DEAR FRIENDS AND COLLEAGUES

It is my honor and privilege to present to you the 2016 Scientific Discoveries report from UC San Diego (UCSD), Department of Pediatrics and Rady Children’s Hospital (RCH).

I am very proud of the achievements of our clinician-investigators and scientists. The work highlighted here is a just a summary and a cross-section of the science that takes place in our Department rather than a comprehensive view. It is also an illustration of the spectacular quality of the research that goes on in the corridors of the Department of Pediatrics at both the School of Medicine at University of California 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.

One major reason to be excited about putting together such a summary is the hope that this will open the doors a little wider to potential collaborations with faculty around the nation and the world. I trust that such collaborations and networking can bring investigators closer to solving diseases of childhood and hence building a better society for the future.

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Researchers in the Department of Pediatrics at UC San Diego/Rady Children’s Hospital are using “mini-brains” to test experimental drugs able to revert, ameliorate or compensate the genetic defects.
Dr. Alysson Muotri’s lab creates cerebral organoids or “mini-brains” as a tool to investigate complex neurological disorders. These brain organoids can be created by reprogramming peripheral cells from individuals with neurological disorders, such as Autism or Williams syndrome (WS), and recapitulate their embryonic neurodevelopment in a dish. Interestingly, the process happens in a three-dimensional environment and the cells will self-assemble, forming an organized layered cortical structure that resembles a fetal brain on the first gestational trimester.

At the functional level, Dr. Muotri observed an increase in complexity of the neuronal networks, giving raise to sophisticated synchronization of electrical activity, indicating that these organoids can mature similar to the human brain. By comparing and contrasting patient-derived brain organoids to neurotypical individuals, it is possible to understand the molecular and cellular mechanisms related to the disease pathology. The Muotri lab applied this methodology to study WS and found a gene responsible for a reduction on cortical surface that is linked to the hypersociability observed on these individuals (1). Brain organoids were also used to demonstrate how the circulating Brazilian Zika virus causes microcephaly and other birth defects (2).

Also important, these cerebral organoids are being used to test experimental drugs able to revert, ameliorate or compensate the genetic defects. The beauty of this system is that thousands of drugs can be tried at the same time, optimizing for efficacy and dosage, for each individual. This type of personalized medicine is still restricted to Dr. Muotri’s academic lab but they are hopeful this technology can become accessible to patients all over the world in a near future.

References:

Advances in embryonic stem cell and induced pluripotent stem cell technology have opened up new avenues of disease modeling in vitro. Recently, stem cells and iPSC have been differentiated into three dimensional organoid systems to study the development of the intestine, retina, liver, kidney and even the brain. These organoids are able to differentiate, self-organize and form distinct, complex, biologically relevant structures, thus making them ideal in vitro models of development, disease pathogenesis and drug discovery. Several groups have developed cerebral organoid models that generate functional cortical neurons and can recapitulate forebrain, midbrain and hindbrain regions with functional electrophysiological properties to probe the mechanisms of neurodevelopment, autism and microcephaly.

To investigate how ZIKA virus infection leads to microcephaly, Dr. Tariq Rana and his team generated human embryonic stem cell-derived cerebral organoids to recapitulate early stage, first trimester fetal brain development. To further characterize the cerebral organoid models at the molecular level, the coding and non-coding transcriptome of human embryonic stem cells and their derived cerebral organoids were compared. They found that a prototype strain of ZIKA MR766 efficiently infects organoids and causes a decrease in overall organoid size that correlates with the kinetics of viral copy number. The innate immune receptor Toll-Like-Receptor 3 (TLR3) was upregulated after ZIKV infection of human organoids and TLR3 inhibition reduced the phenotypic effects of ZIKV infection. Pathway analysis of gene expression changes during TLR3 activation highlighted 41 genes also related to neuronal development, suggesting a mechanistic connection to disrupted neurogenesis. Dr. Rana’s research identified a link between ZIKV-mediated TLR3 activation, perturbed cell fate and a reduction in organoid volume reminiscent of microcephaly. Consequently, TLR3 competitive inhibitor attenuated the severe ZIKV-mediated apoptosis and organoid shrinkage seen in ZIKV only treated organoids.

These discoveries led to a drug development project in the laboratory where several classes of TLR3 inhibitors are being designed, synthesized, and evaluated for their effects on ZIKA virus inhibition in in vitro and in vivo models, as well as wide-ranging effects of TLR activation on immune regulation in CNS disorders.

Children can suffer from a range of early-onset neurodegenerative diseases, in which specific parts of the brain show evidence of cell- and volume-loss. Pontocerebellar atrophy was first described over a century ago, with onset during fetal life, in which the pons and cerebellum show dramatic loss of cells. In this condition, the cell loss occurs so early as to overlap with periods of neurogenesis, so the condition is alternatively called pontocerebellar hypoplasia (PCH).

Clinically, patients present with absence or loss of brainstem reflexes and swallowing, and lifespan is severely compromised. Dr. Joseph Gleeson previously published 6 genetic forms of disease, implicating the factors involved in maturation of tRNAs or protein synthesis. Of the clinically defined subtypes, only PCH type 7 showed ambiguous genitalia, and cause was unknown.

From 13 recessive PCH7 families PCH7, the Gleeson lab cloned the TOE1 with biallelic mutations in every case. Jens Lykke-Anderson at UC San Diego had reported TOE1 as an RNA 3’ exonuclease, localized to nuclear Cajal bodies (CBs). But Dr. Gleeson and his team wondered how a RNA exonuclease, which typically localize in the cytoplasm to degrade mRNAs, could be localized to CBs, and what could be its RNA substrate.

To understand disease mechanism, the Gleeson lab immunoprecipitated TOE1 and found association with pre-small nuclear RNAs (snRNAs) U1, U2, U4 and U6. snRNAs are the core catalytic components of the spliceosome, mediating pre-mRNA splicing in CBs. In patient iPSC-derived neurons and mutant zebrafish, they found accumulation of untrimmed-snRNAs. For decades the field has been searching for the factor acting after Integrator, to complete 3’ snRNAs trimming. In their paper, the Gleeson lab demonstrate TOE1 as a long sought component, both necessary and sufficient for this trimming.

Their discovery identified the cause of a mysterious pediatric neurodegenerative syndrome, linked it to defects in snRNA maturation, and identified the key factor involved in maturation of snRNA 3’ ends. These results suggest snRNA maturation, and by implication RNA splicing, as defective in PCH.

FIGURE. A. MRI of control and patient with PCH7, showing reduced volume of the pons and cerebellum (arrow). B. Schematic of proposed role for TOE1, functioning on trimming of 3’ tail of pre-snRNA following Integrator-mediated cleavage of Pol II transcribed pri-snRNA.
STEM CELL DIFFERENTIATION AND DISEASE

UC San Diego, Department of Pediatrics laboratories are deciphering mechanisms that not only help explain how the mature human body plan is formed, but could also explain why certain disease processes involve multiple cell types.
For the human body to take shape properly, multiple cell types from different lineages and germ layers must coordinate their patterning and maturation, processes that persist into adulthood. As reported in a 2015 paper in Cell Press’s *Stem Cell Reports*, the Snyder lab wished to study how this fundamental developmental program is launched in earliest embryogenesis. The team recognized that the co-patterning of ectoderm-derived neural cells with mesoderm-derived blood vessels provides a prototype for this process. They exploited the fact that human embryonic stem cells (hESCs) can model the developing human epiblast. By imaging these early stages of development in real time, they observed, surprisingly, that the neural crest (NC)—the source of the autonomic, peripheral, and enteric nervous system—not the brain and spinal cord—initiates co-patterning with emerging vasculature.

Blood vessel-secreted nitric oxide and direct contact with vascular smooth muscle cells via an adhesion molecule called T-cadherin causes the NC to become the type of neuron (autonomic neurons) that regulate the function of the blood vessels (as well as other cardiovascular functions). Conversely, mature blood vessel patterning depends on contact with the NC. If this cross-talk is blocked, both systems regress. Once this neurovascular template is established, then central nervous system (CNS) neurons—neurons that comprise the brain and spinal cord—can comport to that pattern secondarily.

This kind of mechanism not only helps explain how the mature human body plan is formed, but could also explain why certain disease processes involve multiple cell types—for example, why cancers metastasize along blood vessels, why certain birth defects take the form they do, how stroke becomes manifest, etc.

Reference:
Discoveries in Dr. Stephanie Cherqui’s lab demonstrate that Hematopoietic Stem and Progenitor Cell (HSPC) transplantation could rescue the multi-systemic lysosomal storage disorder, cystinosis, characterized by tissue cystine accumulation and multi-organ failure. HSPC transplantation led to the dramatic reduction of cystine in all organs in the mouse model, the Ctns<sup>−/−</sup> mice, and the long-term structural and functional preservation of the kidneys, eyes, and thyroid. Investigations in the Cherqui lab are now preparing for a phase I clinical trial for cystinosis consisting in autologous transplantation of gene-corrected HSPCs using a lentivirus vector.

The extent of efficacy of HSPCs to rescue cystinosis was surprising especially considering that cystinosin is a transmembrane lysosomal protein. The study of the mechanism showed that a large subset of HSPCs differentiated into macrophages that could transfer cystinosin-bearing lysosomes to the deficient host cells via long tubular extensions known as tunneling nanotubes (TNTs). This is the first demonstration of cross-correction in the context of a lysosomal transmembrane protein. It was also found that TNTs could cross the renal tubular basement membrane in vivo and transfer cystinosin-bearing lysosomes to the proximal tubular cells. Similar mechanism was shown for the ocular and thyroid rescue in the Ctns<sup>−/−</sup> mice.

Because TNTs can also transfer mitochondria, Dr. Cherqui tested HSPC transplantation in a mouse model of Friedreich’s Ataxia (FRDA), the YG8R mice. FRDA is an autosomal recessive neuromuscular degenerative disorder caused by reduced expression of the mitochondrial protein frataxin. HSPC transplantation completely prevented the development of the locomotor deficits and muscle weakness in the YG8R mice. Degeneration of the large sensory neurons in the dorsal root ganglia (DRG) was prevented as well as mitochondrial dysfunction in tissues. Abundant HSPC-derived cells were differentiated into microglial cells in the brain and spinal cord, and macrophages in DRGs, heart and skeletal muscle. In vivo transfer of frataxin-GFP and cox8-GFP mitochondrial proteins were observed from HSPC-derived microglial/macrophages to diseased neurons and cardiac muscular myocytes.

References:
Through comprehensive mapping of the epigenome and experimental validation, UC San Diego/Rady Children’s Hospital investigators in the Department of Pediatrics uncover mechanisms of how liver and pancreas arise from common endodermal progenitor cells. This work has significant implications for the targeted programming of stem cells and other cell sources.
In the last few decades, the prevalence of type 1 diabetes (T1D) has increased dramatically, particularly in children under the age of 5 years. It is estimated that more than 40,000 children in the U.S. are diagnosed with T1D each year. T1D is an autoimmune disease that develops when the body’s self-immune system mistakenly attacks pancreatic beta cells, the only insulin-producing cells. An ultimate cure for T1D relies on the termination of the attack of beta cells by self-immune system, a process called the restoration of immune tolerance.

With the support from the JDRF, Dr. Wenxian Fu has developed a new strategy to restore immune tolerance in T1D, with the goal to stop diabetes. Using a monoclonal antibody to block the signal of a cytokine receptor called interleukin-2 receptor beta (IL-2Rβ), Dr. Fu’s lab has demonstrated that this modulation is very effective to increase the ratio between pathogenic cells (which promote the development T1D) and regulatory cells (which suppress T1D), as illustrated in the schematic diagram to the right.

This approach has great potential for translational application to restore a long-lasting immune tolerance in T1D patients. Dr. Fu is currently collaborating with Dr. Michael Gottschalk (Chief of Endocrinology Division at Rady Children’s Hospital) to test the effect of IL-2Rβ blockade in human T1D patients.

Reference:

Maternal obesity increases birth weight and adiposity in humans. Compelling epidemiology data have also demonstrated that high birth weight is a key risk factor for the development of obesity in later life. However, the underlying mechanism through which maternal obesity increases fetal body fat is largely unknown.

By using high-fat diet and mouse models, Dr. Jianhua Shao has discovered that maternal obesity increases expression of lipoprotein lipase (LPL) expression and placental lipid transport. The key finding of these studies is the identification of silent mating type information regulation 2 homology 1 (SIRT1)/peroxisome proliferator-activated receptor γ (PPARγ) as a new cellular signaling pathway in the placentas.

SIRT1 is a NAD-dependent protein deacetylase that regulates energy metabolism, aging, and other cellular processes. The Shao lab has revealed that SIRT1 is highly expressed in the placenta and maternal high fat feeding suppressed SIRT1 expression. Furthermore, they found that decreased SIRT1 attenuates its inhibitory effect on PPARγ transactivity, which directly regulates LPL gene transcription. Therefore, Dr. Shao’s studies unveiled that SIRT1 serves as a nutrient sensor in trophoblasts and through its downstream transcript factor PPARγ regulates placental nutrient transporters expression.

This discovery significantly improves the understanding of placental nutrient transport and provides a foundation to prevent maternal obesity-induced excessive fetal fat accumulation.

Reference:
Understanding how organs, such as the liver and pancreas, develop will aid in the generation of new regenerative therapies for diseases associated with these organs, such as diabetes. Yet, how and when organ-specific transcriptional programs are initiated during developmental progression remains poorly understood.

To better understand this process, Dr. Maike Sander’s group generated maps of chromosomal modifications over time as embryonic stem cells differentiate toward mature pancreas and liver cells. From these maps, they discovered links between the accessibility of certain regions of the chromosome and the ability of the cell to respond to organ-inductive signals from their environment, known as developmental competence. Moreover, they found that FOXA pioneer transcription factors, which regulate the development of endodermal organs, occupy these open regions on the chromosome and are required for the acquisition of developmental competence. These pioneer transcription factors then recruit lineage-specific transcription factors, such as the pancreas-specific transcription factor PDX-1, to complete organ specification.

Through comprehensive mapping of the epigenome and experimental validation, investigators in the Sander lab uncovered mechanisms of how liver and pancreas arise from common endodermal progenitor cells. This work has significant implications for the targeted programming of stem cells and other cell sources to generate pancreatic beta cells for replacement therapy in diabetes.
KIDNEY DEVELOPMENT AND DISEASE

UC San Diego/Rady Children's Hospital investigators using zebrafish kidneys discover ways to enable more targeted interventions that should improve diagnostic, prognostic and therapeutic options for children with kidney birth defects.
Congenital anomalies of the kidney and urinary tract constitute approximately 20 percent of the major birth defects identified in the prenatal period and are the most common cause of chronic kidney disease in children. Dr. Elliot Perens and his colleagues have identified a gene regulatory network responsible in kidney development. The first step in kidney and urinary tract development is the formation of the intermediate mesoderm (IM). Because of the genetic connections between IM development, congenital renal anomalies and regenerative medicine, the researchers’ goal was to elucidate the genetic networks that control IM specification and differentiation.

Using zebrafish kidneys, Dr. Perens studies demonstrated that the transcription factor Hand2 limits IM dimensions by controlling cell fate decisions along the lateral border of the IM. More specifically, they showed that hand2 promotes venous progenitor development while inhibiting IM formation at this interface. These studies also suggest that hand2 and osr1, a zinc-finger transcription factor gene previously implicated in promoting kidney formation, have functionally antagonistic roles during kidney development.

Together, this data sheds light on a previously unrecognized genetic network regulating IM dimensions. The discovery will help to enable more targeted and personalized interventions that should improve diagnostic, prognostic and therapeutic options for children with kidney birth defects. Such therapeutic options may include novel renal replacement therapies using stem cell and regenerative technologies.

Cystinosis is an autosomal recessive disorder caused by mutations of the CTNS gene (17p13) encoding the cystine. This results in the intralysosomal accumulation of cystine in all tissues, most notably the kidneys. Patients with infantile nephropathic cystinosis (INC) exhibit signs and symptoms of renal Fanconi syndrome and chronic kidney disease (CKD) in early childhood.

Muscle wasting is a common complication in patients with cystinosis. The lab of Dr. Robert Mak defines the metabolic phenotype in Ctns−/− mice, an established murine model of INC, with focus on muscle wasting and energy homeostasis. Lower body weight, increased energy homeostasis and muscle wasting was demonstrated in Ctns−/− mice. They studied the effects of cystinosis on skeletal muscle histomorphometry (Figure 2). Mean soleus and tibias anterior fiber cross-sectional area in Ctns−/− mice was significantly decreased than that in wild type (WT) controls. Muscle function, as assessed by forelimb grip strength and rotarod controls. Muscle function, as assessed by forelimb grip strength and rotarod activity, was significantly decreased in Ctns−/− mice vs WT or CKD controls.

Importantly, the Mak lab reports novel discoveries in the development of beige adipocytes in Ctns−/− mice. They observed elevated expression of beige adipocyte surface markers (CD137, Tmem26, and Tbx1) in inguinal WAT in Ctns−/− mice than in WT controls (Figure 1). Furthermore, inguinal WAT CD137 and Tbx1 expression was higher in Ctns−/− mice than in CKD controls. Another important marker for beige adipocyte in inguinal WAT is UCP-1, which is usually not detected in WAT. UCP-1 protein was detected in inguinal WAT of Ctns−/− and CKD mice but was undetectable in WT controls. UCP-1 protein level in inguinal WAT of Ctns−/− was higher than that in CKD mice.

Further studies are required to investigate the underlying mechanisms of these metabolic defects in INC, which are associated with poor quality of life and mortality, and for which there is no current therapy.

**Reference:**
Using novel technology to grow 3D “mini guts” to further understand how EpCAM (epithelial cell adhesion molecule) contributes to the health of the intestine, researchers at UC San Diego/ Rady Children’s Hospital in the Department of Pediatrics are leading the way to discover new treatments for a set of patients without treatment options at this time.
Congenital tufting enteropathy (CTE) is one of several intractable diarrheal diseases of infancy that typically presents in the neonatal period with chronic watery diarrhea, electrolyte imbalances, and impaired growth. Patients suffer from intestinal failure necessitating parental 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 crypt hyperplasia, but the most characteristic pathologic abnormalities are focal epithelial tufts in the small intestine. Dr. Sivagnanam discovered mutations in the gene, EpCAM (epithelial cell adhesion molecule), to be the cause of this disease. Using models of disease, investigators in the Sivagnanam lab have gone on to understand that EpCAM plays a role in the underlying structure of CTE, where the crypt villus axis. Thus, they serve as a powerful tool for understanding the dynamics diseases such as CTE, where structure is altered. The knowledge acquired from these discoveries will allow a paradigm shift in the understanding of EpCAM, CTE, diarrheal diseases, and intestinal failure and may eventually aid in development of new treatments for a set of patients without treatment options at this time.

Mini-Guts Provide Insights into an Intestinal Failure Disease

These results point out the relevance of RNA-based therapy for the treatment of serious diseases such as liver fibrosis and provide new evidence on the link between hepatocyte apoptosis and liver fibrosis. These discoveries open new venues to develop novel anti-fibrotic approaches that would significantly aid in decreasing the global burden of liver disease.

**Figure.** Functional model of disease. Mutation of EpCAM and subsequent reduction of EpCAM and claudin-7 causes features of CTE, including tufting, intercellular gaps, increased desmosomes, and villous atrophy. This leads to functional consequences of increased proliferation, migration, and permeability with diarrhea in patients.

**References:**

**Chronic Liver Disease**

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’s lab targeted hepatocyte cell death, through the selective knockdown of Bid protein, a key pro-apoptotic molecule as a novel anti-fibrotic strategy. The reduction of hepatic expression of Bid in vivo was achieved using a next-generation RNA-based technology that allows an efficacy accumulation in the liver. They observed that Bid siRNA treatment effectively reduced hepatic stellate cell activation and liver fibrosis.

These studies point out the relevance of RNA-based therapy for the treatment of serious diseases such as liver fibrosis and provide new evidence on the link between hepatocyte apoptosis and liver fibrosis. These discoveries open new venues to develop novel anti-fibrotic approaches that would significantly aid in decreasing the global burden of liver disease.

**References:**
Eosinophilic esophagitis (EoE) is an antigen-driven allergic disease of increasing worldwide incidence and prevalence. Inflammatory cells and allergic and pro-fibrotic cytokines are pivotal to the disease process. Unbridled inflammation causes chronic end-organ dysfunction with esophageal rigidity, strictures, and smooth muscle dysmotility with resultant clinical dysphagia and food impactions. Tissue remodeling is the molecular underpinning for EoE complications, begins early in childhood, and is variably responsive to standard therapies. Dr. Seema Aceves studies the molecular mechanisms and clinical impacts of esophageal remodeling. While it is accepted that inflammation is an integral driving force in EoE, her lab has recently demonstrated the potentially paradigm-shifting observation that a rigid matrix alters esophageal structural cell function to propagate fibrosis and inflammation and to promote abnormal contraction of the smooth muscle independently of inflammation.

The clinical implication of these findings is that there is a pressing need for therapies that target inflammation-independent, extracellular matrix-dependent changes in structural cells such as fibroblasts and smooth muscle cells. Until it is better understood how the molecular mechanisms by which EoE cells sense their mechanical environment, it will not be known what these target are and may impede the ability to effectively target disease complications. To study these processes, the Aceves lab uses primary human model systems including isolated cells, multicellular “organoids”, and, most recently through collaborative efforts with the University of Arkansas, a novel intact ex vivo human esophageal mucosal platform.

References:

FIGURE. Primary human esophageal smooth muscle cells have increased size (hypertrophy) on rigid matrix (A, B) and altered gene expression of contractile agents such as phospholamban (PLN) (C).
Discoveries at UC San Diego/Rady Children’s Hospital in the Department of Pediatrics are paving the way to better understand congenital heart disease.
In the care of patients with congenital heart disease, less invasive, percutaneous interventional treatments have supplanted many surgical approaches for simple lesions, such as atrial septal defect. By contrast, complex congenital heart defects continue to require open-heart surgery. In single ventricle (children born with one main pumping chamber rather than the normal two-ventricle circulation) patients, a staged approach is employed, which requires multiple open-heart surgeries with significant attendant morbidity and mortality.

In pre-clinical testing over a decade, Dr. Kanishka Ratnayaka and his team have developed the techniques and technology needed for non-surgical transcatheter anastomosis of two separate blood vessels. [1] They can now perform precise crossing from a donor to a recipient vessel and endovascular stent based anastomosis of those blood vessels. They undertook this transcatheter approach for an adult with untreated congenital heart disease with severe cyanosis and significant surgical risk. The procedure was rehearsed using a 3-dimensional patient specific printed heart model based on contrast-enhanced cardiac computed tomography.

Dr. Ratnayaka performed a first-in-human, fully percutaneous closed chest large vessel anastomosis joining the superior vena cava and pulmonary artery (superior cavo-pulmonary anastomosis or bi-directional Glenn operation equivalent). [2] With the percutaneous procedure, the patient recovered uneventfully and remains significantly improved clinically after 6 months. Large vessel anastomosis is a mainstay of open-heart surgery. It has a long history but has significant concomitant morbidity and mortality. This procedure may provide a viable alternative to one of the foundational open-heart surgeries currently performed to treat single ventricle congenital heart disease.

**FIRST-IN-HUMAN: CLOSED CHEST TRANSCATHETER SUPERIOR CAVO-PULMONARY ANASTOMOSIS**

**References:**


**FIGURE.** Pre- and post-intervention contrast enhanced cardiac computed tomography (CT): Transcatheter Superior Cavo-Pulmonary Anastomosis

**Figure:** Pre- and post-intervention contrast enhanced cardiac computed tomography (CT): Transcatheter Superior Cavo-Pulmonary Anastomosis
Hypoplastic left heart syndrome (HLHS) is one of the most severe human congenital heart defects (CHDs), accounting for the most frequent cause of death in infants with CHDs. To date only a few disease-causing genes have been identified. Progress towards understanding HLHS has been hindered by the lack of genetically engineered animal models. Nothing is known about the earliest embryonic events during heart development underlying HLHS.

Jacobsen syndrome (JS, OMIM #147791) is a rare chromosomal disorder caused by deletions in distal 11q. 5-10% of all infants with JS are born with HLHS, higher than for any known human genetic syndrome. Dr. Paul Grossfeld and colleagues have previously identified the ETS-1 transcription factor as the likely gene for causing CHDs in JS. Knockdown of ETS-1 in Xenopus leads to an embryonic lethal cardiac phenotype reminiscent of HLHS: a hypoplastic outflow tract and a thickened ventricular chamber with diminished chamber volume.

These discoveries indicate loss of ETS-1 causes an HLHS-like phenotype through a multi-hit model involving cardiac neural crest and endocardium suggesting the possibility of early intervention for the prevention of HLHS.

References:


FIGURE 1. Histological section of embryos receiving Ets1-MO (left panel). (A) At stage 28 when the primitive heart tube is formed, loss of Ets-1 in the heart mesoderm not only disrupted the folding of myocardium, but also abolished the development of endocardial cells. (B) At stage 45 when chambered heart is formed, loss of Ets-1 in the neural crest affected the development of aortic arches, while the loss of Ets-1 in heart mesoderm affected the development of aortic arches and severely inhibited the development of the ventricle. The thickened myocardial layer and reduced ventricle volume resembles HLHS. Grafting of heart field tissue partially rescued the cardiac defect (center panel). Immunohistochemistry analysis with myocardium and OFT markers (MF20 & SMA) shows that the morphology of the heart is partially restored with HF graft. The survival of the embryos is also improved (right panel).

FIGURE 2. RT-PCR analysis for potential downstream targets of Ets-1. BMP10 expression is significantly increased by the loss of Ets-1 at tailbud stages (St22) (left), which is also verified by in situ hybridization experiment. Top panel demonstrates BMP-10 expression on side of embryo injected with ETS-1 MO. Bottom panel demonstrates BMP-10 expression on non-injected control side.
UC San Diego/Rady Children’s Hospital investigators are providing proof of concept that the gut microbiome early in life is crucial for future health and disease.
Partial Restoration of the Microbiota of Cesarean-Born Infants via Vaginal Microbial Transfer

In a pilot clinical study, researchers at UC San Diego Department of Pediatrics and Icahn School of Medicine at Mount Sinai determined that a simple swab to transfer vaginal microbes from a mother to her C-section-delivered newborn can alter the baby’s microbial makeup (microbiome) in a way that more closely resembles the microbiome of a vaginally delivered baby.

Babies delivered by C-section differ from babies delivered vaginally in the makeup of the microbes that live in and on their bodies. These early microbiomes help educate the baby’s developing immune system. Previous research suggests a link between C-section delivery and increased subsequent risk of obesity, asthma, allergies, atopic disease and other immune deficiencies. Many of these diseases have also been linked to the microbiome, though the role a newborn’s microbiome plays in current or long-term health is not yet well-understood.

According to Rob Knight, PhD, Professor of Pediatrics and Computer Science and a member of the Division of Host-Microbe Systems & Therapeutics, “This study points the way to how we would do that, and provides the proof-of-concept that microbiome modification early in life is possible. In fact, we already have more than 10,000 additional samples collected as part of this study that still await analysis.”

Reference:

FIGURE 1: Restoring the maternal microbiota in infants born by C-section (A). Infants born by C-section were swabbed with a gauze that was incubated in the maternal vagina 30-60 min prior to the C-section. All mothers delivering by C-section received antibiotics (ABX) as part of standard of care. The gauze was extracted prior to the procedure, kept in a sterile container, and used to swab the newborn within the first one to three minutes after birth, starting with the mouth, face, and rest of the body. (B) Proportion of each sample estimated to originate from different maternal sources (using bacterial source tracking) of anal (top row), oral (middle), and skin (bottom) samples in infants delivered either vaginally (left column, n = 7 subjects sampled at six time points), by C-section (unexposed) (right, n = 8 × 6), or by C-section and exposed to vaginal fluids (middle, n = 4 × 6). (C) Proportional bacterial community distances in anal (left), oral (middle), and skin (right) samples between vaginally delivered and C-section-delivered exposed (I-V) and not exposed (C-V) to the vaginal gauze, during the first month of life (Unweighted UniFrac distances). Bars indicate standard deviation from the mean. Distances between vaginal and exposed C-section infants were significantly smaller than from unexposed C-section infants (ANOVA and Tukey’s honest significant difference test. *P < 0.01) (D) Representative bacterial taxa enriched in infants with perinatal exposure to vaginal fluids during the first month of life. Bars indicate standard deviation from the mean.
Contrary to current medical dogma, researchers at UC San Diego Department of Pediatrics and Skaggs School of Pharmacy and Pharmaceutical Sciences report that the common antibiotic azithromycin kills many multidrug-resistant bacteria very effectively—when tested under conditions that closely resemble the human body and its natural antimicrobial factors.

“Unquestioning adherence to a single standardized lab practice may be keeping doctors from considering potentially life-saving antibiotics—therapies that are proven safe and readily available in any hospital or pharmacy,” said senior author Victor Nizet, MD, of the Division of Host-Microbe Systems & Therapeutics.

In this study, Dr. Nizet’s team found that simply growing these Gram-negative rod bacteria in mammalian tissue culture media—the same stuff used to sustain human cells in the lab—instead of standard bacteriologic media made a huge difference in their sensitivity to azithromycin. Even more striking, the drug-resistant superbugs were completely wiped out when azithromycin was paired antimicrobial peptides produced naturally by the human body during infection. In a mouse model of multidrug-resistant A.baumannii pneumonia, 24 hours after infection, azithromycin-treated mice had 99 percent fewer bacteria in their lungs than untreated mice. The finding, published June 10, 2015 in EBioMedicine prompted a reconsideration of the current standard of care for patients with so-called “superbug” infections.

Reference:
HIV survival is dependent on its ability to evade intrinsic cellular processes and defenses including macroautophagy (autophagy). Autophagy is a degradation pathway whereby cytosolic autophagosomes engulf and sequester cytoplasmic constituents such as sub-cellular organelles and microbial pathogens. These autophagosomes then fuse with lysosomes forming autolysosomes, resulting in the degradation of the engulfed components. Evidence of integrated and co-regulated roles of lysosomes and autophagosomes has emerged from the discovery of an overarching lysosomal regulatory gene network (CLEAR, Coordinated Lysosomal Expression and Regulation) and its master regulator, the basic helix-loop-helix leucine zipper transcription factor EB (TFEB).

HIV Nef acts as an anti-autophagic maturation factor through interaction with beclin-1 (BECN1). In this research, the Spector laboratory reported that exposure of macrophages to HIV induces toll-like receptor 8 (TLR8) and BECN1-dependent dephosphorylation and nuclear translocation of TFEB associated with an increase in autophagy. RNA interference for ATG13, BECN1 dependent dephosphorylation and nuclear translocation of TFEB. During permissive infection, Nef binds BECN1 resulting in MTOC activation, TFEB phosphorylation and cytosolic sequestration, and the inhibition of autophagy. This is the first discovery demonstrating viral regulation of the autophagy master regulator TFEB. Understanding how HIV and other viruses such as influenza and herpes viruses inhibit autophagy can lead to the development of broad-spectrum antiviral drugs that restore autophagy through pharmacological means during viral infection with the aim of eliminating the virus.

**FIGURE.** Schematic diagram of how HIV induces autophagy during initial infection. Under basal conditions, TFEB is phosphorylated by MTORC1, resulting in the cytoplasmic retention of TFEB through interactions with 14-3-3 proteins. Inhibition of MTOC1 through TLR8 signaling leads to TFEB dephosphorylation, TFEB translocation to the nucleus with induction of autophagosome formation, lysosomal proteins and induction of autophagy.

**Human Immunodeficiency Virus Type 1 NEF Inhibits Autophagy through Transcription Factor EB Sequestration**

HIV infected persons frequently lose THT responses, fail to mount protective immune responses and show decreased auto phagosome formation. Conversely, although increased resulting in decreased auto phagy markers. Nevertheless, despite restoration of T cells and DC numbers following successful combination antiretroviral therapy (ART), abnormalities in myeloid DCs (mDCs) persist and are thought to be a major cause of abnormal T-cell stimulatory capacity and function.

In this study, Dr. Stephen Spector and his team examined the effect of mDC induced IL-23 on T-cell effector functions against viral pathogens during HIV infection. They observed that HIV or HIV gp120 blocked LPS induced mDC maturation; gp120 impairs IL-12p70 and increases IL-23 production from mDCs. These aberrant mDCs inhibited the proliferation and IFNy production of alloreactive T cells and recall response to viral pathogens in the presence of a peptide pool consisting of cytomegalo virus, Epstein-Barr virus and influenza (CEF). Further, they showed that gp120 induced IL-23 upregulates SOCS1 protein expression in T cells. Partial knockdown of SOCS1 in T cells enhanced IFNy production and loss of CEF peptide pool stimulated monocytes. Finally, they showed that IL-23 induced SOCS1 binds to the IFNy transcription complex. Importantly, in this study, expression of SOCS1 in primary human T cells was present in untreated HIV-infected persons. Thus, they showed for the first time the mechanism by which HIV associated dysregulation of mDCs can cause T-cell dysfunction.

**FIGURE.** Simplified model of SOCS1 mediated regulation of IFNy synthesis. (i) IFNy gene expression is activated by the binding of trans cription factor Tbet to the proximal promoter, facilitated by chromatin remodeling. Additional transcription factors (CREB, AFT2, AP1) recruit co-activators and HATs (CBP) that enhances the opening of the chromatin structure and maximizes IFNy gene transcription. Of these, CREB is the positive regulator of IFNy transcription in primary human T cells in response to microbial pathogens. (ii) Based on these results, we propose that HIV induced IL-23 upregulates SOCS1 in T cells; SOCS1 interacts with CREB and negatively regulates IFNy production. Additionally, SOCS1 might also down regulate pSTAT1 further dampening the IFNy activity. (Abbreviations: CREB, cAMP response element binding protein, CBP; CREB binding protein, HAT; histone acetyltransferase, Tbet; T-box expressed in T cells)

**IL-23 Produced by Myeloid Dendritic Cells Contributes to T-Cell Dysfunction in HIV-1 Infection by Inducing SOCS1 Expression**

This research highlights the contribution of dysregulated innate immune responses in the failure of HIV-infected persons to fully immune reconstitute despite apparent virologic suppression. Moreover, these findings establish a link between high SOCS1 expression and defective T effector responses, provide new insights into how SOCS1 antagonists might improve immune reconstitution of HIV-infected persons on ART with sustained viral suppression.

**Reference:**
INFLAMMATION AND IMMUNITY

The developmental expression of the inflammasome and IL-1β released by fetal lung macrophages have been identified by UC San Diego/Rady Children’s Hospital researchers in the Department of Pediatrics as key mechanisms and potential therapeutic targets for neonatal lung disease.
The laboratory of Dr. Alessandra Franco discovered that the heavy constant region of IgG stimulates antigen-specific, HLA-restricted natural regulatory T cells (nTreg) that control immune homeostasis. These T cells can be found in circulation in 100% of healthy human subjects studied. Moreover, Fc-specific nTreg expand after IVIG therapy in Kawasaki disease (KD) subjects, an acute pediatric vasculitis of the coronary arteries, suggesting that IVIG boosts immune regulation via nTreg stimulation. They identified 16 immunodominant short Fc-derived peptides (15 amino acids long) tailored to bind the HLA of antigen presenting cells without the need for antigen processing. Peptide-specific nTreg responses were documented even in KD patients who developed arterial complications (CAA) and who failed to respond to the intact Fc after IVIG treatment (US Patent 0321.119402WO/SD2015-050-1).

Importantly, 6 of 16 peptides bind multiple class II HLA alleles and appear to be recognized by the majority healthy donors and KD patients after IVIG studied, including KD patients with CAA. Dr. Franco and her team are consolidating these results in a large cohort of healthy donors and KD patients after IVIG treatment. Their results suggest that immunodominant Fc-derived peptides may be a valid alternative to IVIG and may have application in reducing vascular inflammation in a broad range of diseases.

It was discovered that the application of immunodominant pan-HLA Fc peptides in the clinic can be extended to a variety of inflammatory conditions including autoimmune. These findings also indicate that pan-HLA immunodominant Fc peptides would be an excellent personalized therapy for Kawasaki disease patients and a low cost, stable and feasible alternative to IVIG.

Reference:
Therapeutic Tonsillectomy in Children with Periodic Fever, Aphthous Stomatitis, Pharyngitis, Adenitis (PFAPA) Syndrome

Periodic Fever, Aphthous stomatitis, Pharyngitis and Adenitis (PFAPA) syndrome is an inflammatory disorder of childhood classically characterized by recurrent fevers, pharyngitis, stomatitis, cervical adenitis and leukocytosis. Little is known about the true incidence, natural course, pathogenesis, and appropriate therapy in this recently described syndrome. While the mechanism is unclear, previous studies have shown that tonsillectomy can be a therapeutic option with improvement in quality of life in many patients with PFAPA, but long-term clinical follow up is lacking.

Dr. Lori Broderick and her team collected patient data and detailed family histories for over 200 children with recurrent fevers including 94 patients with PFAPA to create a prospective cohort of children treated at a tertiary care center in San Diego, California, USA. Patient data was collected under an IRB-approved protocol using patient charts and a standardized questionnaire, demographic data, including age, gender, and ethnicity, clinical profiles (presence of symptoms, fever profile, treatments) and detailed family histories over a 7-year period.

To date, 63 patients with PFAPA and 11 patients with other non-infectious recurrent fevers have undergone tonsillectomy. Forty-four patients with PFAPA syndrome have had complete resolution of symptoms after surgery, with the average length of follow up is 31.6 months (range 1-111 months). In 2 patients, there has been a relapse of symptoms, defined as fevers persisting for more than 6 months, which remain responsive to medical therapy. Our cohort of patients demonstrates clinical characteristics consistent with PFAPA. This discovery demonstrates that tonsillectomy is an effective surgical treatment option for management of children with PFAPA syndrome.

IL-1β and Inflammasome Activity Link Inflammation to Abnormal Fetal Airway Development

Inflammation in the developing preterm lung leads to disrupted airway morphogenesis and chronic lung disease in human neonates. However, the molecular mechanisms linking inflammation and the pathways controlling airway morphogenesis remain unclear.

Dr. Lawrence Prince and his team have shown that IL-1β released by activated fetal lung macrophages is the key inflammatory mediator that disrupts airway morphogenesis. In mouse lung explants, blocking IL-1β expression, post-translational processing, and signaling protected the formation of new airways from the inhibitory effects of Escherichia coli LPS. Consistent with a critical role for IL-1β, mice expressing a gain-of-function Nlrp3 allele and subsequent overactive inflammasome activity displayed abnormal saccular-stage lung morphogenesis and died soon after birth. Although the early-stage fetal lung appeared capable of mounting an NF-κB-mediated immune response, airway formation became more sensitive to inflammation later in development. This period of susceptibility coincided to inflammation later in development.

These data identify developmental expression of the inflammasome and IL-1β release by fetal lung macrophages as key mechanisms and potential therapeutic targets for neonatal lung disease.

References:

References:

LORI BRODERICK, MD, PhD
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LAWRENCE S. PRINCE, MD, PhD
Associate Professor of Pediatrics
Neonatology
Researchers at UC San Diego/Rady Children’s Hospital in the Department of Pediatrics are describing new methods for the analysis of whole-genome sequences in humans.
Discovery of CDX2 as a Prognostic Biomarker in Colon Cancer

Dr. Debashis Sahoo used publicly available gene-expression data to identify CDX2 expression as a prognostic biomarker in patients with colon cancer [1]. Additionally, lack of CDX2 expression identified a subgroup of patients with high-risk stage II colon cancer who appeared to benefit from adjuvant chemotherapy.

The Sahoo lab searched for genes that fulfilled the “X-negative implies ALCAM-positive” Boolean relationship in a collection of 2329 human colon gene-expression array experiments. This collection was downloaded from the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO) repository. The search was conducted with the use of BooleanNet software [2] with a false discovery rate of less than 0.0001 as a cutoff point for positive results (Figure). Candidate genes were ranked according to the dynamic range of their expression levels. The search identified 16 candidate genes. Of these, one encoded for a transcription factor, CDX2, with expression levels that were lower in more immature cancers and could also be studied with the use of a clinical-grade diagnostic test. The study identified a small population of colon cancers—about 4%—that lacked CDX2 expression. CDX2-negative tumors, as compared with CDX2-positive tumors, were associated with lower rates of 5-year disease-free survival, overall survival, and disease-specific survival.

But in a large, pooled database that included patients from multiple institutions and a large prospective, randomized adjuvant-therapy trial in which CDX2 expression was not assessed at the time of treatment, patients with stage II CDX2-negative tumors who were treated with adjuvant chemotherapy had a higher rate of 5-year disease-free survival (Figure). CDX2-negative disease, in contrast, was unaffected by adjuvant chemotherapy.

Prognostic biomarkers are key to the risk stratification of patients with colon cancer and the decision to recommend adjuvant chemotherapy in patients with early-stage disease. The discoveries published by Dr. Sahoo and his team indicate that patients with stage II or stage III CDX2-negative colon cancer might benefit from adjuvant chemotherapy and that adjuvant chemotherapy might be a treatment option for patients with stage II CDX2-negative disease, who are commonly treated with surgery alone.

References:

Dr. Debashis Sahoo
Assistant Professor of Pediatrics
and Computer, Science and Engineering
Genome Information Sciences

FIGURE. Boolean analysis in colon cancer. Boolean implication: CDX2 low => ALCAM high. CDX2-negative stage II colon cancer respond to chemotherapy.
Induced pluripotent stem cells (iPSCs) show variable methylation patterns between lines, some of which reflect aberrant differences relative to embryonic stem cells (ESCs). To examine whether this aberrant methylation results from genetic variation or non-genetic mechanisms, Dr. Kelly Frazer’s lab generated human iPSCs from monozygotic twins to investigate how genetic background, clone, and passage number contribute. They found that aberrantly methylated CpGs are enriched in regulatory regions associated with MYC protein motifs and affect gene expression. Differentially methylated CpGs were classified as being associated with genetic and/or non-genetic factors (clone and passage), and it was found that aberrant methylation preferentially occurs at CpGs associated with clone-specific effects.

The Frazer lab further found that clone-specific effects play a strong role in recurrent aberrant methylation at specific CpG sites across different studies. Their discoveries argue that a non-genetic biological mechanism underlies aberrant methylation in iPSCs and that it is likely based on a probabilistic process involving MYC that takes place during or shortly after reprogramming.

I iPSCs could potentially revolutionize transplantation medicine. In the future, they may provide patients a copious supply of immune-matched tissues. However, before this becomes a reality additional knowledge must be gained about the molecular nature of iPSCs such as how the epigenome of iPSCs vary from normal tissues. Dr. Frazer’s work provides a foundation for future studies aimed at elucidating the specific molecular mechanisms underlying aberrancy as well as the physiological consequences of these epigenetic modifications.

**References:**


The impact of prenatal vitamin intervention initiated during pregnancy is evaluated by UC San Diego/Rady Children’s Hospital in the Department of Pediatrics research teams in drinking women and demonstrated improvement in neurobehavioral outcomes in infants.
In collaboration with researchers from the University of California Davis, Emory University and the OMNI-Net for Children International Charitable Fund in Ukraine, Dr. Christina Chambers and her team evaluated the impact of prenatal vitamin intervention initiated during pregnancy in a sample of moderate to heavy drinking women and demonstrated improvement in neurobehavioral outcomes in infants as young as six months of age.

Alcohol-using and nondrinking women were randomized to one of three multivitamin/mineral supplement groups: multivitamins/minerals (MVM), multivitamins/minerals plus choline, or no supplements. Three hundred and sixty-seven children were tested at six months of age with the Bayley Scales of Infant Development, 2nd ED (BSID-II) yielding standard scores for Mental Development Index (MDI), Psychomotor Development Index (PDI) and Behavior. Generalized linear modeling was used: (1) for factorial analysis of effects of alcohol group, multivitamin/minerals, and choline supplementation; and (2) to examine the relationship between amount and timing of alcohol (ounces of absolute alcohol/day [ozAA/day] peri-conception and on average in the second trimester) and MVM supplementation on developmental outcomes while controlling sex, social class, and smoking. MDI was significantly impacted by peri-conceptual alcohol dose ($X^2(1) \ p < .001$) with more alcohol associated with lower scores and males more negatively affected than females ($X^2(1) \ p < .002$). Micronutrient supplementation had a protective effect; those receiving supplements performed better ($p < .005$). The PDI motor scores did not differ by group but were affected by peri-conceptual alcohol dose ($X^2(1) < .04$).

Choline showed no effect on cognitive scores, but was associated with more negative motor outcomes. Although women are advised to avoid alcohol entirely throughout pregnancy, this work showed that in the common situation where women have consumed alcohol before recognition of pregnancy, following advice to take prenatal vitamin supplements may help ameliorate some of the adverse effects of prenatal alcohol exposure on their offspring. The discoveries from this study are the first to show this apparent benefit, and reinforce the need for appropriate nutritional prenatal care for women worldwide.

Reference:

FIGURE. Bayley infant development, 2nd edition psychomotor and mental development scores at a mean of 6 months of age as a function of child sex, alcohol exposure in gestation and multivitamin/mineral supplementation.

A. Psychomotor Development Index (PDI) (M = 100, SD = 15) No groups means significantly different. B. Mental Development Index (MDI) (M = 100, SD = 15)
Establishment of a Research-Only Human Milk Biorepository

In 2014, the first human milk biorepository (HMB) dedicated to research was established. Mommy’s Milk HMB is a multidisciplinary research initiative of the UC San Diego’s Department of Pediatrics Division of Dysmorphology and Teratology’s Center for Better Beginnings and the UC San Diego Altman Clinical and Translation Research Institute’s Center for Life Course Research, with the support of Rady Children’s Hospital-San Diego. The goal of the HMB is to enable scientists from diverse fields to answer a wide variety of questions about human milk, including its unique biochemical properties and the extent to which pharmaceuticals and substances to which nursing mothers are exposed can be passed to the infant during breastfeeding. This latter goal is of critical importance as there are few data on the passage of most agents into breast milk, while the extensive benefits of breastfeeding are well established. As a result, many women are advised either to avoid a medication while breastfeeding, or to stop breastfeeding entirely if a medication is needed.

Currently, there are over 700 women enrolled in the biorepository who have provided milk samples and completed questionnaires covering a broad range of information such as maternal demographic and health data, exposure data regarding medications, recreational substances, nutritional supplements, diet, exercise and measures of maternal stress, anxiety and depression. Infant health data on growth, neurodevelopment, illnesses, and signs and symptoms of toxicity are also collected by interview and medical records review and linked to breast milk samples.

The HMB has established a local partnership with the San Diego Blood Bank to collect samples within the broader community, and initiated a national framework for accepting samples from their MotherToBaby study participants throughout the U.S. and Canada. At the U.S. FDA Public Meeting on Drugs in Breastfeeding held in April 2016, the HMB was highlighted as a unique and valuable resource for critically needed research.

Reference:


TABLE. Prevalence of exposure by category in women enrolled in the Human Milk Biorepository (HMB) between 2014-2017

<table>
<thead>
<tr>
<th>Exposure Category</th>
<th>N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamins/Supplements</td>
<td>526 (85)</td>
</tr>
<tr>
<td>Prescription Medications</td>
<td>232 (38)</td>
</tr>
<tr>
<td>Over-the-Counter Medications</td>
<td>288 (47)</td>
</tr>
<tr>
<td>Birth Control</td>
<td>166 (27)</td>
</tr>
<tr>
<td>Caffeine</td>
<td>520 (84)</td>
</tr>
<tr>
<td>Alcohol</td>
<td>357 (58)</td>
</tr>
<tr>
<td>Recreational Drugs</td>
<td>57 (9)</td>
</tr>
<tr>
<td>Cigarette Smoke</td>
<td>19 (3)</td>
</tr>
</tbody>
</table>
Breastfeeding is associated with decreased risk of childhood obesity. However, there is a strong correlation between maternal pre-pregnancy weight status and childhood obesity, and it is unclear whether or not breastfeeding among overweight mothers could mitigate this risk. The goal of Dr. Kyung Rhee’s research was to examine whether or not exclusive breastfeeding (compared to formula feeding) among overweight and obese mothers is associated with a decreased risk of higher infant weight-for-length percentile at one year.

Using data from the Infant Feeding Practices II study, Dr. Rhee and her team examined the effects of exclusive breastfeeding on infant weight-for-length (W/L) percentile at one year. Infants who were preterm or underweight at one year, and mothers who were underweight prior to pregnancy were excluded from the analysis. A total of 915 subjects met inclusion criteria. There was a significant interaction between exclusive breastfeeding and infant W/L percentiles at 12 months (p<0.02). This interaction may help to decrease the risk of childhood obesity in this at-risk population.

Dr. Rhee found that normal weight and obese mothers who exclusively breastfed for four months also had lower W/L percentile at one year than those who used formula. Infants of overweight and obese mothers who exclusively breastfed for four months had a significantly slower growth trajectory if they were exclusively breastfed for 4 months (p<0.001), while those who used formula had lower W/L percentile at 12 months (p=0.29). The goal was to examine the interaction between these intrinsic factors and their association with weight status in early childhood.

FIGURE 1A. Growth model examining the effect of exclusive breastfeeding for 4 months on infant weight-for-length percentile over the first 12 months of life, stratified by maternal pre-pregnancy weight status.

FIGURE 1B. Growth model examining the effect of exclusive breastfeeding for 4 months on infant weight-for-length percentile over the first 12 months of life, stratified by maternal pre-pregnancy weight status.

FIGURE 1C. Growth model examining the effect of exclusive breastfeeding for 4 months on infant weight-for-length percentile over the first 12 months of life, stratified by maternal pre-pregnancy weight status.

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FIGURE 1C. Growth model examining the effect of exclusive breastfeeding for 4 months on infant weight-for-length percentile over the first 12 months of life, stratified by maternal pre-pregnancy weight status.

Being highly responsive to environmental food cues (high food responsivity) putes one at risk for increased caloric intake, excess weight gain, and obesity. Those who are unable to inhibit impulses (poor effortful control) also have greater consumption of snack foods and excess weight gain. Our interaction between FR x EC existed in children ≤ 61 months (p=0.37).

These results suggest that intrinsic factors such as food responsivity and inhibition control interact to contribute to early childhood BMI in children > 61 months old, but may not manifest in BMI changes prior to that time point.

Additional work is needed to examine prevention strategies for children prior to this age.

Research Team: Strong, D., Rhee, K.E.

Mean BMI of group 3 (M = 18.0, SD = 3.8) differs from mean BMI of group 2 (M = 15.7, SD = 1.5, p = .005), and group 1 (M = 12.2, SD = 2.0, p = .070). There was a significant main effect of FR (F=5.5, p<.002), SR (F=4.0, p<.05), and EC (F=4.6, p<.002) on child BMI. Controlling for covariates, there was a significant 3-way interaction between FR x EC x Age (F=7.26, p<.001). Among children >61 months, those with low EC and high FR had the highest BMI (21.0, S.E. 0.86) while those with high EC and low FR had the lowest BMI (13.7, S.E. 0.66) (p=0.01). No interaction between FR x EC existed in children ≤ 61 months (p=0.37).

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Iron deficiency affects 273 million children worldwide and 2.4 million children in the U.S. Iron-deficiency anemia in infancy interferes with early brain development and can have serious, long-lasting effects on children’s development. For example, iron-deficiency anemia in infancy has been associated with difficulties in emotion and attention regulation, but it is currently not known how these regulatory deficits might contribute to “downstream” problem behaviors.

This study, based on over 1,000 Chilean children, examined whether iron-deficiency anemia in infancy leads to poor emotional and attentional control in childhood and whether these deficits lead to problem behaviors in adolescence. Study results showed that, when compared to children who had sufficient iron status in infancy, those who had iron-deficiency anemia had poorer emotional and attentional regulation at age 10 (they exhibited more emotional dysregulation and daydreaming), and these associations were related to more risk-taking, problematic alcohol use, and daydreaming, and these behavioral characteristics make such children “functionally isolated,” or less able to seek and receive environmental inputs crucial for social and cognitive skill development.

These findings demonstrate that iron-deficiency anemia in infancy contributes to “downstream” problem behaviors in adolescence through its influence on poor regulatory capacities in childhood. Findings underscore the potentially dangerous long-term effects from infant iron-deficiency anemia that emerge at adolescence, when risk-taking and rule-breaking behaviors, as well as excessive alcohol use and risky sexual behavior, can have serious consequences.

This study conducted by Dr. Sheila Gahagan and her team examined functional isolation in children who were iron-deficient anemic in infancy and its effects on social difficulties in middle childhood and problem behaviors in adolescence. Using a sample of 873 Chilean children, 45% of whom were iron deficient or iron-deficient anemic in infancy, results indicated that infant iron-deficiency anemia was associated with children’s social difficulties and poor behavioral characteristics at age 10 (being disliked, excluded from the peer group), which further linked to substance use and delinquent behaviors at adolescence.

Iron Deficiency in Infancy Can Lead to Risky Behaviors in Adolescence

The estimated global prevalence of anemia in children younger than 5 years is 43%, or approximately 273 million children worldwide. In the U.S., approximately 1.5 million children were diagnosed as iron deficient between 2007 and 2010. Like severely malnourished children, iron-deficient anemic children are listless, lethargic, and emotionally dull and disengaged. These atypical behavioral characteristics make such children “functionally isolated,” or less able to seek and receive environmental inputs crucial for social and cognitive skill development.

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Findings indicate that children who were iron-deficient anemic in infancy are functionally isolated in childhood, and that the early limited caregiver responsiveness derived from their anemic status in infancy contributes to long-term social difficulties in adolescence.

Iron Deficiency, Child Affect, and Maternal Unresponsiveness: Testing the Long-Term Effects of Functional Isolation

The persistence of negative outcomes derived from iron deficiency anemia found here, highlights the need for prevention, screening and treatment to lessen the long-term effects of this pervasive nutrient disorder.

Reference:
Teaching Children to Resist Overeating

Childhood obesity affects 1 out of every 3 children, and only 1/3 of children who go through a weight loss program are no longer overweight in adulthood. Emerging research suggests that people who are overweight, compared to people who are not overweight, have decreased ability to detect fullness (satiety responsiveness) and have trouble resisting eating when food is in front of them (food cue responsiveness).

Dr. Boutelle developed a new treatment model focusing on reducing overeating among children, called Regulation of Cues (ROC). ROC is designed to teach children to detect their fullness and resist eating when physically full. In the clinic, children and their parents learn and practice sensing their fullness during meals. After meals, they practice resisting eating their favorite foods. Children and their parents also learn coping skills to help them resist overeating in the clinic and at home.

In 2014, Dr. Boutelle and colleagues published the first pilot randomized clinical trial comparing the ROC program with a control among 8-12 year-old overweight children and their parents. Results of this study showed that the ROC program was well liked by both parents and children. The children in the ROC program decreased their eating when full. Additionally, the ROC children decreased their food cue responsiveness and lost more weight, although these only approached significance. Dr. Boutelle is now conducting a study further developing the food cue responsiveness part of the ROC program and is planning on conducting a larger clinical trial testing ROC.

References:

Research at UC San Diego/Rady Children’s Hospital in the Department of Pediatrics shows that UBE4B gene expression is strongly associated with neuroblastoma patient outcomes.
Targeted Therapeutics using Dual Targeted PI-3 Kinas/BRD4 Inhibitors; Discovery and Development of SF1126 and SF2523 “Bench to Bedside”

It is now clear that a greater understanding of the PTEN, PI-3 kinase and epigenetic signaling networks and the downstream targets of this tumor suppressor-oncogene pair will identify new therapeutic targets for clinical intervention since these pathways are mutated and/or altered at high frequency in human cancer. Based on these studies we have developed an RGD integrin targeted small molecule inhibitor, SF1126 for clinical application (Cancer Research 68: 206, 2008) (structure shown right). It was one of the first pan PI-3 kinase inhibitors to enter human clinical trials in cancer. Dr. Donald Durden and his team have now executed and completed the Phase I trial of SF1126 (Eur. J. Cancer, 2012). In 2015, they executed the first Phase I trial of a this dual PI-3K/BRD4 inhibitor in pediatric oncology via the NANT consortium. This was first time a child was treated with this class of a therapeutic agent.

The Durden lab has gone on to develop in silico a large number of dual targeted small molecules in a pipeline for the treatment of pediatric diseases. Currently, they have a large in silico drug discovery effort fueled by X-ray crystallography/molecular modeling and built around the PI-3k signaling pathway. Hundreds of small molecules are in their pipeline which inhibit these targets at nM potency with good safety profiles in animal models for toxicity (PNAS, 2017). Dual inhibitory chemotypes developed so far include: 1) PI3K/MEK 2) PI3K-PARP 3) PI3K/BRD4 (SF2523 (Fig.)) 4) PI3K/HDAC 5) PI3K/CDK4/6 and others. Dr. Durden’s lab has solved the co-crystalized structure of their dual PI-3K/BRD4 inhibitor, SF2523, bound to the active site of BRD4 at 1.8 Angstroms (BD1) (Fig.).

The in silico design and synthesis of small molecule which inhibit more than one therapeutic targets represents a paradigm shift in drug development and will exert orthogonal control over synthetic lethalities in cancer and other diseases and provide safer and more efficacious therapeutics for the treatment of pediatric diseases.

Reference:
There is a critical need for new treatment options for children with high-risk and relapsed neuroblastoma, and therapies directed against biologically relevant pathways are likely to be more effective with less toxicity. Aberrant growth factor receptor expression and function has been shown to be important in the pathogenesis of a variety of malignancies, and altered expression and signaling of number of growth factor receptors (GFRs) have been associated with neuroblastoma pathogenesis. GFR expression and activity levels are regulated by the processes of intracellular trafficking and degradation, which in turn are directed by ubiquitination of target proteins by ubiquitin ligases. Ubiquitin ligases represent both potential critical regulators of neuroblastoma tumor growth and survival and potentially important targets for the development of novel therapies.

UBE4B is an E3/E4 ubiquitin ligase involved in the regulation of GFR trafficking and degradation (Sirisaengtaksin et al, J Biol Chem, 2014). The UBE4B gene is located in the chromosome 1p36 region that is deleted in approximately one-third of neuroblastoma tumors, and 1p36 deletion is associated with poor neuroblastoma patient prognosis. Dr. Peter Zage and his team have shown that UBE4B gene expression is strongly associated with neuroblastoma patient outcomes (Figure). They have also established a key role for the ubiquitin ligase UBE4B in the regulation of GFR-mediated intracellular signaling in neuroblastoma cells, responses of neuroblastoma cells to therapy, and neuroblastoma tumor differentiation (Woodfield et al, Genes & Cancer, 2016; Figure), suggesting it may function as a novel tumor suppressor, prognostic marker, and therapeutic target.

Continued research in the Zage lab looks to build on these findings through a better understanding of UBE4B-mediated growth factor receptor trafficking and its link to responses and resistance to targeted therapies and through direct targeting of receptor trafficking as a unique approach to pediatric solid tumor therapy.

**FIGURE.** Association of UBE4B Expression with Neuroblastoma Pathogenesis. Using neuroblastoma patient data-sets in the R2 Genomics Analysis and Visualization Platform (http://r2.amc.nl), patients were divided into high (blue) and low (red) UBE4B gene expression groups and event-free survival curves generated (top left). Relative UBE4B expression levels were plotted in patients with and without relapse from the same patient data-sets (middle left). Numbers of patients with high or low UBE4B expression among patients either alive or dead at the time of data censoring for the data-sets are also shown (bottom left). Neuroblastoma patient samples were lysed and Western blots for UBE4B and phosphorylated ERK were performed and quantified (top right; Spearman’s correlation R = -0.7381 and p = 0.03655). Neuroblastoma tumor samples were evaluated by immunohistochemistry for UBE4B expression, with differentiated tumors demonstrating high levels of expression (middle right) and undifferentiated tumors demonstrating low or absent expression (bottom right).
Substantial institutional variability in testing and treatment of bronchiolitis, a major cause of pediatric hospitalization is being studied by researchers at UC San Diego/Rady Children’s Hospital in the Department of Pediatrics.
BRIAN WILLIAMS, MD 
Assistant Clinical Professor of Pediatrics and Medicine 
Hospital Medicine 

This project, conducted by Dr. Brian Williams and his team, includes a quality improvement initiative as well as a focused research component. They have a goal of screening >95% of children hospitalized at Rady Children’s Hospital – San Diego and have been able to achieve this goal through changing nursing admission workflow.

Referral to the California Smokers’ Helpline. This aim of the project is to improve the documented rates of “passive” (second or third-hand) smoke exposure. The current rate is 9%, however, based on national data Dr. Williams expects that at least 25% of children are exposed to passive smoke. His project includes automating a referral to the California smokers’ Helpline (goal referral rate of 50%). Future goals include providing in-person counseling (goal referral rate of 50%).

Research: Dr. Williams is conducting two research studies. One study compares attitudes and practices of bedside nurses and respiratory therapists in the hospital. The other study focuses on smoking cessation education of pediatric residents. Both studies are multi-center studies including Rady Children’s Hospital/San Diego, Mattel Children’s Hospital UCLA, UCSF Benioff Children’s Hospitals at San Francisco and Oakland, UC-Davis Children’s Hospital.

This project is clinically important as eliminating smoke exposure can improve child health, caregiver health, and potentially reduce the chance of the child becoming a smoker later in life.

Research Team: 
Brian Williams, MD (UCSD), Pr. - Joshua Melvin, MD (UCD), Amanda Kussak, MD (UCLA); Camila Gavel, MD (UCI); Cindy Nelson Pungy, RN, PNP (UCSF-Oakland); Kathleen Tedh, Ph.D. (UCSF), Elisa Tong, MD (UC-Davis), Ulfat Shaikh, MD (UC-Davis), Lisa Shiek, MD (UC-Davis); Winnie I., RN, FNP (UCSF-Oakland).

References: 


HEATHER C. PIERCE, MD 
Associate Clinical Professor of Pediatrics 
Hospital Medicine 

Variability of Intensive Care Management for Children with Bronchiolitis

Bronchiolitis is the leading cause of hospitalization in infants, with ~125,000 to 150,000 hospitalizations each year in the United States. Despite the prevalence of this disease, the management of children with bronchiolitis varies widely. Both Continuous positive airway pressure (CPAP) and High Flow Nasal Cannula (HFNC) improved respiratory rates, oxygenation, and work of breathing in children with severe bronchiolitis. Unfortunately, current data to identify a subgroup that confers the greatest benefit are not available, nor is there a consensus on objective measures to guide escalation of therapy.

This prospective, multicenter 16 site cohort study was conducted for 3 consecutive years during the 2007–2010 winter seasons, as part of the Multicenter Airway Research Collaboration (MARC), a program of the Emergency Medicine Network (EMNet) at UCNet (www.ucnet-asa.org). Data on children ~2 years old hospitalized with bronchiolitis who received admission to the ICU and/or continuous positive airway pressure (CPAP) within 24 hours of admission were examined. Among the 2207 enrolled patients with bronchiolitis, 342 children met inclusion criteria. Clinical data and nasopharyngeal aspirates were collected. Respiratory distress severity scores and intraclass correlation coefficients were calculated. The median value of the percentages for all sites using CPAP was 15% (range: 3%-100%), the HFNC was 24% (range: 0%-94%). Adjusting for site-specific random effects (as well as children’s demographic characteristics and severity of bronchiolitis), the intraclass correlation coefficient for CPAP and/or intubation was 27% (95% confidence interval: 8.4–44); for HFNC, it was 44.7% (95% confidence interval: 24.7–67).

In this multicenter study of children requiring intensive care for bronchiolitis, Dr. Heather Pierce and her team identified substantial institutional variability in testing and treatment, including use of CPAP, intubation, and HFNC. These differences were not explained by between-site differences in patient characteristics, including severity of illness. Further research is needed to identify best practices for intensive care interventions for this major cause of pediatric hospitalization.

Reference: 

### TABLE. ICC Assessing Between-Site Variability in the Use of Tests and Treatments Given to Children With Bronchiolitis Who Received Intensive Care With and Without Adjustment for Children’s Characteristics

<table>
<thead>
<tr>
<th>Test and Treatment</th>
<th>Visit</th>
<th>Adjusted for Site Random Effects Only</th>
<th>Adjusted for Site Random Effects and Children’s Characteristics*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ICC</td>
<td>90% CI</td>
<td>90% CI</td>
</tr>
<tr>
<td>CPR</td>
<td>20.4</td>
<td>3.9-37.9</td>
<td>24.7</td>
</tr>
<tr>
<td>CBC</td>
<td>11.8</td>
<td>2.4-24.6</td>
<td>12.0</td>
</tr>
<tr>
<td>Antibiotics</td>
<td>17.1</td>
<td>-0.1-34.3</td>
<td>17.1</td>
</tr>
<tr>
<td>Antibiotics</td>
<td>27.0</td>
<td>13.7-40.3</td>
<td>27.0</td>
</tr>
<tr>
<td>Antibiotics</td>
<td>17.4</td>
<td>-0.3-35.6</td>
<td>17.4</td>
</tr>
<tr>
<td>Antibiotics</td>
<td>0.8</td>
<td>0.0-60.8</td>
<td>0.8</td>
</tr>
<tr>
<td>Antibiotics</td>
<td>9.7</td>
<td>0.3-29.8</td>
<td>9.7</td>
</tr>
<tr>
<td>Antibiotics</td>
<td>23.8</td>
<td>9.8-47.2</td>
<td>23.8</td>
</tr>
<tr>
<td>Antibiotics</td>
<td>26.8</td>
<td>13.3-40.1</td>
<td>26.8</td>
</tr>
<tr>
<td>Antibiotics</td>
<td>16.8</td>
<td>6.2-26.0</td>
<td>16.8</td>
</tr>
<tr>
<td>Antibiotics</td>
<td>21.3</td>
<td>8.4-43.9</td>
<td>21.3</td>
</tr>
<tr>
<td>Antibiotics</td>
<td>42.3</td>
<td>26.6-58.1</td>
<td>42.3</td>
</tr>
</tbody>
</table>

CPC: chest radiograph; NC: not calculable.
*Age, gender, race, insurance, median household income according to zip code, EDSS, and presence of other acute care and intensive care research.
Because the performance of automated flow cytometric urinalysis is not well described in pediatric urinary tract infection, Dr. John Kanegaye and colleagues determined the diagnostic performance of the automated cell counts and emergency department (ED) point-of-care (POC) dipstick urinalyses in the evaluation of young febrile children. From a convenience sample of febrile pediatric ED patients ≥48 months of age who underwent orchiid catheterization to obtain POC and automated urinalyses and urine cultures, they performed receiver operating characteristic (ROC) analyses and calculated diagnostic indices for POC dipstick and automated cell counts at different cut points.

Of 342 eligible children, 42 (12%) had urinary bacterial growth ≥50,000/mL. Concurrent manual hemocytometer and automated WBC counts performed on 323 samples (94%) were within 10 cell/μL for 90% (Figure). The areas under the ROC curves were: automated WBC, 0.97; automated bacterial count, 0.998; POC leukocyte esterase, 0.94; and POC nitrite, 0.76. Automated bacterial counts had the best ability to diagnose UTI. A bacterial count of 100/μL had sensitivity of 95% and specificity of 98% at the cutoff of ≥1 leukocyte esterase or positive nitrite. Combinations of WBC and bacterial counts did not outperform bacterial counts alone. ROC and automated tests performed as well in the subset of patients 0-90 days of age as in older patients.

In this study, Dr. Kanegaye reports the excellent diagnostic performances of automated cytometry and POC dipstick among young febrile children evaluated for UTI in a pediatric ED. Using automated flow cytometry on catheterized urine samples from febrile young ED patients at risk for UTI, bacterial count ≥250 cell/μL has the best ability to diagnose UTI. A flow cytometric urinalysis is not well described in pediatric urinary tract infections in children in the emergency department.

Automated Urinalysis and Urine Dipstick in the Emergency Evaluation of Young Febrile Children

References:


John Kanegaye, MD
Clinical Professor of Pediatrics
Emergency Medicine

The Prevalence of Bruising Among Infants in Pediatric Emergency Departments

Nominate data are necessary to guide the evaluation for abuse among infants with bruising. However, previously published prevalence data from well-child care clinics, general EDs, and abuse clinics may not apply to pediatric ED settings where patients present with a full spectrum of medical, surgical, traumatic, and social complaints. Therefore, a team of investigators led by Dr. John Kanegaye determined the prevalence of bruising in the first year of life in previously healthy infants presenting to pediatric EDs. They also determined the prevalence of bruising among age and chief complaint and the frequency of child abuse evaluations of infants with bruising.

In this prospective, observational, multicenter study, Dr. Kanegaye’s team used structured sampling to assemble a cohort of 2,488 infants aged 12 months or younger presenting to pediatric EDs. Pediatric emergency medicine clinicians performed complete skin examinations to screen for bruising, and investigators documented skin findings, age, chief complaint, and abuse evaluation. Bruising was present in 88 infants (3.5%, 95% CI 2.9% to 4.4%), and prevalence increased with age (Figure). The prevalence of bruising was lower among infants ≤5 months of age than among those >5 months of age (1.3% vs. 6.4%, difference 5.1%, 95% CI 3.6% to 6.8%). The majority (87%) of infants with bruising had a trauma chief complaint. However, 69% of infants with a trauma chief complaint lacked bruising. Premobile infants rarely had bruises (18/1,395; 1.3%), and the majority of those with bruises (15/18; 83%) had a trauma chief complaint. Pre-mobile infants with medical chief complaints were rarely bruised (3/1,294; 0.2%). Abuse evaluations occurred for 38 infants (1.5%) and were most common (30/1; 91%) among pre-mobile bruised infants.

These discoveries reveal that the prevalence of bruising is low among previously healthy infants evaluated in the pediatric ED but increases through the first year of life. Bruising is especially uncommon among infants with medical chief complaints, and clinicians must be vigilant to identify and elucidate the cause of bruising in these infants. In order to inform guidelines and practice, future investigation will determine the specific bruise characteristics resulting from abusive and accidental injury.

References:


John Kanegaye, MD

FIGURE. Number of patients with and without bruises and bruise prevalence by patient’s age (in months).
Bacteremia is a potentially fatal disease. Although the reported rates of bacteremia in febrile children less than three years of age over the past ten years is less than 1%, the rate is still of great clinical concern, leading to blood culture testing in previously healthy, young, febrile children. False positive blood cultures vastly outnumber true positive blood cultures by 2-5 times. Multiple studies found increased financial costs due to false positive cultures, as well as patient and family stressors. Identifying true versus false bacteremia at the time of a positive blood culture result could decrease the costs associated with false positives.

Dr. Elizabeth Mannino Avila and her team conducted a retrospective record review and found no significant difference between true bacteremia (TB) and false bacteremia (FB) in: Age, gender, days of fever, total white blood cell count, absolute neutrophil count, localizing symptoms, positive urine culture, admission, lethargy, localizing symptoms, vomiting, or diarrhea. In the final, multivariate model, only sick contact exposure and hours to positive culture were significantly associated with true bacteremia.

Despite reductions in vaccine-related serious bacterial infections, clinicians still struggle to identify children at risk. Providers aim to reduce unnecessary care, while promptly addressing true bacteremia. Dr. Avila’s study offers variables to inform clinical decisions and family discussions around bacteremia risk.

**Research Team:** Elizabeth Mannino Avila (PI); Erin Stucky Fisher, MD, MHM; Kyung Rhee, MD, MSc, MA

**True versus False Bacteremia in Febrile Children Under 3 Years of Age**

**FIGURE.** Average Hours to Positive Blood Culture by Organism

**Other includes:** Aerobic non-spore forming GPR, Chryseobacterium meningosepticum, Corynebacterium Sp not jeikeium, diptheroids, micrococcus species, Propionibacterium, Saphrophytic neisseria.
Researchers at UC San Diego/Rady Children’s Hospital in the Department of Pediatrics have discovered a signaling pathway in macrophages that regulate the pro-fibrotic response to lung injury and identify novel drugs.
By studying human extreme phenotypes and taking advantage of experiments in nature, Dr. Haddad and his team will learn about not only disease but also normal biology and physiology. Furthermore, although this disease occurs only at high altitude when humans are exposed to hypoxia, the response of humans to various environmental conditions will provide more information about how mechanisms go astray at sea level.

Reference:

FIGURE 1. Production of erythroid cells from human iPSC. (A) Schematic representation of the successive culture steps for production of cultured RBCs (rRBCs) from fibroblasts. Fibroblasts were transformed to iPSCs using the Yamanaka factors. (B) CD analysis of various CD markers (CD34, CD45, CD71, and CD235a) under normoxia for sea-level samples in time in culture. Each experiment was done in three replicates, and the experiment was done at least three times. Error bars represent the mean ± SEM of at least two to three measurements. The experiment was repeated at least three times.

FIGURE 2. Hypoxic response of sea-level, non-CMS, and CMS cells. Marked response of CMS samples. A. Flow cytometric analysis using CD235a (glycophorin A) as a marker after culturing as detailed in Fig. 1 at the EB stage at day 28. Representative FACS plots of sea level, non-CMS, and CMS in normoxia (left) and in hypoxia (right). The dot plot represents the live cells as gated through propidium iodide. CD235a+ cells are shown in red along the y axis, and CD235a− cells are shown in blue. The percentage in each figure represents the relative proportion of CD235a cells. There are major differences between CMS (bottom) versus the non-CMS (middle) and sea-level (top) samples. The FACS plots are representative of one experiment. Similar results were obtained in all the experimental repeats. B. Summary of hypoxic response of CMS patients (n = 5 subjects) and non-CMS (n = 4 subjects) and sea-level (n = 3 subjects) control subjects. The graph depicts the relative proportion of CD235a cells. There are significant differences between CMS (bottom) versus the non-CMS (middle) and sea-level (top) samples. The FACS plots are representative of one experiment. Similar results were obtained in all the experimental repeats. C. Summary of interclonal variability among the subjects: three clones (clones 1, 2, and 3) were tested for three subjects (subjects 1, 2, and 3) for each group: CMS, non-CMS, and sea level. The y axis depicts the relative proportion of CD235a under hypoxia (5% O2). There is a significantly striking difference between CMS, non-CMS, and CMS under hypoxia. *** P < 0.001. D. Summary of interclonal variability among the subjects: three clones (clones 1, 2, and 3) were tested for three subjects (subjects 1, 2, and 3) for each group: CMS, non-CMS, and sea level. The y axis depicts the relative proportion of CD235a under hypoxia for different clones. D. Dose response. Graph represents the relative response of CMS, non-CMS, and sea-level cells to 21%, 10%, and 1.5% O2 levels. CMS shows hyper-responsiveness at 10, 5, and 1.5% O2.
Given almost no credit for keeping humans alive, the small airways dance incessantly to keep clean the lungs of the debris and pathogens that are inhaled with every breath. While microanatomists have reported for more than half a century that the luminal surface of the small airways (bronchioles) are highly plicated with furrows and ridges that run axially along their length, these structures appeared more decorative than functional. But well beyond decoration, these little furrows and ridges assume the vital responsibility for producing and maintaining the soap and water, aka ‘ASL’ (airway surface liquid), essential to keep pulmonary infections from taking over.

Dr. Paul Quinton and his team were surprised to discover that 1) the epithelium of the small airway is constantly secreting and absorbing ASL simultaneously (4) and 2) it is the unique plicated structure of the luminal surface that permits such apparently paradoxical behavior; that is, they found using immunocytochemistry that a biomarker (NKCC1) for secretory cells, consistently localized to cells in the fundal region of the furrows, but not in the ridges, of plications (Figs. 1, 2) (1). The Quinton lab also observed in preliminary investigations that a marker (β-ENaC) for absorbing cells appeared mainly in cells of the ridges of the plications. Hence, the cells in the furrows secrete while cells in the ridges concurrently absorb ASL so that the luminal surfaces are constitutively washed without ever leaving the airway dehydrated or flooded with fluid. They also found evidence that these airways secrete HCO$_3^-$ (2), essential to thin secreted mucins as what might poetically be called ‘soap’.

Articulated, picture a two-step dance in time with breathing as the airways widen, and then contract, repetitively. Obligatorily, the furrows of the plications keep in step with these changes so that ASL is physically squeezed out of the furrow space and then allowed to return (Fig. 1). These discoveries provide a new basis for determining novel approaches to treating airway diseases—Physiology choreographs the best dances.

**References:**

**The Texas Two Step**

Given almost no credit for keeping humans alive, the small airways dance incessantly to keep clean the lungs of the debris and pathogens that are inhaled with every breath. While microanatomists have reported for more than half a century that the luminal surface of the small airways (bronchioles) are highly plicated with furrows and ridges that run axially along their length, these structures appeared more decorative than functional. But well beyond decoration, these little furrows and ridges assume the vital responsibility for producing and maintaining the soap and water, aka ‘ASL’ (airway surface liquid), essential to keep pulmonary infections from taking over.

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With a little imagination, picture a two-step dance in time with breathing as the airways widen, and then contract, repetitively. Obligatorily, the furrows of the plications keep in step with these changes so that ASL is physically squeezed out of the furrow space and then allowed to return (Fig. 1). These discoveries provide a new basis for determining novel approaches to treating airway diseases—Physiology choreographs the best dances.

**References:**

**FIGURE 1.** Integrated functions and structure of the small airways: The plications of the surface of the small airway recycle the airway surface liquid (ASL) by actively secreting fluids from secretory cells (blue) within the fundal zone of the furrows. As the secreted fluid replenishes the furrow space, it is reabsorbed by the absorptive cells (red) at the ridges of the plications and recycled back to the serosal fluid from where it originated. In addition, active HCO$_3^-$ secretion by the epithelium into the ASL provides the essential HCO$_3^-$ rich environment for the release, expansion, and thinning of mucins to provide the mucus blanket for the ciliated escalator to entrap and convey pathogens and debris out of the airway.

**FIGURE 2.** Distribution of Secretory Cells: Immunocytochemical localization of the T84 antibody (green) for NKCC1 identifies secretory cells in the epithelial surface. NKCC1 labeled (green) cells are distributed predominately within the fundal zone of the furrows of the plications. Inset shows cross sections of small airway illustrating cut out of plications as shown in immuno-stained image (Blue: DAPI nuclear stain) (taken from AJRCMB 54:3, 2016).
A research team led by Dr. Donald Durden and Dr. James Hagood in the Department of Pediatrics at UC San Diego/Rady Children’s Hospital have uncovered a new target to treat fibrotic diseases including lung fibrosis. Lung fibrosis occurs in idiopathic pulmonary fibrosis (IPF) and other chronic lung diseases of children e.g. BPD which are characterized by the deposition of fibrous tissue. These diseases lack effective therapy and contribute to significant morbidity and mortality in the world.

Using genetically altered mice, researchers unearth a druggable signaling pathway in macrophages involving a kinase, Syk and its downstream target Rac2 that regulates the pro-fibrotic response to lung injury. The study was orchestrated by junior faculty members, Drs. Shweta Joshi and Simon Wong in the Divisions of Pediatric Hematology/Oncology and Pulmonary Medicine in the Department of Pediatrics.

Mice deficient in Rac2 were protected against bleomycin-induced fibrosis, normal lung function and displayed diminished collagen deposition in association with lower expression of alternatively activated profibrotic macrophage markers. Findings demonstrated a macrophage-dependent process by which the injection of pro-fibrotic macrophages from the normal mice restored the bleomycin induced pulmonary fibrosis susceptibility in Rac2 knockout mice, establishing a critical role for a macrophage-Rac2 signaling axis in the regulation of macrophage induced lung fibrosis in vivo. They also demonstrate that markers of alternative macrophage activation are increased in patients with IPF.

Taken together, these studies define an important role for this pathway in tissue fibrosis. Moreover, Drs. Durden and Hagood and their research team have identified novel drugs that attack this pathway to block fibrotic diseases.

RAC2 is Required for Alternative Macrophage Activation and Bleomycin Induced Pulmonary Fibrosis; A Macrophage Autonomous Phenotype

**Research Team:**
Shweta Joshi, Alok R. Singh, Muamer Zulcic. UCSD Department of Pediatrics, Moores UCSD Cancer Center, University of California, San Diego, 92093.

**Graphs in A and C represent mean ± SEM with n = 3–4 samples/group. Data was analyzed by One-way ANOVA with post-hoc Tukey’s multiple comparison tests, **p ≤ 0.01 and ***p ≤ 0.001 when WT bleomycin treated group was compared to saline treated groups or the Rac2-/- bleomycin treated group. Data shown here is representative of one experiment, but all experiments were repeated three times with similar results."
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