages in repair of different models of colitis. Second, they identified novel inflammatory expression patterns across these models of colitis and lastly, these findings show distinct patterns of stem cell induction in response to different causes of colitis. This work advances the field by defining new targets for cellular therapy in repair of intestinal ulceration seen in IBD.

Intestinal stem cells responsible for day-to-day maintenance on the lining of the intestine express the marker Lgr5. Based on prior work it was thought that these cells were temporarily lost during colitis. Using four different models of colitis in mice, Dr. Polk and his team showed that only in one of those models were the stem cells completely lost, but not the other three. Using a novel approach to address the importance of Lgr5+ cells in repair they deleted these cells using diptheria toxin and showed that during chronic inflammation they limit the amount of inflammation and injury but had no effect on limiting acute ulceration, which was repaired by a cell population previously described during intestinal development but thought to be absent in adolescent and adult animals. Thus, the Lgr5+ stem cell is differently lost during different causes of colitis and shows previously unknown regulation of inflammation during chronic colitis, that is seen in human IBD. The next steps for these studies will be to understand why the different choice of stem cells is necessary and how to expand them during colitis as a novel therapy.

**Legend:** The schematic drawing shows normal renewal during homeostasis compared to different forms of intestinal injury. These findings show fetal-like cells and classical homestatic stem cells (Lgr5+) are distinctly important in repair and in limiting injury, respectively.
A comprehensive understanding of mechanisms that underlie the development and function of human cells requires human cell models. For the pancreatic lineage, protocols have been developed to differentiate human pluripotent stem cells (hPSCs) into pancreatic endocrine and exocrine cells through intermediates resembling in vivo development. In recent years, this differentiation system has been employed to decipher mechanisms of pancreatic development, congenital defects of the pancreas, as well as genetic forms of diabetes and exocrine diseases.

Dr. Sander and her team summarized recent insights gained from studies of pancreatic hPSC models. They discuss how genome-scale analyses of the differentiation system have helped elucidate roles of chromatin state, transcription factors, and noncoding RNAs in pancreatic development and how the analysis of cells with disease-relevant mutations has provided insight into the molecular underpinnings of genetically determined diseases of the pancreas.

**IMPLICATION:** This study provides an outlook to future applications of stem cell models for providing insight into disease mechanisms of diabetes and facilitating a precision medicine approach to treat type 1 and type 2 diabetes.

**Reference:**