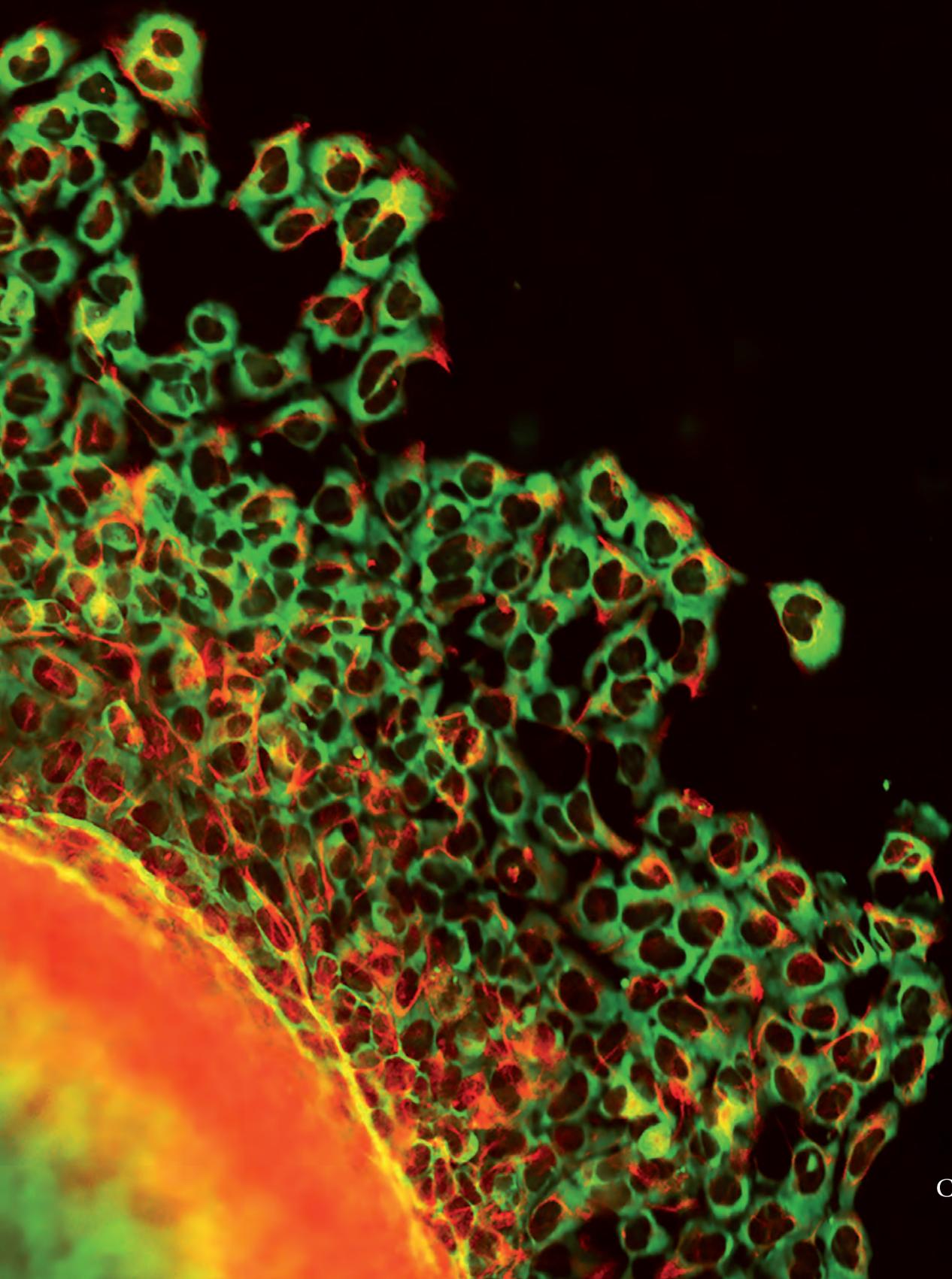


CHILDREN'S FOUNDATION RESEARCH INSTITUTE

Scientific Report



LeBonheur
Children's Hospital

UT THE UNIVERSITY OF
TENNESSEE
HEALTH SCIENCE CENTER



Children's Foundation
Research Institute



In the past 25 years, the Children's Foundation Research Institute (CFRI) and its impact have grown substantially, enabling investigators at Le Bonheur Children's Hospital to conduct groundbreaking research that will improve the lives of children in our community and beyond. This scientific report provides a glimpse into our investigators' work in critical areas of research, such as congenital heart disease, epilepsy, obesity, tuberous sclerosis complex and diabetes.

The CFRI facilitates all basic and translational research at Le Bonheur by providing critical infrastructure. In the CFRI, investigators have access to state-of-the-art equipment and guidance for all aspects of the research process. The CFRI supports all of Le Bonheur's service lines, and we have also strengthened relationships with other departments and colleges at the University of Tennessee Health Science Center, as well as St. Jude Children's Research Hospital and the University of Memphis.

The high productivity of CFRI investigators is evidenced by more than 220 peer-reviewed publications in 2019, with multiple articles in high impact journals, such as *Acta Neuropathologica*, *American Journal of Respiratory and Critical Care Medicine* and *Nature Communications*. In the past year, our scientists have made breakthroughs in treating obesity and diabetes in children, influenza and respiratory syncytial virus infections in infants, cystic fibrosis, kidney cysts and tumors in patients with tuberous sclerosis, patent ductus arteriosus in infants, life-threatening cardiomyopathies, liver and gastrointestinal diseases, complex orthopedic problems, severe trauma and pediatric neurological disorders, including complex seizures and brain tumors.

The generosity of our donors makes these achievements possible. The Children's Foundation of Memphis played a pivotal role in establishing the CFRI and has continued to generously support our efforts. The Assisi Foundation of Memphis, Plough Foundation, Brinkley Foundation and Belz Foundation have also made significant contributions to the CFRI.

The CFRI is dedicated to providing investigators with expertise, services, technology and resources to conduct research that will impact the children of today, as well as future generations. Moving forward, the efforts of the CFRI will enable Le Bonheur to become a nationally recognized pediatric research center and make significant contributions to the understanding, prevention and treatment of childhood disease and injury. We anticipate these contributions will continue to better the health and lives of children.

With gratitude,

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ABOUT THE CFRI

The Children's Foundation Research Institute (CFRI) was established in 1995 as a partnership among Le Bonheur Children's Hospital, the University of Tennessee Health Science Center (UTHSC) and the Children's Foundation of Memphis (CFOM) to provide infrastructure, expertise, support and coordination

to facilitate basic, clinical and translational research with the goal of improving the health of children.

In December 2010, Le Bonheur opened a new state-of-the-art hospital, and the old hospital building was renovated into a Research Tower to house the CFRI. The Research Tower includes more than 30,000 square feet of basic laboratory space and 12,000 square feet of pediatric clinical research space, as well as administrative, clinical and basic research core facilities, faculty and staff offices, an auditorium and meeting rooms. This facility reflects Le Bonheur's emphasis on and investment in research efforts.

The CFRI provides resources and personnel to Le Bonheur and the UTHSC Department of Pediatrics faculty for grant preparation and submission, scientific manuscript writing and publishing, clinical trial budgeting, contracting, invoicing and collections, clinical research studies and IRB and regulatory approval and implementation. All of these services provide comprehensive support to accomplish quality basic and clinical research.

Le Bonheur focuses research on areas that are major health problems for the population and community it serves, including pediatric obesity, asthma and health care disparities. Additionally, Le Bonheur has multiple centers of excellence, including our Neuroscience Institute, Tuberous Sclerosis Center of Excellence and Heart Institute that attract patients from all over the world. Le Bonheur has also developed cutting-edge research programs, including the Biorepository and Integrative Genomics (BIG) and microbiome projects that benefit research in several subspecialties.





CRITICAL CARE



CRITICAL CARE INTENSIVISTS STUDY IMMUNOPARALYSIS, MODS IN MULTICENTER STUDY

Critical Care Intensivists Samir Shah, MD, Alina Nico West, MD, PhD, and Sachin Tadphale, MD, joined researchers at Nationwide Children's Hospital on an NIH-funded multicenter study to investigate immunoparalysis in multiorgan dysfunction syndrome (MODS). This study, named Pediatric Research of Drugs, Immunoparalysis and Genetics during MODS (PARADIGM), will involve 1,400 children across multiple sites in the United States and aims to reduce the incidence of immunoparalysis, associated complications and death in patients with MODS. The investigators postulate the risk of developing immunoparalysis in MODS can be predicted using patient-specific, diagnosis-specific and treatment-specific factors.

Pediatric MODS is a common diagnosis among critically ill children in the intensive care unit (ICU). MODS is defined by abnormal function of organ systems that develops following acute illness or injury. This condition is associated with an immunosuppressed state that can progress to immunoparalysis. In immunoparalysis, the innate immune system, which provides the initial response to an infection, is severely impaired. As a functional immune system is critical to prevent infection, organ failure and death, immunoparalysis can have deadly consequences. Immunoparalysis is confirmed by

exposing a sample of whole blood from a patient to lipopolysaccharide (an endotoxin present on the outer membrane of some bacteria) and measuring the ability of whole blood to produce TNF- α (an inflammatory cytokine) in response. If the sample produces little to no TNF- α , then the patient is diagnosed with immunoparalysis.



Despite the prevalence of immunoparalysis in MODS, determining which patients will develop this serious complication is difficult. The investigators predict this study will reveal TNF- α thresholds, diagnoses, treatments and genetic factors associated with increased morbidity and mortality. This study may also produce tailored therapies and a TNF- α assay suitable for identifying patients at risk of severe outcomes.

A NEW MODEL OF PULMONARY HYPERTENSION IN RSV INFECTION

Respiratory syncytial virus (RSV) commonly induces pulmonary hypertension in infants, appearing

in up to 75% of infants with moderate to severe RSV bronchiolitis. Pulmonary hypertension secondary to RSV infection is a risk factor for increased morbidity and mortality, especially among infants with congenital heart disease. Despite the prevalence of pulmonary hypertension in RSV bronchiolitis, the exact mechanisms are unclear. To address this knowledge gap, Critical Care Intensivist Dai Kimura, MD, FAAP, and colleagues established the first mouse model of RSV-induced pulmonary hypertension.

The investigators infected mice at 5 days of age and again at 4 weeks of age to reproduce the initial infection and later reinfection often observed in human infants. They included multiple measures to assess the impact of RSV infection on pulmonary

function and characteristics, such as oxygen saturation, echocardiography, right ventricular systolic pressure (RVSP) and histology.

Findings from echocardiography and RVSP were consistent with pulmonary hypertension in mice infected with RSV. Lung histology also revealed inflammation, fibrosis, increased arterial thickness and muscularization, indicative of pulmonary hypertension.

Based on these findings, Kimura and his colleagues successfully established the first model of pulmonary hypertension secondary to RSV. This new model will provide fresh opportunities for researchers to investigate the pathogenesis of RSV-induced hypertension and explore potential therapeutic interventions.





A FRESH START AND NEW BEGINNINGS FOR PATIENTS WITH TYPE 1 DIABETES

To help children and adolescents with poorly controlled type 1 diabetes and a history of recurrent diabetic ketoacidosis (DKA) admissions, Clinical Psychologist Angelica Eddington, PhD, and Endocrinologist Kathryn Sumpter, MD, see patients in the Fresh Start Clinic on a monthly basis.

Type 1 diabetes is a prevalent chronic disease among children and adolescents in the United States with more than 15,000 individuals diagnosed each year. To prevent serious complications, patients must meet glycemic control targets defined by the American Diabetes Association, i.e., hemoglobin A1c (HbA1c) \leq 7.5%. Poorly controlled type 1 diabetes can cause DKA, a life-threatening condition in which the blood becomes acidic. Unfortunately, many children struggle to maintain glycemic control and require costly medical intervention.

The goal of the Fresh Start Clinic is to improve glycemic control and lower hospitalization rates among patients with type 1 diabetes. Sumpter and Eddington partner with

certified diabetes educators, registered social workers and registered dietitians to help children overcome barriers in their care. This multidisciplinary team uses motivational interviewing, a technique that helps families identify areas of opportunity and form goals to work toward until the next clinic visit.

Sumpter has also developed the Be Empowered Grown Intentionally Now (BEGIN) program in collaboration with Le Bonheur Community Outreach. BEGIN is supported by funding from the



Endocrinologist Kathryn Sumpter, MD, started two initiatives to help children with poorly controlled type 1 diabetes. The Fresh Start Clinic and Be Empowered Grow Intentionally Now (BEGIN) programs aim to prevent diabetic ketoacidosis admissions to the hospital.

Memphis-based Urban Child Institute and focuses on children and adolescents with poorly controlled type 1 diabetes throughout Shelby County. The BEGIN program launched in February 2020 to provide support for patients with type 1 diabetes in real-life settings.

Sumpter and her colleagues are currently enrolling patients aged 8-17 years living in Shelby County and insured through Medicaid (TennCare) with A1c > 10% or a history of DKA admission in the preceding 12 months. Patients in the BEGIN program will receive more clinical care in addition to comprehensive psychosocial and practical support, including a continuous glucose monitor, dedicated social worker and therapist, easy access to diabetes providers via texting and free transportation to clinic.

LE BONHEUR ENDOCRINOLOGIST HELPS BUILD HEALTHY BONES

When Endocrinologist Alicia Diaz-Thomas, MD, first came to Le Bonheur, she encountered a young patient with bone disease. Her experience assisting the child's physician with a bisphosphonate infusion (a bone-strengthening treatment) alerted her to the need for management of bone disease and promotion of bone health in pediatric endocrinology.

"Treatment of bone disease is an emerging field in pediatric endocrinology. A large amount of work is being done now to determine best practices. It's not something you're trained for," said Diaz-Thomas.

Over time, Diaz-Thomas began encountering more children with bone disease through referrals from orthopedics and neurology.



"I saw children with mobility problems due to epilepsy or cerebral palsy who developed disease osteoporosis. Children on long-term steroid treatment for Duchenne muscular dystrophy or asthma can also develop secondary bone conditions," said Diaz-Thomas. "I also saw patients with osteoporosis. It is a leading cause of morbidity in adults, but the underpinnings of the disease are in childhood."

These encounters made it clear to Diaz-Thomas that supporting proper bone growth in children is critical to ensure healthy bones in adults. To meet this growing need, Diaz-Thomas founded the Bone Health Clinic at Le Bonheur in April 2019. In the Bone Health Clinic, physicians see patients with a variety of primary and secondary bone conditions who often require care from a multidisciplinary team. As the clinic grows, the goal is to have patients see every specialist on the same day to reduce the burden of multiple appointments on families and enable collaboration among physicians on a child's care team. Bone disease refers to a family of conditions affecting bone strength, divided into primary and secondary bone conditions. Primary bone

conditions are caused by genetic mutations, whereas secondary bone conditions are acquired through nutritional deficits, steroid treatment or restricted mobility. In osteogenesis imperfecta, a primary bone disease, bones become brittle and prone to fractures. Osteogenesis imperfecta also causes a host of systemic issues, including growth problems, hearing difficulties and cardiopulmonary issues. In rickets, a secondary bone condition, bones become soft and weak. Rickets is usually caused by nutritional deficits due to restricted diets or prolonged breastfeeding without vitamin D supplementation. Rickets can be resolved by vitamin D and/or calcium supplementation, but it can slow or reduce bone growth during childhood, a critical period for bone growth.

“The Bone Health Clinic is meeting a previously unrecognized need. Bone diseases are becoming more common, and we need more people working in this space. Our hope is to move toward preventive care provided by primary care physicians to help children at risk of bone disease,” said Diaz-Thomas.

EMPOWERING YOUTH WITH TYPE 2 DIABETES

In January 2019, Endocrinologist Amit Lahoti, MD, initiated the multidisciplinary EMPOWER clinic for youth with type 2 diabetes to address the unique

clinical and research needs of this growing population in Memphis. Lahoti leads this endeavor with registered dietitians, diabetes educators, nursing staff and a



social worker. Clinical Psychologist Angelica Eddington, PhD, has contributed to evaluation and monitoring tools for the clinic based on evidence-based research.

Type 2 diabetes is an increasing problem in adolescents with the highest rates observed among adolescents aged 15-19 years in minority populations. At Le Bonheur’s diabetes clinic, approximately one in every five diabetes patients has type 2 diabetes. Management of type 2 diabetes is different from management of type 1 diabetes in multiple ways, including a greater

emphasis on lifestyle changes for patients and their families, different insulin titration protocols, earlier screenings and management of diabetes complications and comorbidities.

Prior to the EMPOWER clinic, type 2 diabetes was frequently managed the same way as type 1 diabetes, creating an unmet need. The EMPOWER clinic at Le Bonheur provides a unique opportunity to contribute to the health of children with type 2 diabetes locally by improving clinical care. This clinic also impacts the care of children with type 2 diabetes at regional and national levels by increasing their representation in multicenter research, national consortiums and databases.





THERAPY FOR PRIMARY SCLEROSING CHOLANGITIS

In primary sclerosing cholangitis (PSC), a rare liver disorder, chronic inflammation and ensuing fibrosis damage the bile ducts in the liver, which results in scarring and liver damage and leads to cirrhosis and end-stage liver disease. Treatments for PSC are limited, and liver transplantation is the only cure. Ursodeoxycholic acid (UCDA), a primary bile acid in bears used to treat several liver disorders in adults and children, is often used off-label to treat PSC. However, data supporting the efficacy of UCDA in PSC are limited. Furthermore, recent data from adults with PSC revealed potential adverse outcomes with high doses of UCDA.



Pediatric Hepatologist Dennis Black, MD, who has a long-standing interest in PSC, led a novel multicenter prospective trial to evaluate the impact of UCDA treatment and withdrawal on PSC in children, funded by a grant from the FDA Office of Orphan

Product Development. This disease is especially rare in children, and parents had to agree to temporarily discontinue a medication that might be helping their child.

Black and colleagues at 12 major pediatric liver programs across the country recruited 27 participants with PSC who took UCDA between 2011 and 2016. To examine the impact of UCDA withdrawal and reinstatement on PSC, the investigators divided the trial into four phases. In phase I, patients maintained their normal prescribed dose of UCDA. In phase II, the dose of UCDA was reduced by 50%. In phase III, UCDA was stopped completely. In phase IV, UCDA was restarted at a standardized dose.

Liver enzymes alanine aminotransferase (ALT)

and gamma-glutamyl transferase (GGT), adverse events, inflammatory biomarkers, bile acids, UDCA levels and complete blood counts were monitored. Based on changes in ALT and GGT in response to UCDA withdrawal, the investigators placed patients into three UCDA response subgroups: null,

ALT and GGT <29 IU/L; flare, ALT and/or GGT >100 IU/L during phase II and/or III; and indeterminant, ALT and/or GGT >29 IU/L and <100 IU/L during phase II and/or phase III. Approximately one-third of study participants were in each group.

A total of 22 patients completed the study. Among these patients, three experienced serious adverse events. Two of these patients experienced a significant disease flare and were moved to phase IV of the study with subsequent remission. All patients in the flare group showed remission upon reinstatement of UCDA. Notably, GGT levels were significantly higher and albumin levels were significantly lower at baseline in the flare and indeterminant groups than in the null group. Baseline plasma levels of IL-8 and TNF- α were higher in the flare group than in the null and indeterminant groups. Despite remaining within the normal range, GGT significantly increased in the null group during or after UCDA withdrawal.

Although the results of this trial are limited by the small number of participants and brief period of UCDA withdrawal, they indicated UCDA may have a therapeutic effect in some children with PSC, and plans are underway for a more definitive study. The investigators also noted that elevations in baseline GGT, IL-8 and TNF- α may help predict which patients are at risk of experiencing a flare. The study has been published in *Hepatology Communications*.

When this study was presented at the annual meeting of the North American Society for Pediatric



Pediatric Hepatologist and CFRI Scientific Director Dennis Black, MD, led a trial to evaluate ursodeoxycholic acid in the treatment of primary sclerosing cholangitis.

Gastroenterology, Hepatology and Nutrition, Black was awarded the Gerard Odell Prize for Excellence in Hepatology Research.

BLACK WINS MENTORING AWARD FROM THE AMERICAN GASTROENTEROLOGICAL ASSOCIATION

The American Gastroenterological Association (AGA) honored Dennis Black, MD, with the AGA Institute Council Obesity, Metabolism & Nutrition Section Research Mentor Award. This award is for AGA council members who have made outstanding contributions to mentoring in specific fields of research. Throughout his career, Black has specifically dedicated his time to the mentoring and training of new investigators. Black will receive this award during Digestive Disease Week, one of the largest conferences for physicians and researchers in gastroenterology, hepatology, endoscopy and gastrointestinal surgery.



BIOREPOSITORY AND INTEGRATIVE GENOMICS (BIG) INITIATIVE SURPASSES 10,000 UNIQUE SAMPLES

Now in its fifth year, the Biorepository and Integrative Genomics (BIG) Initiative, a collection of pediatric DNA samples, continued its growth as a resource for precision medicine research that is directly relevant to children in the local community. BIG has enrolled more than 20,000 participants and archived more than 10,000 unique DNA samples, approaching numbers large enough for genetic association studies on complex medical conditions.

During the last two years, BIG enrollment operations expanded from Le Bonheur inpatient rooms and the outpatient center phlebotomy station

to include multiple outpatient clinics, the Neonatal Intensive Care Unit and the Emergency Department. With restrictions due to the COVID-19 pandemic, BIG investigators have introduced a remote procedure to safely contact Le Bonheur patients and their families to obtain secure electronic informed consent for their participation.

Two large studies have started the application process to obtain and analyze BIG samples: one is a multistate study of genetic determinants for the risk of recurrent urinary tract infections; the second will combine samples from BIG with those from other repositories to perform large-scale genotype-phenotype association analyses to identify novel genes associated with a wide range of diseases and related



quantitative traits that affect diverse populations in Tennessee. To facilitate smaller studies, BIG has assembled a genomics analysis pipeline that includes a next-generation sequencer and an informatics server system for analysis, storage and management of sequencing data.

EXPLORING THE GENETICS OF OBESITY

Geneticist Chester Brown, MD, is determining the roles that specific genes play in regulating body composition. Specifically, Brown is interested in why some individuals are more prone to develop

obesity than others and hopes to gain insight into the long-observed link between obesity and diabetes by studying mouse models developed in his laboratory. The overall aim of these studies is to provide alternative strategies to treat obesity.

Brown's laboratory focuses on the activin and bone morphogenetic protein signaling pathways through which GDF3 and activins signal or impact signaling. Brown plans to assess the PI3K-AKT pathway, which plays a major role in diabetes and rare genetic disorders including Congenital Lipomatous Overgrowth, Vascular Malformations, Epidermal Nevis, Spinal/Skeletal



Anomalies/Scoliosis (CLOVES) syndrome, due to recurrent somatic mutations in PIK3CA that result in abnormal fat deposition. In addition to the PI3K-AKT pathway, Brown has multiple projects focused on GDF3, Smad proteins and activin proteins.

The PI3K-AKT signaling pathway plays important roles in cell growth and glucose metabolism. Patients within the congenital lipomatosis spectrum (Proteus syndrome, CLOVES and congenital lipomatosis) have somatic activating mutations in components of the PI3K-AKT signaling pathway that lead to asymmetric, lipomatous overgrowth. In CLOVES patients, due to somatic mutations in PIK3CA, lipomas grow dramatically at the expense of normal fat stores, conferring an overall emaciated appearance to the patient but sparing glucose and lipid metabolism. Removal of the lipomas results in return to a normal body fat distribution and appearance. In this project, Brown seeks to determine the metabolic consequences of activating PIK3CA mutations by generating a tamoxifen-inducible CLOVES mouse model and whether metabolic properties of lipomas derived from the CLOVES model can rescue diet and genetically induced obesity.

Expression of GDF3 is highly induced in white adipose tissues under high fat diet conditions. *Gdf3* null mice are protected against diet-induced obesity due to failure of adipocyte hypertrophy and higher metabolic rates as compared to wild-type mice fed a regular diet or high fat diet. In this project, Brown seeks to understand the mechanisms that contribute to these phenomena, using a variety of approaches in vitro and in vivo.

Brown and colleagues are also exploring Smad2,



Genetist Chester Brown, MD, is investigating the role of genes in regulating body composition with the aim of providing alternative strategies for treating obesity.

Smad3 and other downstream effector proteins in the activin signaling pathway. In the nucleus, these downstream effector proteins bind to DNA and control the expression of many target genes involved in adipogenesis. Smad3 plays a role in adipocyte fate decisions, including the appearance of brown adipocytes in white adipose tissues from Smad3 global knockout mice and protection from diet-induced obesity.

Brown will investigate the adipose tissue selective effects of downstream mediators of activin signaling, specifically Smad2 and Smad3. These studies will delineate the molecular pathways that regulate metabolism in response to activin signaling.

Finally, Brown is investigating the role of activins A and B in obesity. Loss of activins A and B in adipose tissues alters the phenotypic characteristics of white adipose tissue depots and adipocytes in a cell-autonomous fashion including smaller adipocytes, smaller white adipose depots and histologic and molecular features typically associated with brown adipose, including protection from diet-induced obesity. This study will further characterize the mechanisms underlying this phenomenon and inform treatment of extreme obesity. 



HEART INSTITUTE



IDENTIFYING MODIFIER GENES IN CARDIOMYOPATHY

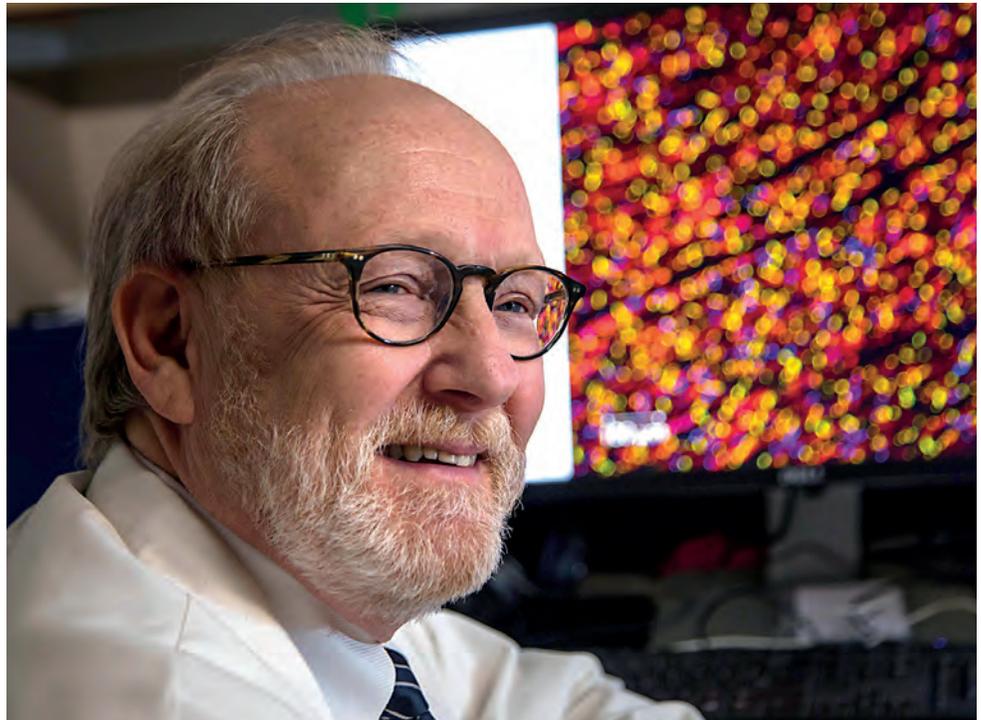
Jeffrey A. Towbin, MD, Enkhsaikhan Purevjav, MD, and Lu Lu, PhD, are leading a new NIH-funded project to uncover modifier genes involved in cardiomyopathies. Unlike causal gene variants, modifier gene variants are difficult to detect using traditional genomics approaches such as genome wide association studies. Their innovative approach includes the largest mouse genetic reference population, BXD, and samples from patients with cardiomyopathies.

Cardiomyopathies are a family of heritable and acquired diseases of heart muscle that carry a high risk of heart failure and death. Mutations in several cytoskeletal genes cause cardiomyopathies. However, among patients carrying the same causal mutations, the presentation, severity and outcomes are highly variable. The high variability in cardiomyopathies indicates potential genetic modifiers, i.e., modifier genes. Modifier gene variants could interact with causal gene variants through epistasis to drive unique cardiomyopathy phenotypes.

The BXD family was founded by crossing B6 and D2 mice into 152 diverse lines. These mice are ideal for studying genetic interactions in cardiomyopathies because one parent

strain (D2) exhibits cardiomyopathy phenotypes, while the other (B6) has a normal heart. BXD mice are also fully sequenced and exhibit diverse cardiomyopathy phenotypes.

Using this novel approach, the investigators have chosen to focus on Z-disk myopalladin. Mutations in Z-disk myopalladin cause all major types of cardiomyopathies. Therefore, Z-disk myopalladin is a strong causal gene that can be harnessed to detect potential modifier gene variants. The investigators plan to identify modifier variants relevant to Z-disk myopalladin and create maps of genetic loci associated with cardiomyopathy phenotypes. Once candidate modifier genes are identified, the researchers will



Co-Director of the Heart Institute Jeffrey A. Towbin, MD, received an NIH R01 grant to explore modifier genes in cardiomyopathy. The project aims to provide better patient care and prevent poor outcomes for cardiomyopathies.

sequence samples from patients with cardiomyopathy and unaffected family members to confirm mutations or expression changes in the candidate genes.

With this project, Towbin, Purevjav and Lu aim to improve personalized care in cardiomyopathies. Identification of modifier gene variants could lead to comprehensive molecular screening and the ability to predict disease progression. Ultimately, this knowledge could help physicians make informed decisions about patient care and possibly prevent poor outcomes such as sudden death.

NON-SURGICAL CLOSURE OF PATENT DUCTUS ARTERIOSUS

Interventional Cardiologist Shyam Sathanandam, MD, is pioneering a study on non-surgical catheterization patent ductus arteriosus (PDA) device closure in micro-premature newborns. Micro-premature



Interventional Cardiologist Shyam Sathanandam, MD, is pioneering a study on non-surgical closure of patent ductus arteriosus in premature newborns in an effort to limit complications that arise with other treatment options.

newborns weighing as little as 600 grams and retaining a PDA are at high risk of developing heart failure and lung dysfunction. Therapies in the past have included medications such as indomethacin or surgical ligation, which can result in a prolonged recovery period. This study by Sathanandam and colleagues utilizes a PDA closure device to close the PDA by cardiac catheterization, limiting the potential complications of previous treatment options. 



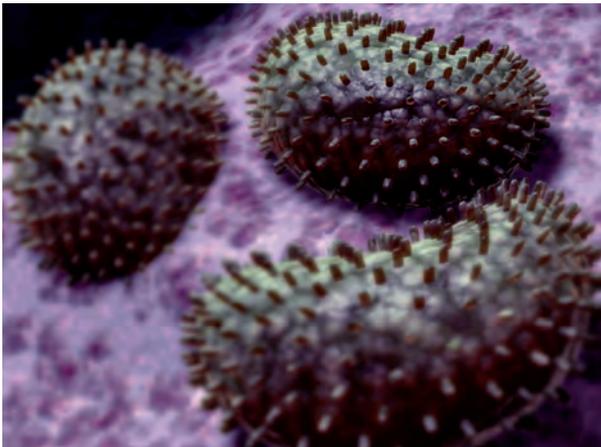


CONGENITAL CMV SCREENING

In 2019, the Division of Infectious Diseases continued its congenital CMV screening program. In this program, patients are screened using saliva swabs at Methodist birthing hospitals. Newborns who screen positive are brought in for confirmatory testing for infection as well as further laboratory and imaging assessments to determine need and eligibility for treatment. Results of the first several years of screening will be submitted for publication this year.

ALLERGIC ASTHMA PROTECTS FROM H1N1 INFLUENZA VIRUS AND STREPTOCOCCUS PNEUMONIAE

Pre-existing allergic asthma protects from severe morbidity from influenza A virus (IAV) and *Streptococcus pneumoniae* (*Spn*) co-infection because of extensive alterations in the respiratory tract including immunological and microbiological differences.



Influenza A virus

This Le Bonheur research, published in *Scientific Reports*, was prompted by the results from the 2009 swine flu pandemic during which asthmatics had

less severe outcomes of influenza including reduced bacterial pneumonia and intensive care unit (ICU) admittance as compared to non-asthmatics.

“Asthma is a complicated syndrome that develops through intricate gene and environment interaction,” said Le Bonheur Researcher Amali Samarasinghe, PhD. “Our study aimed to understand the possible mechanisms at play in asthmatics during respiratory infections to determine how each asthmatic may respond.”

Researchers developed a mouse model of asthma, influenza and pneumococcal pneumonia in order to study host-pathogen interactions in live tissue, which is unable to be observed in humans.

The results of the study revealed several ways in which allergic airways differ from non-allergic during co-infection of IAV and *Spn* including:

1. The inflammation of allergic airways delayed or protected against severe disease from co-infection.
2. Allergic airways had a more diverse immune cell signature during co-infection.
3. Antibiotic treatment impeded protection from infection-induced morbidity in allergic mice.
4. Lung mucosal microbiome was more diverse in allergic airways, and antibiotic-induced dysbiosis rendered the allergic mice susceptible to severe disease associated with co-infection.

“Underlying conditions present unique challenges and opportunities for invading pathogens,” said Samarasinghe. “The extensive alterations in the respiratory tract during allergic asthma encompass both

immunological and microbiological differences that can have a profound impact on susceptibility to infection.”

The results show that asthmatics have a distinct microbial signature that may contribute to the protective capacity of asthma during IAV and *Spn* co-infection. Any antibiotics should be prescribed

with caution, especially in patients with underlying chronic conditions.

This study was conducted in collaboration with St. Jude Children’s Research Hospital Researchers Jason Rosch, PhD, Ti-Cheng Chang, PhD, and Peter Vogel, DVM, PhD. 





GUT MICROBIAL DYSBIOSIS AND GUT-LUNG AXIS IN NEWBORN MICE

Researchers Joseph Pierre, PhD, Ajay Talati, MD, and Kent Willis, MD, study host-microbiome interactions at the beginning of life with a focus on the gut-lung axis in bronchopulmonary dysplasia, a chronic lung disease of preterm infants. Their translational work utilizes a mixed approach of gnotobiotic and antibiotic-exposure animal models and human cohort studies to gain insight into how commensal microbes alter newborn physiology.

Premature infants, particularly those who receive oxygen treatment soon after birth, are at high risk of developing lung problems characterized by fibrosis and inflammation. Emerging research suggests that the communities of bacteria in the gut can impair development of the immune system and may also



affect inflammation, which in turn plays an important role in lung disease. The interaction among these body systems is called the gut-lung axis.

Antibiotics are known to change the makeup of the gut microbiome and are linked to an increased risk of lung injury. Antibiotic treatment is common in premature babies, but how the gut is involved is not clear. Looking at how antibiotics affect offspring — even before birth — may help researchers better understand the gut-lung axis.



In a recent study, Pierre, Talati and Willis exposed mice to antibiotics before birth. These mice had more fibrosis with oxygen treatment than mice exposed only to antibiotics after birth. Offspring exposed to penicillin in the womb also had lower body mass and reduced capillary size than those not exposed before birth. Additionally, prenatal exposure altered levels of proteins that promote inflammation and immune function as well as those that affect microbial signaling in the lungs.

This study provides valuable experimental evidence that manipulation of gut microbiota by antibiotic exposure influences the progression of oxygen exposure-related lung injury and may assist in the interpretation of future observational studies in human newborns examining the role of the gut-lung axis in bronchopulmonary dysplasia.

COLONIZATION OF THE FUNGAL MICROBIOME IN HUMAN NEONATES

Previous research on the microbiome has focused on the bacterial communities but has neglected to examine the contributions of fungal communities. To address this gap, Researchers Joseph Pierre, PhD, Ajay Talati, MD, and Kent Willis, MD, are exploring how the fungal microbiome assembles in the human gut after birth.

The team is determining how fungi begin to colonize the newborn gut and what happens when this process goes awry. While there are many possibilities that remain to be explored, if the formation of early fungal communities does not proceed as usual, it could lead to asthma and potentially obesity. For scientists to understand if this process is unfolding improperly, knowledge of how the first fungal communities are

supposed to form in newborns is needed. Recent research by this group is a key first step down this path. 





LE BONHEUR NEPHROLOGIST EXAMINES RISK FACTORS FOR ACUTE KIDNEY INJURY WITH AWAKEN

AWAKEN (Assessment of Worldwide Acute Kidney Injury Epidemiology in Neonates) is a multisite observational study focused on neonatal acute kidney injury (AKI), a serious condition affecting 15-70% of critically ill neonates admitted to the Neonatal Intensive Care Unit. The investigators at Le Bonheur, including Nephrologist Arwa Nada, MD, are focused on identifying risk factors that predispose infants to AKI as well as the impact of fluid balance on clinical outcomes. In the past year, the AWAKEN study group has published a number of studies showing

associations between AKI, hemoglobin levels and fluid balance.

Based on findings in the AWAKEN cohort, positive fluid balance was common among neonates with AKI in the first postnatal week. Positive fluid balance in the first postnatal week was associated with the need for mechanical ventilation, indicating the deleterious effects of excess fluid on lung function. Reduced hemoglobin was also common in the first postnatal week among neonates with AKI, demonstrating a moderate ability to predict later development of AKI. Overall, these findings indicate fluid balance and hemoglobin levels may be risk factors for the development of AKI in critically ill neonates.



GENETIC RISK FACTORS AND TREATMENT OF PEDIATRIC SICKLE CELL DISEASE

Nephrologist Rima Zahr, MD, is working on ways to predict the severity of sickle cell disease (SCD) and identify new treatments to slow the progression of kidney damage in SCD. Zahr has published three studies in the past year exploring various aspects of SCD including genetic risk factors for albuminuria in SCD, the efficacy of hydroxyurea in SCD and renal biopsy findings from children and adolescents with SCD.

Patients with sickle cell anemia, a common manifestation of SCD, often progress to chronic kidney disease, which ultimately results in end-stage renal disease. Albuminuria is considered a marker of renal damage, and current screening recommendations for albuminuria start at 10 years of age. However, Zahr postulates that genetic screening may help identify patients with sickle cell anemia who are at risk of progressing to chronic kidney disease before albuminuria screening takes place. Early identification of these patients could hasten therapeutic interventions and prevent severe kidney damage, which Zahr and colleagues have observed in kidney biopsies from patients as young as 4 years old.

To explore the impact of genetic variations on the progression of sickle cell renal disease, Zahr and collaborators used whole genome sequencing data from the Sickle Cell Clinical Research and Intervention Program (SCCRIP), a lifelong longitudinal study of patients with SCD at St. Jude Children's Research Hospital. The investigators specifically focused on variants in the apolipoprotein L-1 (*APOL1*) gene, which are associated with kidney damage and

disease. These *APOL1* variants were also associated with albuminuria in sickle cell anemia in previous research. The investigators examined these variants in the SCCRIP cohort to see if they could predict the development of albuminuria and progression of kidney disease.

Within the SCCRIP cohort, 22% of participants 10 years of age or older developed albuminuria. For participants carrying high-risk *APOL1* variants, the time to develop albuminuria was shortened by 41%, with those taking hydroxyurea showing a 7% delay in the time to develop albuminuria. Some participants with these high-risk variants also developed albuminuria prior to 10 years of age. These findings indicate early screening for high-risk *APOL1* variants among children with SCD could identify those at risk of early-onset albuminuria and kidney damage.

In another study, Zahr and colleagues examined the effects of hydroxyurea in patients with sickle cell anemia from two different cohorts, the Hydroxyurea Study of Long-term Effects and SCCRIP. The investigators found children who initiated hydroxyurea prior to 10 years of age were less likely to develop albuminuria than those who initiated hydroxyurea at 10 years of age or older. However, children with higher baseline albumin to creatine ratios showed a greater risk of developing albuminuria even after initiating hydroxyurea, while children with lower baseline albumin to creatine ratios were more likely to experience resolution of albuminuria after taking hydroxyurea for one year. The investigators speculated that placing children on the maximum tolerable dose of hydroxyurea at an earlier age could prevent the onset of albuminuria or ameliorate existing albuminuria.

Overall, Zahr's recent studies make a case for earlier screening and intervention in children with SCD. In future studies, Zahr and colleagues plan to examine the progression of albuminuria and kidney disease in children with sickle cell anemia, investigate the influence of genetic risk factors on the progression of kidney disease and test novel therapeutic strategies in at-risk patients. Such studies may eventually provide a precision medicine approach to preventing and ameliorating renal damage in SCD.

A NOVEL MECHANISM IN TUBEROUS SCLEROSIS COMPLEX RENAL DISEASE

Tuberous sclerosis complex (TSC) renal disease affects more than 80% of individuals with TSC, with

50% of these individuals exhibiting renal cystic disease. Among individuals with TSC, 40% exhibit reduced glomerular filtration rates, indicative of declining renal function. As renal cysts multiply and grow, they place a significant burden on neighboring functional tissue, which increases blood pressure, reduces renal function and can ultimately lead to chronic kidney disease or kidney failure.

Nephrologist John Bissler, MD, director of the Tuberous Sclerosis Center of Excellence, continues to investigate the mechanisms of TSC renal disease by examining the role of extracellular vesicles in renal cyst formation. Extracellular vesicles enable cell-to-cell communication and participate in both normal and



Chief of Pediatric Nephrology and Director of the Tuberous Sclerosis Center of Excellence John Bissler, MD, is investigating the mechanisms that cause tuberous sclerosis complex (TSC) patients with renal disease to develop kidney cysts. This research paves the way for new therapeutics in TSC renal disease.

pathogenic processes throughout the body. Based on Bissler's recent research, extracellular vesicles may be the missing link underlying a critical process in TSC renal disease: phenotypic induction.

Previous research in TSC renal disease showed that cells comprising angiomyolipomata, a renal lesion caused by TSC, exhibit an inactivating mutation and loss of *Tsc* gene expression. By contrast, cells comprising renal cysts show largely intact *Tsc* gene expression with no mutations. Therefore, Bissler and colleagues hypothesized that phenotypic induction, or tissue reprogramming, drive renal cells to take on *Tsc* mutant cell phenotypes and form renal cysts. To investigate this possibility, Bissler examined two new animal models of TSC renal cystic disease, renal tissue from patients with TSC polycystic disease and extracellular vesicle release in cell culture.

Bissler and colleagues found type A intercalated cells were the predominant cell type in renal cysts from both mouse and human tissue samples. Despite intact *Tsc* gene expression, these cells proliferated to form cysts throughout the mouse kidney. Next, the investigators determined whether type A intercalated cells showed activation of the mTORC1 pathway, which is regulated by the *Tsc1* and *Tsc2* genes. Even with an intact *Tsc* gene locus, these cells showed



increased activation of the mTORC1 pathway.

Additionally, when the investigators treated mice with rapamycin, an mTORC1 inhibitor, they observed

increased survival in *Tsc* mutant mice. Patients with polycystic renal disease also responded positively to mTORC1 inhibitor treatment, showing reduced cyst burden after one year of treatment.

In a final set of experiments, Bissler and colleagues tested whether extracellular vesicles mediated phenotypic induction in type A intercalated cells. To test this idea, they used a cell culture approach. First, the investigators mutated *Tsc* genes in an inner medullary collecting duct cell line and then isolated extracellular

vesicles produced by the cells. The isolated extracellular vesicles were then applied to an intercalated cell line, which increased expression of phosphorylated S6, an indicator of mTORC1 activity.

Altogether, these experiments indicate that neighboring mutant cells secrete extracellular vesicles to drive phenotypic induction in normal type A intercalated cells. In response, the intercalated cells hyperactivate the mTORC1 signaling axis and proliferate, creating renal cysts. These cells respond to mTORC1 inhibition in both mice and humans. This research may also lead to new therapeutic strategies targeting the extracellular vesicles involved in the generation of renal cysts.





NEUROSCIENCE INSTITUTE



STUDIES DEMONSTRATE THE UTILITY OF TRANSCRANIAL MAGNETIC STIMULATION IN LANGUAGE MAPPING

Preserving brain cortical regions involved in language is critical to prevent postoperative language deficits in patients undergoing neurosurgery for brain tumors and epilepsy. While invasive techniques such as direct cortical stimulation and the Wada procedure are effective in identifying language areas, they are also associated with significant risks. Shalini Narayana, MBBS, PhD, director of Le Bonheur's Transcranial Magnetic Stimulation (TMS) Laboratory, and colleagues have assessed the use of TMS, a non-invasive technique developed as an alternative to direct cortical stimulation, in language mapping.

Narayana and investigators performed two studies comparing TMS to two commonly used noninvasive techniques, functional magnetic resonance imaging (fMRI) and magnetoencephalography (MEG). fMRI is widely used as a validated alternative to more invasive methods. fMRI cannot be used with patients who have contraindications such as metal plates or pacemakers, and brain tumors can create signal artifacts due to vascular abnormalities. Patients are required to lie still inside a large, loud machine, which can be difficult for young children. Similarly, MEG requires patients to remain still. Some children must be sedated to undergo fMRI and MEG, which limits the types of language tasks that can be performed. By contrast, TMS has a frameless stereotaxic navigation system, shows fewer





Director of the Infantile Epilepsy Center Sarah Weatherspoon, MD, is working to improve quality and clinical research for children with infantile epilepsy.

signal artifacts and does not require patients to remain still. TMS also offers the ability to perform a wider variety of language tasks.

In these studies, the investigators determined the sensitivity, specificity and accuracy of TMS in preoperative language mapping for patients with epilepsy and brain tumors. These patients included both children and adults, allowing the investigators to look for differences among age groups. Narayana and colleagues also examined the agreement between results from TMS and those from fMRI and MEG.

The study comparing TMS and fMRI revealed TMS had overall accuracy of 71%, specificity of 83% and sensitivity of 20% in localizing all cortical language areas among all patients. TMS was slightly better at localizing language areas in the left hemisphere than in the right but showed good specificity for areas

within both hemispheres. However, the overall agreement between TMS and fMRI was only slight, indicating a need for further optimization of TMS.

In the MEG versus TMS study, the investigators uncovered similar findings. Compared to MEG, TMS showed overall accuracy of 73%, specificity of 72% and sensitivity of 74%. In identifying hemispheric dominance for language, MEG and TMS showed 63% agreement. While the agreement between MEG and TMS was stronger than that between fMRI and TMS, the investigators still noted room for improvement in TMS parameters and recommended TMS

and MEG be used in tandem to accurately locate language-specific cortex.

NEUROSCIENCE INSTITUTE LAUNCHES THE INFANTILE EPILEPSY CENTER

In October 2019, Pediatric Epileptologist Sarah Weatherspoon, MD, founded the Infantile Epilepsy Center in the Neuroscience Institute. This new center has a unique focus on infantile spasms and rare forms of epilepsy diagnosed in children younger than 2 years. Infantile epilepsies can have devastating effects on child development if not treated early and often require multidisciplinary care.

Providers across multiple disciplines in the Infantile Epilepsy Center use a collaborative approach to provide comprehensive care to infants and children with epilepsy. Weatherspoon, who also serves as

director of the Infantile Epilepsy Center, is focused on improving clinical outcomes through quality improvement initiatives and clinical research.

INNOVATIVE GENETIC THERAPY FOR DRAVET SYNDROME

Neuroscience Institute Co-director James Wheless, MD, is undertaking a study of an innovative new treatment for Dravet syndrome, a rare and severe form of childhood epilepsy. Dravet syndrome is caused by mutations in a single gene in the brain. This disease is a devastating diagnosis for families as these children appear healthy at birth but develop seizures with febrile illnesses between 6 and 12 months of life. As these children develop, the disease progresses, ultimately resulting in marked mental impairment, inability to walk and persistent seizures.



Le Bonheur Neurologist and Co-director of the Neuroscience Institute James Wheless, MD, is studying a new treatment for Dravet syndrome, a rare and severe form of epilepsy.

To date, the only available treatments address the symptoms of the disease, not the cause. Beginning this year, Wheless and colleagues will administer a new treatment into the spinal fluid to correct the disease-causing mutation. Dravet syndrome will be the first genetic epilepsy treated with gene therapy to cure the condition. Le Bonheur is one of 18 Dravet Syndrome Comprehensive Care Centers in the country

and one of the few centers that will be involved with this novel treatment strategy. Treatment early in life may prevent the progression of this disease.

PREVENTING SEIZURE CLUSTERS WITH MIDAZOLAM NASAL SPRAY

Neuroscience Institute Co-director James Wheless, MD, completed a phase III clinical trial and open-label extension trial examining the efficacy of midazolam nasal spray in children experiencing seizure clusters. These trials showed midazolam nasal spray effectively stopped and prevented seizures for up to six hours after the initial dose with a small number of participants requiring a second dose to achieve these effects. Midazolam nasal spray is also easier to administer to children experiencing seizures than injectable midazolam.

LE BONHEUR NEUROSURGEON WINS CNS PEDIATRICS PAPER OF THE YEAR FOR SECOND CONSECUTIVE YEAR

Le Bonheur Neurosurgeon Paul Klimo, MD, MPH, was awarded

the Pediatrics Paper of the Year by the Congress of Neurological Surgeons (CNS) during the annual meeting in San Francisco, Calif., for the second year in a row. The paper, “The Preventable Shunt Revision Rate: A Multicenter Evaluation,” was published in *Neurosurgery* in March 2019.

The Preventable Shunt Revision Rate (PSRR) was introduced in 2016 as a quality metric to determine

which shunt failures were avoidable. Shunt surgery is the most common procedure performed in neurosurgery, and shunt malfunction is the second most common cause of rehospitalization in children.

“PSRR goes directly to the heart of the current quality movement in health care,” said Klimo. “Quality metrics lead to the implementation of processes to avert negative consequences and maximize positive results.”

The paper evaluated two years of consecutive shunt operations data from nine participating centers in North America to determine the PSRR across institutions as well as the most common etiologies that lead to shunt failure. Of the 5,092 shunt

operations performed, 861 failed within 90 days, an overall failure rate of 16.9%.

Of the 861 failed shunts, 307 were determined to be potentially preventable – an overall 90-day PSRR of 35.7%. Preventability was defined as cerebrospinal fluid (CSF) infection, wound breakdown or infection, suboptimal position of the proximal or distal catheter or an improperly assembled or inadequately secured shunt that resulted in postoperative disconnection, migration, kinking or obstruction. The most common etiologies of preventable failure were shunt infection, malposition of the proximal catheter and judgment errors.

The study showed that a significant proportion, approximately one-third, of early shunt failures are preventable. The paper suggests that the overall shunt infection rate for any major children’s institution should be 5% or less. Predictors of preventable failure included lack of endoscopy, recent shunt infection, shunt type and participating center.

“Direct involvement and oversight by staff are critical and can help reduce the risk of all causes for preventable shunt failures,” said Klimo. “PSRR allows practitioners and institutions to identify areas that may be improved such as investing in image guidance technology, implementing a shunt surgery checklist and reaching out to general surgeons to assist in accurate distal catheter placement.”

Future efforts with PSRR include creating a real-world registry as well as re-evaluating centers that have implemented changes in an effort to lower PSRR.

Klimo also won the CNS Pediatrics Paper of the Year in 2018 for his research on survival rates for pineoblastoma tumors.



Chief of Pediatric Neurosurgery Paul Klimo, MD, MPH, won CNS Pediatrics Paper of the Year for his work with shunt revisions.

OBESITY



HEALTHY LIFESTYLE CLINIC MAKES GREAT STRIDES

Since the Healthy Lifestyle Clinic was founded in 2014, the faculty and staff have made a number of achievements. The first two years of the clinic were spent establishing reputation in the community and educating people on the nature of weight and obesity management, which resulted in an enormous positive response. Obesity Medicine Specialist Thomaseo Burton, PhD, joined the Healthy Lifestyle Clinic in 2015 and was involved in the initial outreach to the community.

“We emphasized that obesity management isn’t something that just happens in the clinic. The family, community and school need to be involved to make

the necessary lifestyle changes,” said Burton.

The Healthy Lifestyle Clinic’s initial community outreach resulted in numerous referrals that kept providers within the clinic busy but ultimately exposed some growing pains.

“We received an overwhelming response and ended up with a huge waitlist. Patients would be referred to our clinic by their primary care physicians, but they couldn’t get an appointment for six to nine months after the initial referral. It created a lot of frustration for everyone,” said Burton.

Providers in the Healthy Lifestyle Clinic came up with a new triage system and started scheduling waitlisted patients and their families for group orientation. In orientation, patients and their families



learn about the Healthy Lifestyle Clinic’s multidisciplinary approach to weight management and receive basic tips on how to promote a healthy lifestyle, such as drinking more water, avoiding sugary foods and beverages and engaging in regular exercise.

“The triage system allowed us to educate patients on why they were being referred and how the Healthy Lifestyle Clinic can help. It also helped patients decide whether the program was right for them. Some patients chose not to be involved, while others expressed motivation and started counseling. With this approach, we can now manage the flow of patients and contact most new referrals in 30 days or less,” said Burton.

With the success of the triage system, providers in the Healthy Lifestyle Clinic are now looking forward to building relationships with primary care physicians in the Memphis area.

“The rates of pediatric obesity in Memphis are staggering. Realistically, we cannot see every child with obesity in Memphis and the Mid-South region. That’s why we are working to empower pediatricians to do some of this management,” said Burton.

By building partnerships with local pediatricians, the Healthy Lifestyle Clinic is spreading knowledge on simple lifestyle interventions that can improve children’s health. The clinic provides packets with information on healthy foods, simple exercise plans and goal setting. This information can help children



and their families avoid unhealthy beverages such as sugary fruit juice and empower physicians to advise these families in a manner consistent with weight management. With this strategy, the Healthy Lifestyle Clinic is poised for more growth and success in the future.

FAMILIES FIGHT OBESITY WITH MEALS

Obesity Medicine Specialist Thomaseo Burton, PhD, and Exercise Physiologist Webb Smith, PhD, have developed a novel intervention to address obesity in the kitchen. The intervention, Multidisciplinary

Engagement and Learning/Mindful Eating and Active Living (MEALS), combines mindfulness, cooking techniques and education to teach children and their caregivers how to make healthier meals and engage in mindful eating. This program comprises two-hour education, cooking and discussion sessions once a week for three weeks that are tailored to fit the largely African-American and under-resourced communities served by the Healthy Lifestyle Clinic.

During the sessions, child-caregiver pairs receive a brief lesson on healthy living topics, mindfulness



concepts and kitchen safety; a taste test of healthy foods prepared by a facilitator; meal preparation; and a dine-and-discuss session led by a facilitator. The mindfulness component of the program focuses on helping children become more aware of internal states and how to link these states to hunger, cravings and satiety, as well as how to increase awareness of the quantity and quality of food. The taste test portion encourages participants to use mindfulness techniques to explore the appearance, smell, texture and taste of the sample food. During meal preparation, children and caregivers work together to follow recipes and cook the food. The dine-and-discuss session again encourages participants to apply mindfulness techniques to manage portion sizes, eat the food and consider how their experience in the session relates to their experiences at home.

This intervention is unique compared to traditional clinical management, which usually comprises brief counseling without an experiential component. MEALS also takes barriers to healthy eating, such as cost, time, wariness of new foods and access to ingredients, into consideration by using low-cost ingredients and familiar recipes. Following the pilot program, the facilitators plan to assess the impact of the intervention by measuring participant characteristics, caregiver feedback and youth feedback, with the hope of seeing meaningful improvement in participants' health and well-being.

PREDICTING OBESITY BEFORE BIRTH

The Conditions Affecting Neurocognitive Development and Early Learning (CANDLE)

study has produced multiple findings relevant to early childhood development. Supported by the Memphis-based Urban Child Institute, investigators at the University of Tennessee Health Science Center (UTHSC) and Le Bonheur have followed more than 1,000 mother-infant dyads and reported on the impact of maternal dietary patterns, demographic factors and gestational vitamin D levels on the development of infants and children in Memphis and Shelby County, Tenn. The data from CANDLE are being used to identify areas for intervention to promote healthy development in early childhood.

With colleagues at UTHSC and the University of California at San Francisco, Joan Han, MD, pediatric endocrinologist and director of the Pediatric Obesity Program, has published another study on the CANDLE cohort. This study focused on relationships between maternal metabolic factors, early childhood growth trajectories and obesity risk. Han and colleagues specifically focused on pre-pregnancy BMI, gestational weight gain and gestational diabetes based on previous research indicating the potential influence of these factors on long-term obesity risk in children. The investigators also sought to identify potential racial disparities in the effects of maternal metabolic factors. Unlike previous cohorts, the CANDLE cohort included both black and white infant-mother dyads, which allowed the investigators to look for differences.

The researchers grouped the infants based on three BMI growth trajectories (low BMI, moderate BMI and rising-high BMI) to explore potential associations with maternal metabolic factors. They found that mothers who were overweight or obese, had excessive gestational weight gain and gestational diabetes were

more likely to have children in the rising-high BMI group. Next, the investigators computed the relative risk for each maternal metabolic factor associated with the rising-high BMI trajectory. This computation revealed pre-pregnancy obesity and excessive gestational weight gain were significantly associated with the rising-high BMI trajectory and increased risk of obesity at age 4 among the children, whereas gestational diabetes was only associated with increased risk of obesity at age 4. Moreover, as the number of maternal metabolic factors increased, the risk of being in the rising-high BMI group and being obese at age 4 increased. No significant differences were present between white and black subgroups in terms of the effects of maternal metabolic factors on growth trajectories or childhood obesity.

This study demonstrated maternal metabolic factors are related to child growth trajectories and the risk of childhood obesity. This study improved upon previous research by measuring BMI at several time points and adjusting for multiple confounders such as gestational age and birth weight. The investigators did not find racial disparities in the association of maternal metabolic factors, growth trajectories and childhood obesity risk, which may indicate equalization of obesity risk across all races and ethnicities. This finding could also be attributed to the high rates of obesity in the South for both groups or the smaller number of white versus black participants in this study. A promising implication of this research is that interventions targeting maternal metabolic factors may reduce the risk of childhood obesity, but further research is needed to explore this possibility. 



ORTHOPEDICS



COMPARING IMAGING MODALITIES FOR SPINAL COMPRESSION FRACTURES

In adolescents and children, thoracic and lumbar compression fractures are among the most commonly observed spinal fractures. Compression fractures occur when vertebrae collapse due to trauma or conditions such as osteoporosis. Compression fractures are usually detected by computed tomography (CT) because it provides a complete picture of cranial, thoracic and abdominal injuries. However, some centers use magnetic resonance imaging (MRI) instead of CT because it does not involve the use of ionizing radiation. Moreover, unlike CT, MRI can show soft tissue damage in addition to vertebral fractures. However, MRI is more costly than CT, and young children often require sedation for MRI, further exacerbating the cost.

Given the use of both CT and MRI in diagnosing compression fractures, Orthopedist Jeffrey R. Sawyer, MD, and colleagues sought to compare the sensitivity of these imaging modalities in diagnosing thoracic and lumbar compression fractures. They examined data from 52 patients with thoracic or lumbar fractures who underwent both CT and MRI scans at Le Bonheur. A total of 191 fractures were identified with an average of 3.7 fractures per patient. MRI and CT showed complete

agreement in 44% of patients, with CT missing fractures in only 2% of patients. Since the overall sensitivity of CT was 98% and MRI did not detect additional tissue injury, the investigators suggested the use of CT alone is sufficient to diagnose thoracic and lumbar compression fractures.

HEMOGLOBIN AND DISEASE SEVERITY IN EARLY ONSET SCOLIOSIS

In early onset scoliosis, the abnormal curvature of the spine reduces the available space in the chest cavity, effectively restricting the expansion of the lungs



and decreasing pulmonary function. In severe cases, early onset scoliosis results in thoracic insufficiency syndrome, a dangerous condition in which normal breathing and lung growth are restricted. The pulmonary function test is currently considered the best tool for assessing the severity of this disease. However, the pulmonary function test is difficult to perform in children, leaving physicians to rely upon radiographic measures that do not accurately reflect disease severity.



Orthopedist Jeffrey R. Sawyer, MD, is studying how hemoglobin levels in children serve as a marker for scoliosis severity.

Orthopedist Jeffrey R. Sawyer, MD, and collaborators at other children's hospitals decided to examine another potential marker of disease severity: elevated hemoglobin. Elevated hemoglobin correlates with hypoxia and may also reflect the severity of early onset scoliosis prior to surgery. Measuring hemoglobin in children is also easier than pulmonary

function testing because it only requires a blood draw. To examine the relationship between the severity of pre-operative early onset scoliosis and hemoglobin levels, the investigators measured hemoglobin prior to corrective surgery and at 6, 12 or 18 months post-surgery in 268 patients. They also measured the degree of spine curvature before and after surgery.

Sawyer and colleagues identified 48 patients who had elevated hemoglobin levels prior to surgery. The patients with elevated hemoglobin were younger than

those with normal hemoglobin, but no other marked differences between the groups emerged pre-operatively.

Following surgery, hemoglobin was measured in 27 of the 48 patients with elevated hemoglobin. These measurements revealed a significant reduction in postoperative hemoglobin at 6 months and an overall reduction at 18 months.

Patients without elevated hemoglobin before surgery showed no changes in hemoglobin after surgery.

The investigators concluded elevated hemoglobin could potentially serve as a marker of disease severity.

However, elevated hemoglobin appeared in only a subset of patients, indicating it may be useful in only the most severe cases. Ultimately, the investigators concluded that the best approach for evaluating early onset scoliosis may be a battery of assessments, such as radiographic measurements, hemoglobin levels and patient feedback. 

PEDIATRIC SURGERY



EXPLORING THE GUT MICROBIOME IN HIRSCHSPRUNG-ASSOCIATED ENTEROCOLITIS

Pediatric Surgeon Ankush Gosain, MD, PhD, recently received \$2.1 million for his NIH R01 proposal, “Dysbiosis in Hirschsprung-Associated Enterocolitis Pathogenesis.” In Hirschsprung disease, the enteric nervous system, or the “brain of the gut,” does not develop completely reducing motility, water and nutrient absorption and local blood flow. Hirschsprung-associated enterocolitis (HAEC) is a life-threatening complication of Hirschsprung disease



Pediatric Surgeon Ankush Gosain, MD, PhD, received an NIH R01 grant for his work studying Hirschsprung-associated enterocolitis (HAEC) and how the gut microbiome plays a role in patients with HAEC.

that affects 30-60% of infants with the disease and is the leading cause of death among these infants. Dysbiosis occurs when the balance of beneficial to harmful bacteria in the gut microbiome is disrupted leading to loss of beneficial bacteria, increased harmful bacteria and decreased overall diversity of bacteria. Dysbiosis is prevalent in patients with HAEC, but its role in the disease is unclear.

Gosain’s project will test the central hypothesis that dysbiotic gut microbiota drives the development of HAEC. This innovative project will utilize novel preclinical models to establish a causative relationship between the abnormal gut microbiome and HAEC pathogenesis and test potential therapies. The objectives of this project are to establish a causative relationship between dysbiosis – imbalance of the gut microbiome – and HAEC and identify which bacteria are the main drivers of HAEC.

Gosain will also test therapeutic targets and examine how neurotransmitters made by bacteria influence the motility of the intestine. The expected outcome of these studies will be a deeper understanding of the pathophysiology of HAEC and identification of novel therapeutic approaches for prevention or treatment of HAEC.

ATOMAC EXAMINES BLOOD COMPONENT RATIOS IN PEDIATRIC TRAUMA

Trauma is the leading cause of death for children in the United States. In many cases, trauma causes severe hemorrhage, which reduces blood volume below the level necessary to keep

vital organs working. Massive transfusion protocols, which involve transfusion of large units of blood products in fixed ratios, are often implemented to save patients' lives. The correct balance of blood components such as red blood cells, platelets and plasma is critical to avoid exacerbating coagulopathy and acidosis, two frequent comorbidities in severe trauma patients. However, current massive transfusion protocols rely on data from adult trauma patients, which are not applicable to pediatric trauma patients due to differences in hemostasis and coagulation. To address this issue, Le Bonheur Surgeon-in-Chief Trey Eubanks, MD, and other members of the Arizona-Texas-Oklahoma-Memphis-Arkansas Consortium (ATOMAC) conducted a retrospective study of massive transfusion protocols implemented in pediatric trauma patients treated at each ATOMAC site.

Eubanks and colleagues examined data from 110 pediatric patients who received massive transfusions within 24 hours of severe trauma, mostly caused by motor vehicle accidents. The majority of patients survived to discharge from the hospital while 27% died in the ICU, usually within 48 hours of admission.

When the authors grouped all patients by the transfusion ratios of packaged red blood cells to fresh frozen plasma, the data showed that patients who received packaged red blood cells to fresh frozen plasma at a 1:1 ratio were more likely to survive than those who received these blood products at a 1:2 or



Le Bonheur Surgeon-in-Chief Trey Eubanks, MD, conducted a retrospective study to determine the best massive transfusion protocols for pediatric trauma patients.

1:3 ratio. This association also held after the authors controlled for age, suggesting the transfusion ratio does not need to be adjusted between younger and older children.

Based on these findings, the primary ATOMAC site, Children's Medical Center Dallas, has since implemented the 1:1 ratio for massive transfusions in pediatric patients. A standardized massive transfusion protocol utilizing the 1:1 ratio could expedite treatment for children in critical condition and potentially lead to better survival rates. Although prospective randomized trials are needed to confirm these results, the current findings do hold promise for children who experience severe trauma.





NEW THERAPY FOR PEANUT ALLERGY

The estimated prevalence of peanut allergy in the United States is 0.6% with between 1.5-3% prevalence estimates in the western world. The prevalence appears to be increasing, and reactions can be severe and result in death. Moreover, allergic reactions to peanuts cause the majority of food allergy-related deaths. Treatment options for peanut allergy are limited, and the main strategy is avoidance. However, peanuts are very common in food products, and trace amounts can be found in foods due to cross contamination making it difficult to avoid.

Allergist and Immunologist Jay Lieberman, MD, is leading efforts at Le Bonheur to treat peanut allergy

by participating in studies such as PALISADE (Peanut Allergy Oral Immunotherapy Study for Desensitization in Children and Adults) and follow-up studies.

These studies are designed to test a peanut-derived immunotherapy agent that desensitizes subjects to peanut allergen. The targeted outcome is to prevent severe reactions with small exposures. To date, the trials have shown a decrease in overall reactions to exposures, as well as decreased severity of reactions.

CLINICAL TRIALS FOR CYSTIC FIBROSIS

Pulmonologists Patricia Dubin, MD, and Tonia Gardner, MD, are conducting cystic fibrosis-related modulator research with participation in the



Allergist and Immunologist Jay Lieberman, MD, is conducting clinical trials with a peanut-derived immunotherapy to treat children with peanut allergy.

state-of-the-art modulator studies. Cystic fibrosis is an inherited, multi-organ disease, and the current life expectancy is only 37 years old.

The fundamental disease-causing problem is dysfunction in an ion channel (CFTR) caused by a genetic mutation. CFTR mutations can range from very severe with essentially no CFTR being produced to mild with reasonable amounts of dysfunctional CFTR produced. These functional differences are represented by hundreds of different mutations. As a result, CFTR at the genetic level cannot yet be corrected.

Current studies have focused on developing drugs that increase CFTR levels and others that improve the



function. Combinations of these modulator drugs are needed to improve CFTR function. Presently, Dubin and Gardner are leading clinical trials at Le Bonheur of new compounds to improve CFTR function and minimize undesirable side effects. 





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This image shows a common assay used in the laboratory of Pediatric Surgeon Ankush Gosain, MD, PhD, to study migration of neural crest cells (NCC) during development of the enteric nervous system. Here, a slice of mouse intestine (lower right corner) was harvested during mid-gestation and stimulated with glial-derived neurotrophic factor to promote NCC migration. Researchers then measure the distance that NCC migrated out of the gut slice. Image taken by Ming Fu, MD, PhD, senior scientist in the Gosain Laboratory.

