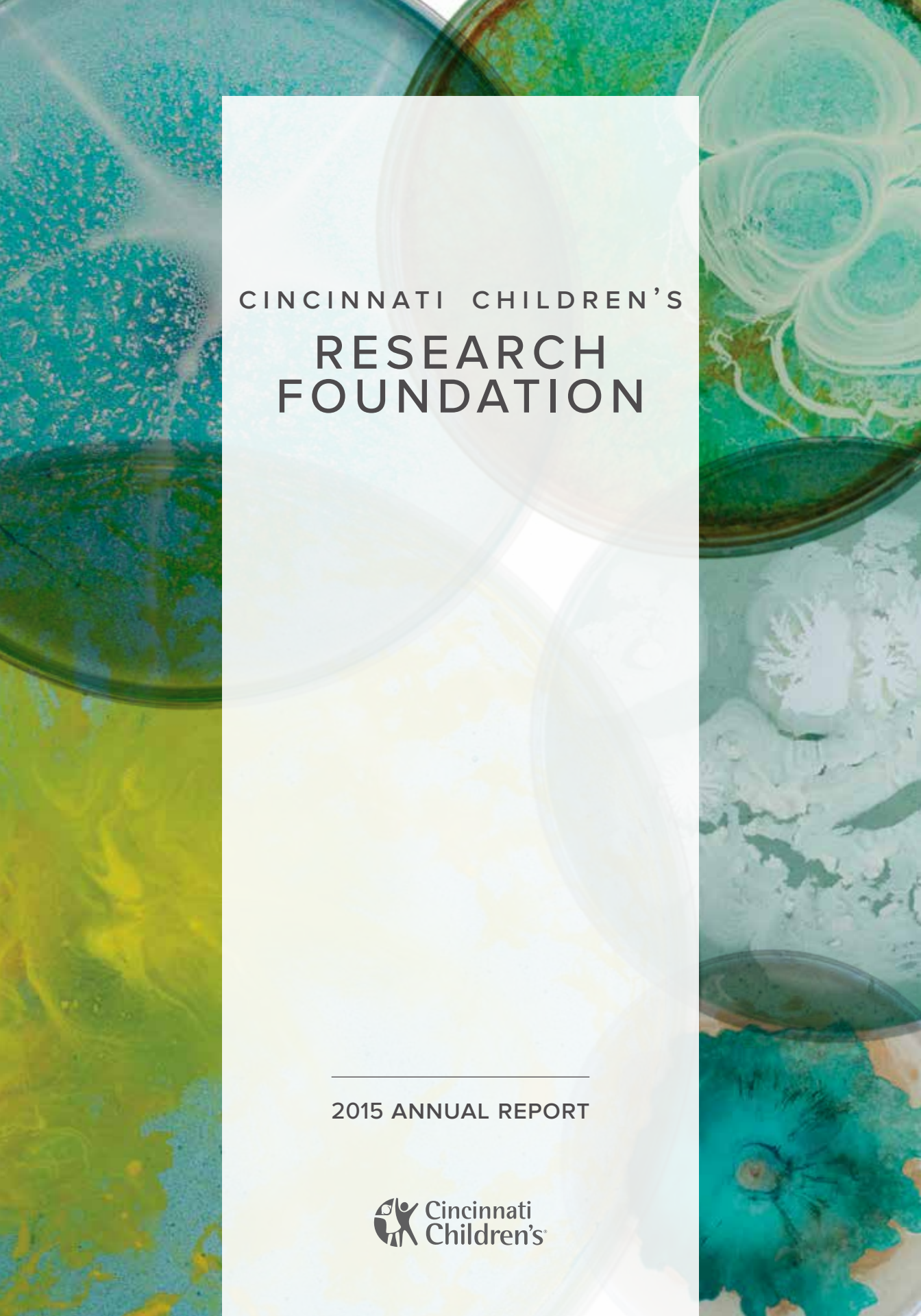
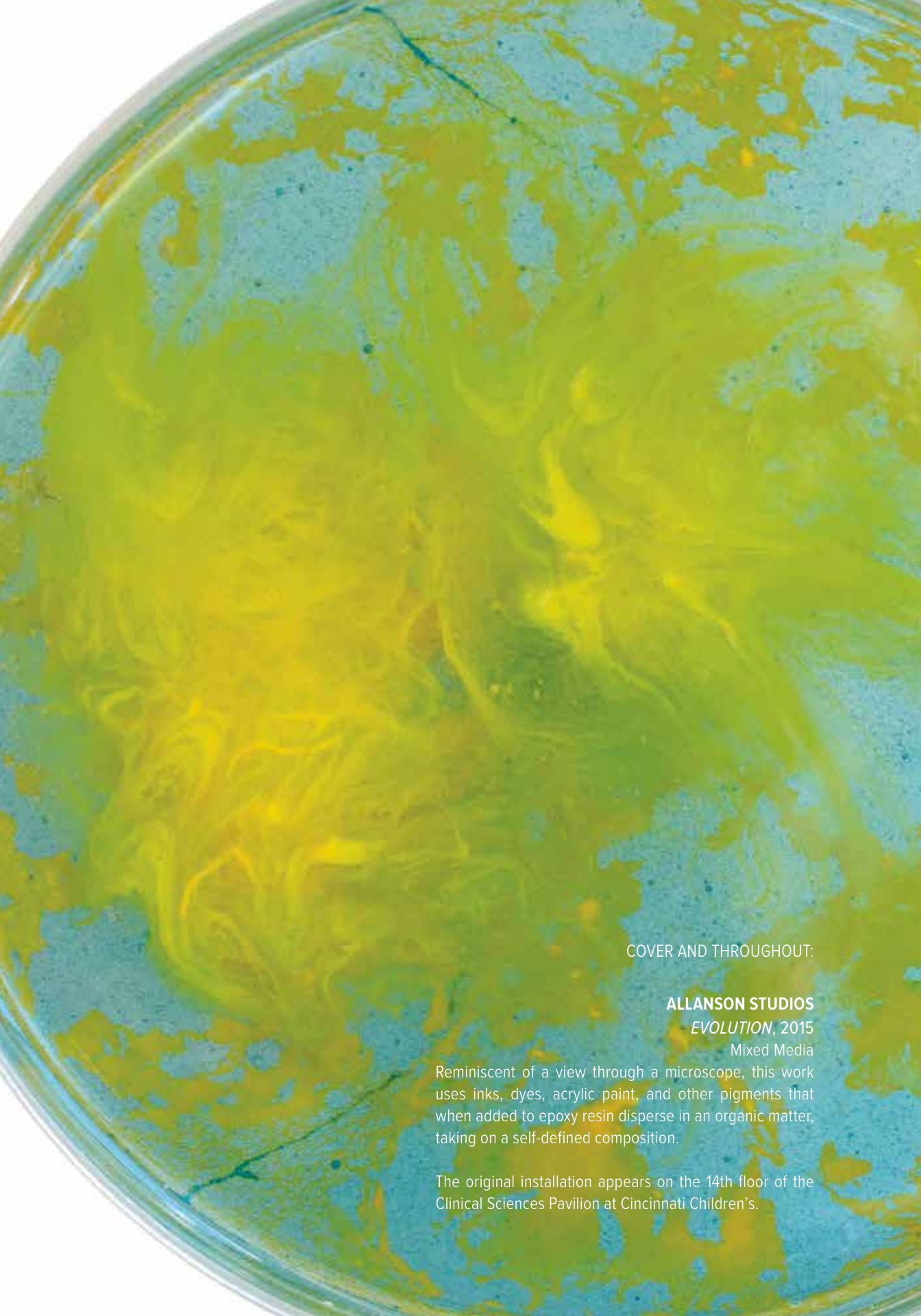


CINCINNATI CHILDREN'S

RESEARCH FOUNDATION

2015 ANNUAL REPORT

Cincinnati Children's



COVER AND THROUGHOUT:

ALLANSON STUDIOS
EVOLUTION, 2015
Mixed Media
Reminiscent of a view through a microscope, this work uses inks, dyes, acrylic paint, and other pigments that when added to epoxy resin disperse in an organic matter, taking on a self-defined composition.

The original installation appears on the 14th floor of the Clinical Sciences Pavilion at Cincinnati Children's.

CONTENTS

DIVISION SUMMARIES
2015 SCIENTIFIC ACHIEVEMENTS

Dear Colleagues,

I am pleased to present this year's annual report of the top scientific achievements of the Cincinnati Children's Research Foundation. As you will soon see, this publication assembles a truly remarkable set of insights, innovations and discoveries produced by our equally remarkable and steadily growing faculty.

In many ways, this report helps establish a baseline for the next five years as we embark upon our strategic objective to lead the world in improving child health through our collaborative culture of discovery, translation and learning. We are accelerating our efforts to expand the impact of our research enterprise across the entire spectrum of discovery, from basic exploration of the mechanisms of human health and disease, through the process of translating innovation into practice, all the way to assessing the impact of research upon community health.

In this publication, you can read about six of the most innovative, farthest reaching breakthroughs achieved at Cincinnati Children's since 2010. You can also read about more than 50 significant discoveries achieved in fiscal 2015. Yet I want to emphasize that these works represent just a portion of the high-impact research happening here, just one slice of the output generated by a scientific infrastructure that took decades to build. In just the past year, our faculty published more than 2,000 peer-reviewed articles, chapters, books and other publications. Very few pediatric medical centers can match this level of productivity.

The cover of this report celebrates the inspiring art installed throughout our new Clinical Sciences Pavilion, the latest physical example of our deep and ongoing commitment to research. The content of this report outlines many other ways Cincinnati Children's supports our mission to improve child health through scientific endeavor. We have established a wide variety of internally funded pilot grant programs to nurture emerging concepts. We invest in more than 20 research core facilities to make the very best technology available to some of the very best minds in pediatrics.

Our ability to invest in outstanding people and our long track record of collaboration have placed Cincinnati Children's in the position to be a significant force in the emerging world of "team science." We look forward to forging new partnerships and collaborations with other leading pediatric institutions. Together, we will make bold progress against the greatest challenges in improving child health. Five years from now, we expect to report upon an even more remarkable set of scientific achievements.

Margaret K. Hostetter, MD

B.K. Rachford Professor
Chair, Department of Pediatrics
Director, Cincinnati Children's Research Foundation
Chief Medical Officer
Cincinnati Children's Hospital Medical Center

INNOVATION IS A WAY OF LIFE AT CINCINNATI CHILDREN'S. IT TRANSCENDS EVERYTHING WE DO.

This year's Research Foundation Annual Report is a comprehensive account of our finest published work over the past year. The breadth and depth of these findings come from the efforts of our diverse and talented scientists and their support staffs, who are from more than 80 countries and make up about one-third of the total workforce at Cincinnati Children's.

The importance we place on innovation at Cincinnati Children's was strongly reinforced this year as we:

- Earned almost $200 million in external grant and industry funding at a time when funding for medical research is, in general, declining.
- Invested $109 million in laboratory and applied research costs that are not covered by external grants or industry support. This significant commitment by our institution underscores our confidence in the people who work here and the quality of their endeavors.
- Launched our 2020 strategic plan, which continues to emphasize our focus on medical research and our drive to transform child health with our collaborative culture of discovery, translation and learning.
- Opened our new 15-story Clinical Sciences Pavilion, a symbol of our longstanding and future commitment to pediatric research. It was designed to elevate our full continuum of discovery, including innovation, translation, community health and improvement science, and accelerate discoveries into practice. The new facility will also help us retain and continue to attract top talent from around the world.

Cincinnati Children's is proud to have some of the best and brightest minds working on some of the most difficult problems in pediatric medicine. Their simple and singular motivation is to be the leader in improving child health.

The advances summarized in this report are inspiring. Yet, we are reminded every day of the gaps that still exist, the cures that remain undiscovered, and the children who face uncertain futures.

As we move into another exciting year filled with promise and anticipation, we thank our partners, funders, donors and the community for the many ways you show your support of our pediatric research efforts. Together, we are changing the outcome for children and families from down the street, across the country, and around the world.

Thomas Cody
Chair, Board of Trustees

Nancy Krieger Eddy
PhD, Research Chair

Michael Fisher
President and CEO

Six Premier Achievements Set Standard for Changing Outcomes Together



Since 2010, the highly talented and productive faculty members of the Cincinnati Children's Research Foundation have produced more than 7,000 studies published in peer-reviewed scientific and medical journals.

These contributions have had far-reaching impact across pediatric medicine, leading to new therapeutics, improved diagnostics, and significant advances in our fundamental understanding of human development and disease.

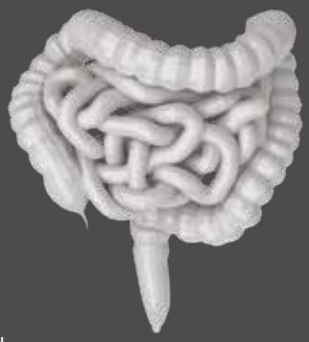
From this impressive body of work, six advances stand out as Cincinnati Children's premier scientific achievements of 2010-15. The following innovations and breakthroughs were selected by committees of senior research leaders, led by Tracy Glauser, MD, and Jeffrey Whitsett, MD. Their selections reflect work with particularly powerful influence upon basic, translational and clinical research — and they set a challenging standard for the work we will pursue in the next five years.

> "These researchers truly exemplify our mission at Cincinnati Children's to improve child health through innovation,"
>
> — Margaret Hostetter, MD, Director, Cincinnati Children's Research Foundation

Organoid Revolution Happening Here

BE THEY TEST PLATFORMS TO EVALUATE NEW MEDICATIONS OR THE FUTURE OF REGENERATIVE MEDICINE, THE TINY INTESTINE AND STOMACH ORGANOIDS DEVELOPED AT CINCINNATI CHILDREN'S BY JAMES WELLS, PhD, and colleagues have only just begun to demonstrate their potential to transform medicine.

In December 2010, a paper posted online in *Nature* revealed that Wells' team had created functional, three-dimensional intestinal tissue from a combination of human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs). With this breakthrough, Cincinnati Children's joined an elite class of medical centers making dramatic progress at growing miniature human brains, kidneys, pancreata, and other complex tissues in laboratory settings.



In a series of cell manipulations, Wells' team coaxed stem cells to transform from definitive endoderm into hindgut progenitor cells, and ultimately into organoids containing all the major intestinal cell types – including enterocytes, goblet, Paneth and enteroendocrine cells. The tissue demonstrated absorptive and secretory functions and even began forming its own intestine-specific stem cells.

At this point, Wells' team had an organ-on-a-chip, a research tool potentially capable of supplanting mice as a human-based model for studying disease. The mini-intestine shows promise as a model to further study necrotizing enterocolitis, inflammatory bowel disease, short bowel syndrome and more.

But the team did not stop here. In a study published online Oct. 19, 2014, in *Nature Medicine*, Wells collaborated with Michael Helmrath, MD, MS, to demonstrate that intestinal organoids can grow into fully mature human tissue once grafted to a mouse kidney to provide a blood supply. This tissue included muscle layers and a self-renewing mucosal lining. The success was an encouraging sign that stem cell-derived organoids might be able to grow on their own once transplanted into the human body.

The very same month, Wells and graduate student Kyle McCracken published another paper in *Nature* announcing success at forming a mini-stomach. This "gastric organoid" specifically resembled the antrum, the portion of the stomach that connects to the intestine. Importantly, the organoids can harbor gut bacteria, which makes them immediately useful in research related to *H. pylori*, the bacterium that causes stomach ulcers.

Further organoid development continues at a rapid pace. Wells and colleagues are working on growing the fundus, the acid-secreting portion of the stomach and several other projects. An exciting aspect of the stomach project is that the organoids were developed from functional anterior foregut spheroids, which also serve as the developmental root of the pancreas and the lung.

In fact, Jason Spence, PhD, one of the colleagues who worked with Wells on the intestine organoid project, has carried on the work at the University of Michigan. In March 2015, Spence reported success at developing a lung organoid with structures resembling bronchi and alveoli that survived in the laboratory for 100 days.

A Study That Changed the Direction of Cardiac Stem Cell Research

SOMETIMES GREAT MEDICINE BOILS DOWN TO FIGURING OUT WHY AN EXCITING NEW TREATMENT APPROACH JUST DOES NOT WORK. THIS IS HOW JEFFERY MOLKENTIN, PHD, ROCKED THE WORLD OF CARDIAC research in 2014. From the instant scientists discovered how to create stem cells that did not require the use of embryonic tissue, experiments began to find a way to use stem cells to regenerate the human heart.

Few missions in medicine have had higher priority. Heart disease is the nation's leading cause of death. Beyond the fatalities, the damage caused by sudden heart attacks and gradual heart failure destroys the quality of life for millions of people while incurring hundreds of billions in health care costs. And yet, despite massive demand for cures, deep investment in research, and great expectations for stem cell technology, cardiac muscle remains stubbornly resistant to repair.

For several years, it seemed that regenerative medicine was poised for a breakthrough. Excitement ran high for the possibility that cardiac progenitor cells or even bone marrow cells expressing the cell surface protein c-kit could be injected into damaged or dead areas of the heart to directly create new beating tissue.

However, what was reported to work so well in mice and rats produced dismal results in human clinical trials in more than 4,000 patients worldwide. Molkentin's team explained why in a paper published May 7, 2014, in *Nature*.

Molkentin is a widely published cardiovascular molecular biologist at Cincinnati Children's Heart Institute who was named a Howard Hughes Medical Institute (HHMI) investigator in 2008. His work to understand the intracellular signaling pathways and transcriptional regulatory circuits that control cell growth and differentiation has significantly advanced understanding of heart disease and muscular dystrophy.

In this study, Molkentin's team developed a genetic strategy in mice so that any new myocyte arising from a potential c-kit stem cell would be labeled green with a fluorescent protein. The lack of color told the story; just 0.027 percent of the murine cardiomyocytes originated from c-kit stem cells. To the extent that c-kit stem cells offered any therapeutic benefit, it was through small improvements to blood vessels, not generation of new muscle tissue.

"What we showed in our study is that c-kit-positive stem cells from the heart can make endothelial cells that form capillaries. But these c-kit positive cells do not like to make cardiomyocytes," Molkentin says. "If they really do generate new cardiomyocytes, the rates are exceptionally low – roughly one in every 3,000 cells – and that becomes meaningless."

These findings shocked the field and stirred extensive scientific debate. Ultimately, Molkentin's work helped reprioritize how future clinical trials are planned, or no longer planned, and freed the research community to begin looking in new directions for a regenerative therapy that can work as intended.

Gene Therapy Advances Raise Hopes for Curing Sickle Cell Disease

For many years, clinicians could do little more than try to manage the pain of sickle cell disease and treat complications as they occurred. For patients, it has been a world of daily antibiotics as young children, blood transfusions, hospital admissions, and — for those lucky enough to find a matching donor — bone marrow transplants. Now, scientists are closing in on a potential cure. Human clinical trials have begun to evaluate a patented treatment that could reshape the future for children born with sickle cell disease and respresent a major step forward for the field of gene therapy.

Developed at Cincinnati Children's by Punam Malik, MD, the treatment employs a modified lentivirus to transfer a healthy fetal hemoglobin gene to people with sickle cell disease, which can allow their bodies to produce normal red blood cells instead of the sickle-shaped cells that define the inherited disorder. If successful, the treatment would effectively cure a disease that affects more than 90,000 people in the United States and millions of people worldwide. It also may serve as a treatment for thalassemia, a related rare blood disease.

A U.S. patent application was filed in 2011 for the gene therapy method after years of research led by Malik demonstrated that the technique was effective in halting blood cell sickling in mice and in human tissue samples. Malik joined Cincinnati Children's in 2007. She received her MBBS in 1985 and her MD in 1989 from Delhi University in New Delhi, India. She earned an MS from the University of Maryland in 1991, then launched a career in Hematology-Oncology at Children's Hospital Los Angeles before moving to Cincinnati.

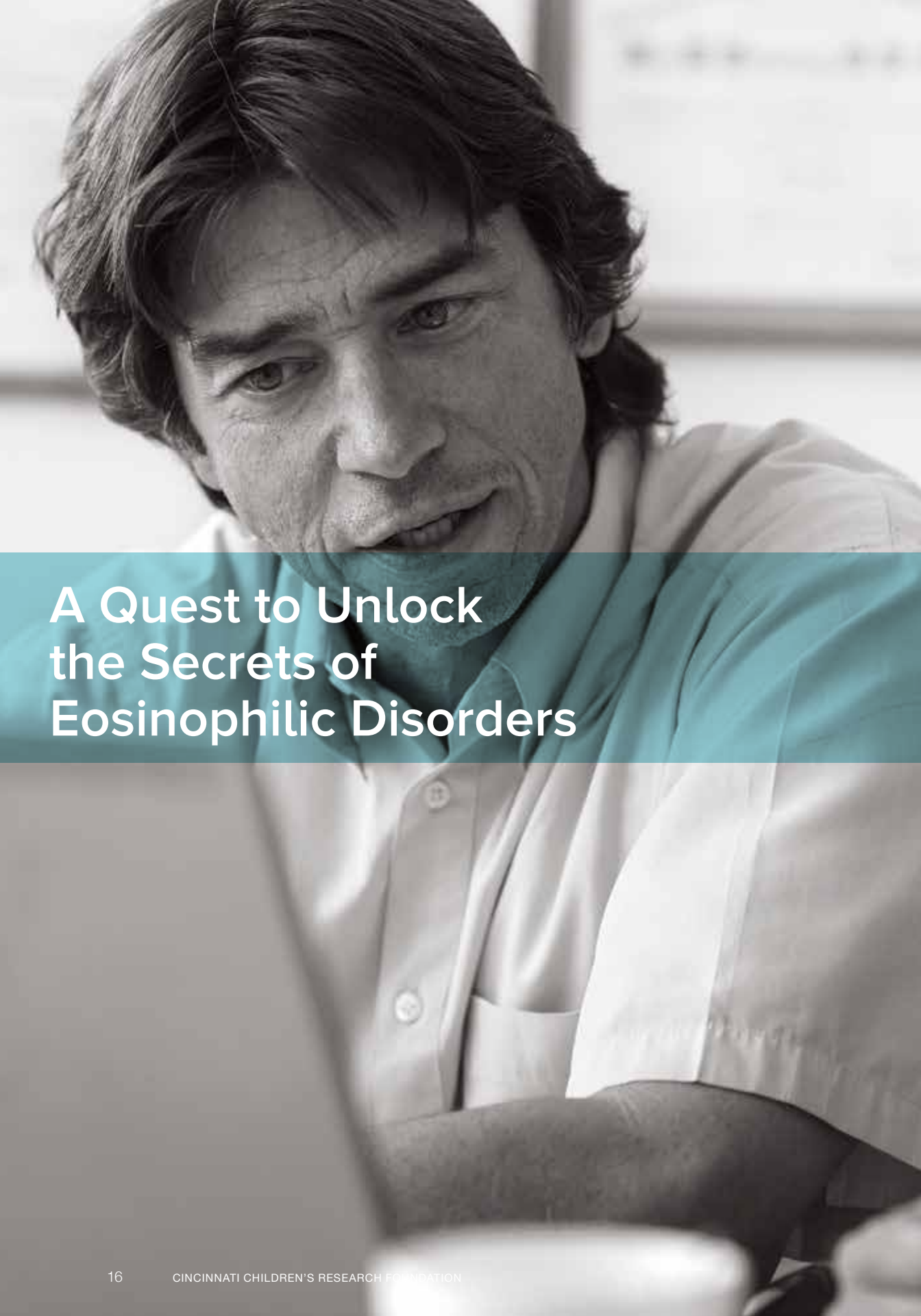
In sickle cell disease, a genetic defect makes red blood cells carry hemoglobin S, which produces misshapen blood cells that cannot efficiently deliver oxygen. Over time, the condition can trigger strokes, kidney failure and other forms of tissue damage.

Malik's innovation flows from the observation that some adults never stop producing fetal hemoglobin (HbF), which prevents red blood cell sickling when present in the body in sufficient quantity. Normally, the fetal hemoglobin gene switches off shortly after birth. The new gene therapy reactivates it.

The clinical trial, which envisions following 10 participants for 15 years, began enrolling participants in 2014. The treatment involves collecting bone marrow stem cells from patients, using a lentivirus vector to deliver a healthy γ-globin gene to the cells, then returning the engineered cells to the patients where they can multiply into permanent HbF factories.

The technique patented at Cincinnati Children's stands out because it restricts transgene expression to maturing erythroid cells, which reduces the cancer risks that have plagued other gene therapy efforts.

It remains unclear whether gene therapy will become the ultimate cure for sickle cell disease. Newer technologies, including the CRISPR/Cas9 gene editing system, may be able to directly correct the sickle cell genetic defect in hematopoietic stem cells without the need for gene insertion therapy.



A Quest to Unlock the Secrets of Eosinophilic Disorders

THE MISSION STARTED BECAUSE SCIENTISTS WANTED TO FIND OUT WHY SOME CHILDREN DEVELOP FOOD ALLERGIES SO SEVERE THAT IT AFFECTS THEIR GROWTH. THESE CHILDREN SUFFER FREQUENT STOMACHACHES, vomiting and diarrhea. They have trouble swallowing and often must endure life on strictly limited diets. The search for answers has changed our understanding of food allergies and established a newly recognized set of conditions, called eosinophilic gastrointestinal disorders (EGID), in which the immune system treats food as a foreign invader.

Marc Rothenberg, MD, PhD, has been a pioneer in the field, and Cincinnati Children's has become a global leader in researching and treating these conditions.

Until the last decade, eosinophils were best known as end-stage cells of the immune system. Their only acknowledged job was to provide host protection against parasites. However, Rothenberg and colleagues have shown that eosinophils are multifunctional leukocytes involved in diverse inflammatory responses. The work progressed from describing a single condition — eosinophilic esophagitis (EoE) — to establishing an entire class of disorders. Rothenberg coined the term EGID in 2004, coinciding with him becoming the Founding Director of the Cincinnati Center for Eosinophilic Disorders, the first center of its kind in the U.S. Rothenberg has published over 300 papers researching these topics, including many that have deeply influenced the field.

Rothenberg's team has characterized several critical pathways that regulate allergic responses, including the eotaxin family of chemokines (eosinophil chemoattractants) and interleukin 5 (eosinophil growth/activating cytokine), which is now a proven target for eosinophilic subtypes of asthma. His laboratory established the first animal models of EoE and identified the EoE transcriptome, a genome-wide set of molecular markers that have been translated into a commercially available EoE diagnostic panel. Rothenberg's team conducted the first controlled clinical trial in EoE, demonstrating the effectiveness of topical glucocorticoid therapy, which is now in clinical use.

In 2010, Rothenberg published the first genome-wide association study of EoE in *Nature Genetics*. This study demonstrated a genetic linkage with chromosome 5q22 and that genetic variants of the immune hormone thymic stromal lymphopoietin were involved in allergen sensitization.

With John Harley, MD, PhD, Leah Kottyan, PhD, and other collaborators at Cincinnati Children's, Rothenberg conducted another genome-wide analysis that interrogated more than 1.5 million genetic variants in EoE. The team published a breakthrough in 2014 in Nature Genetics identifying a powerful association between EoE and chromosome region 2p23, particularly in the gene CAPN14.

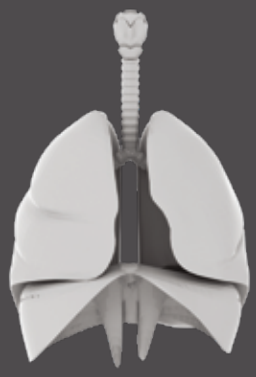
They found that CAPN14 was located in an epigenetic "hotspot" modified by IL-13 and was dynamically upregulated as a function of disease activity. Remarkably, the CAPN14-encoded protein, calpain 14, was specifically and most highly expressed in the esophagus, answering the long-standing question in the allergy field of why patients develop tissue-specific manifestations.

Macrophage Transplantation Could Take Familial PAP Off the Map

PULMONARY ALVEOLAR PROTEINOSIS (PAP) IS A RARE AND BRUTAL PULMONARY DISORDER. PEOPLE WITH THIS CONDITION HAVE LUNGS THAT CANNOT REMOVE USED SURFACTANT, WHICH GRADUALLY BUILDS UP IN

air sacs and erodes the ability to breathe. For years, the only way to relieve symptoms was to perform whole-lung lavage, a surgical procedure introduced in the 1960s that involves flushing as much as 50 liters of saline solution through each lung to clear away accumulated surfactant. However, the effects are temporary. While some patients can go long periods between lavages, others require treatment as often as every one to two months.

In 1999, a research team in Japan discovered that people with idiopathic PAP produce a neutralizing autoantibody against granulocyte-macrophage colony-stimulating factor (GM-CSF). This was important because GM-CSF is crucial for developing the fully mature macrophages needed to clear away used surfactant.



Bruce Trapnell, MD, and colleagues at Cincinnati Children's have dramatically advanced that early work. First, they proved that the antibody was not merely present, but was actually causing the disease; a finding that re-defined idiopathic PAP as an autoimmune disorder. This work led to a diagnostic test for autoimmune PAP currently provided as a clinical research test by Trapnell's team at the Translational Pulmonary Science Center. It also supports their program to develop potentially beneficial alternatives to whole-lung lavage, including inhaled GM-CSF, plasmapheresis, and rituximab therapy.

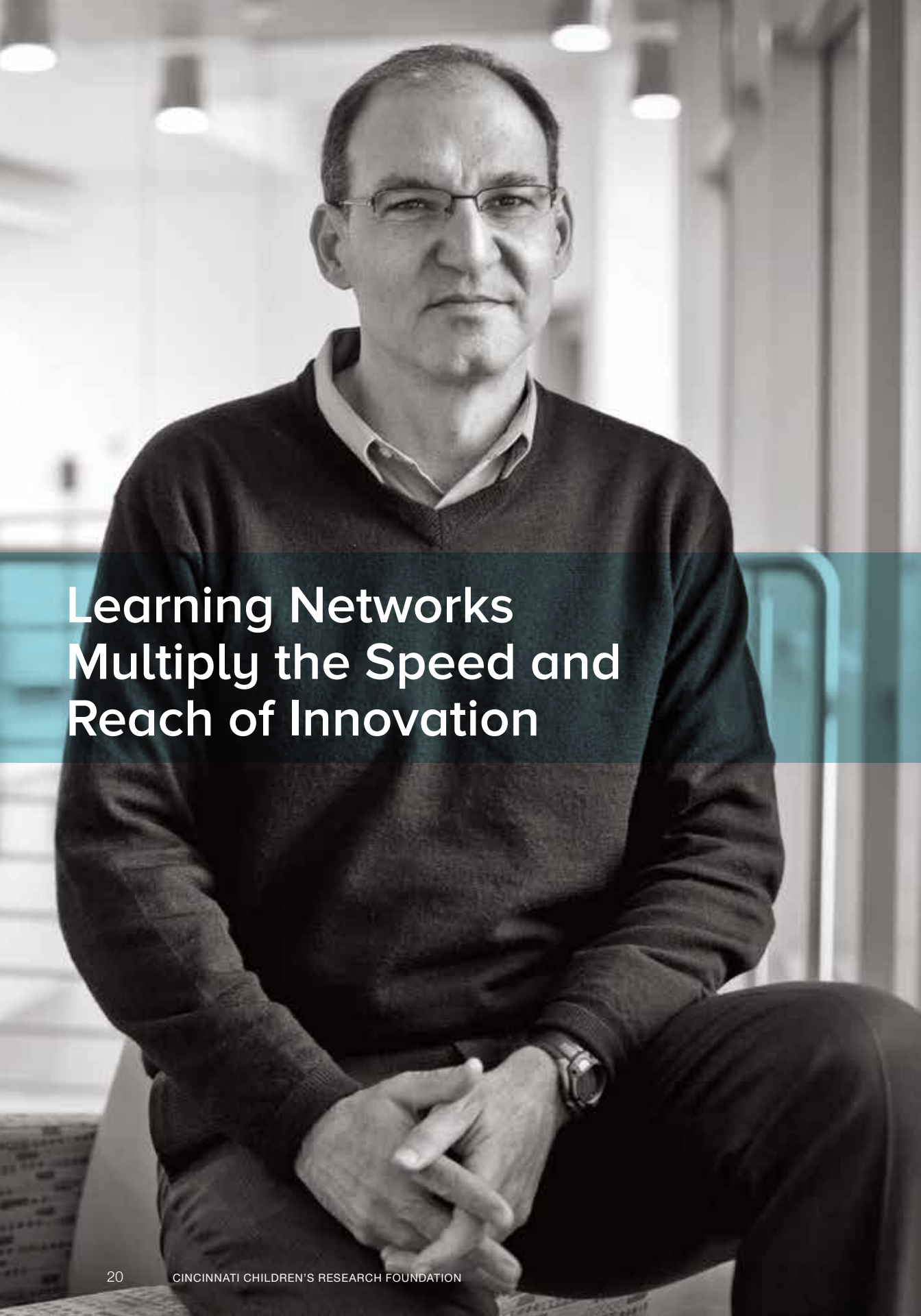
That was just the beginning.

Trapnell and his colleague Takuji Suzuki, MD, PhD, also found that some children have a hereditary form of PAP (hPAP) that does not involve the antibody. Instead, these children had a genetic defect that prevents macrophages from receiving signals from GM-CSF. This finding launched an effort to develop what could be the first successful form of lung gene therapy.

In a breakthrough paper, published in October 2014 in *Nature*, Trapnell and colleagues reported achieving a major milestone. In mice, they successfully performed pulmonary macrophage transplantation (PMT) of both wild-type and Csf2rb-gene-corrected macrophages. Not only was the procedure safe and well-tolerated, the healthy macrophage cells proliferated in the lungs and began doing their job of clearing away the excess surfactant. One administration fully corrected the lung disease as well as the secondary systemic manifestations and restored disease-related biomarkers to normal. Importantly, testing also found that the transplanted macrophages remain confined to the lungs and did not expand into the blood, bone marrow, or spleen. The ability to introduce gene-corrected macrophages in such a controlled fashion has implications that could go well beyond treating hPAP, including potential for treating a variety of serious lung infections.

In humans, Trapnell says transplants could be performed using the patient's own cells, thus eliminating the need for immunosuppression. Delivery can be achieved via bronchoscopic instillation versus much riskier surgical procedures.

With so much potential, many eyes will be watching as Trapnell and colleagues work through the preclinical studies needed to prepare for human clinical trials.



Learning Networks Multiply the Speed and Reach of Innovation

WHAT IF THERE WAS A WAY TO CREATE A VASTLY BETTER CHRONIC ILLNESS CARE SYSTEM BY HARNESSING THE INHERENT MOTIVATION AND COLLECTIVE INTELLIGENCE OF PATIENTS AND CLINICIANS?

Answering that question has been the driving force behind the impressive achievements of Peter Margolis, MD, PhD, and colleagues at Cincinnati Children's whose work is transforming pediatric medicine in fundamental ways.

Margolis is Director of Research for the James M. Anderson Center for Health Systems Excellence at Cincinnati Children's. He has been a pioneer in developing "pediatric learning health systems," including the disease-specific ImproveCareNow network and the wider-scale PEDSnet project. Both projects have assembled massive communities of physicians, families and scientists who can come together to improve outcomes by sharing data, experiences and insights.

Since its launch four years ago, the ImproveCareNow network has grown to include more than 50 gastroenterology sites in the U.S. Their collaborative efforts have resulted in a dramatic 22 percentage point improvement in remission rates for patients in the network, from 55 percent to 77 percent.

Now Margolis is working to expand the lessons learned from the ImproveCareNow project to many more chronic conditions. The new PEDSnet consortium includes eight academic pediatric medical centers, two disease-specific pediatric networks, and two national data partners. Margolis and Christopher Forrest, MD, PhD, Children's Hospital of Philadelphia are the principal investigators. Details about PEDSnet were published in July 2014 in a paper Margolis co-authored for the Journal of the American Medical Informatics Association.

PEDSnet will support the wider goals of the National Pediatric Learning Health System, which seeks to integrate clinical studies done in routine care settings, leverage structured data capture at every encounter, and incorporate quality improvement methods to advance care delivery – including active and meaningful patient participation. By 2018, plans call for expanding PEDSnet to include 10 percent of the nation's children, and to use the network to conduct 25 observational research and 10 interventional studies.

PEDSnet itself is part of a still larger effort known as PCORnet, the National Patient-Centered Clinical Research Network. PCORnet has grown to include 34 research networks like PEDSnet. Margolis was recently chosen to chair the PCORnet Steering Committee.

Already, the participating networks have mapped data for 1 million individuals to the PCORnet Common Data Model. The next phase will include using that information to accelerate discoveries in more than 150 diseases and conditions.

Our 2015 Scientific Achievements

Our research faculty members used nearly $200 million in external funding to produce more than 2,000 peer-reviewed journal articles, book chapters and other publications in FY2015. The following pages feature the most significant publication from each of our research divisions.

Prepubertal Hormone Levels Differ Sharply Between Overweight, Normal-Weight Girls

Frank Biro, MD

RESEARCH AND TRAINING DETAILS

Faculty	15
Joint Appointment Faculty	2
Research Students	7
Support Personnel	13
Direct Annual Grant Support	$1.1M
Direct Annual Industry Support	$78,886
Peer Reviewed Publications	30

Biro FM, Pinney SM, Huang B, Baker ER, Walt Chandler D, Dorn LD. Hormone changes in peripubertal girls. *The Journal of Clinical Endocrinology and Metabolism*. 2014;99(10):3829-3835.

PUBLISHED ONLINE JULY 16, 2014
Journal of Clinical Endocrinology and Metabolism

Using new methods to determine hormone levels in prepubertal girls, a Cincinnati Children's research team has demonstrated that significant changes in hormone levels can be detected prior to clinically evident changes in puberty — a finding that could help explain why breast cancer risks vary among obese and non-obese women later in life.

This longitudinal study examined relationships between adrenal and sex hormones in 252 peripubertal girls in Cincinnati who were recruited between 2004 and 2010. Participants entered the study between ages 6 and 7 and were followed from 30 months before to six months after the appearance of breast development. Detailed findings were published online July 16, 2014, in the *Journal of Clinical Endocrinology and Metabolism.*

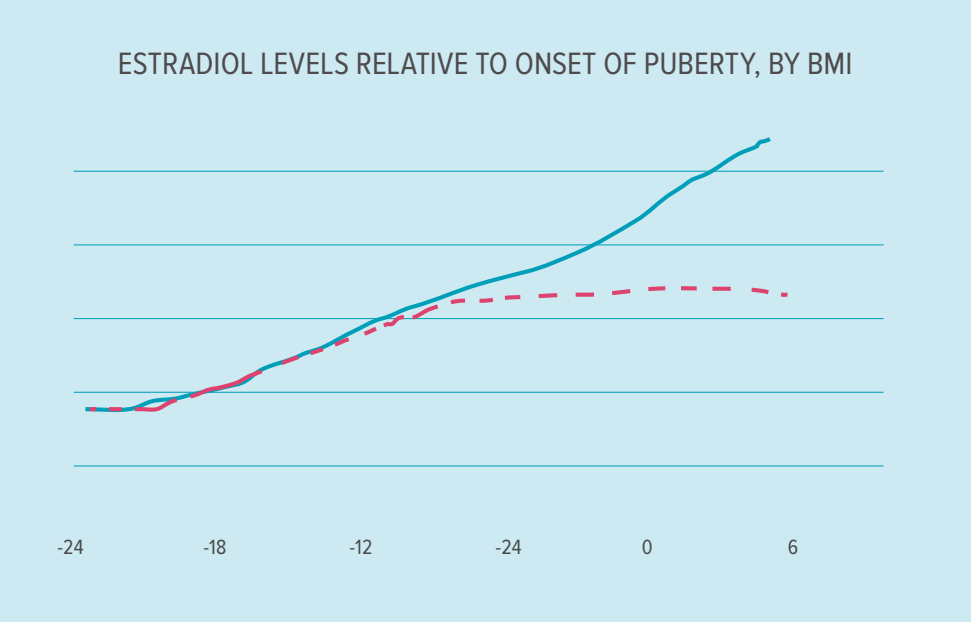
"We noted radical differences between overweight and normal weight girls, and our data suggest that heavy girls are producing some of their circulating estrogens from their adipose tissue, rather than from their ovaries," says Frank Biro, MD, first author of the study. "That finding is consistent with differing rates of breast cancer among postmenopausal women when comparing obese and non-obese."

Gathering data on estradiol, estrone, androstenedione, and T serum concentrations required using high-performance liquid chromatography (HPLC) with tandem mass spectrometry, a newer and highly sensitive analytic approach. Dehydroepiandrosterone sulfate (DHEA-S) and SHBG were measured through established methods.

The research team reports that the average age of breast development in the study group was 8.78 years. Testing revealed that hormone concentrations of DHEA-S increased 30 to 18 months prior to breast development; androstenedione and estrone levels increased 12 and 18 months before onset of breast development; estradiol and T-serum concentrations increased while SHBG levels decreased six to 12 months before breast development.

Heavier peripubertal girls had lower estradiol levels at puberty. These findings suggest a mechanism, especially in heavier girls, for pubertal changes without activating the hypothalamic-pituitary-ovarian axis.

"Our data suggest that heavy girls are producing some of their circulating estrogens from their adipose tissue, rather than from their ovaries."



Serum hormone concentration of estradiol by BMI group relative to onset of puberty. This figure demonstrates that estradiol concentrations are significantly greater at onset of puberty (as defined by onset of breast development), as well as six months after onset, in girls with below-median BMI.

Identification of a Genetic-Molecular Linkage for EoE Opens New Doors for Treatment

Marc Rothenberg, MD, PhD

RESEARCH AND TRAINING DETAILS

Faculty	15
Joint Appointment Faculty	3
Research Fellows	6
Research Students	15
Support Personnel	45
Direct Annual Grant Support	$4M
Direct Annual Industry Support	$322,821
Peer Reviewed Publications	45

Kottyan LC, Davis BP, Sherrill JD, Liu K, Rochman M, Kaufman K, Weirauch MT, Vaughn S, Lazaro S, Rupert AM, Kohram M, Stucke EM, Kemme KA, Magnusen A, He H, Dexheimer P, Chehade M, Wood RA, Pesek RD, Vickery BP, Fleischer DM, Lindbad R, Sampson HA, Mukkada VA, Putnam PE, Abonia JP, Martin LJ, Harley JB, Rothenberg ME. Genome-wide association analysis of eosinophilic esophagitis provides insight into the tissue specificity of this allergic disease. *Nat Genet.* 2014;46(8):895-900.

PUBLISHED ONLINE JULY 13, 2014
Nature Genetics

A gene called CAPN14 has been identified as a novel genetic component in epithelial tissue in the esophagus, and the gene's interaction with the immune hormones thymic stromal lymphopoietin (TSLP) and interleukin 13 (IL-13) may explain why some patients develop eosinophilic esophagitis (EoE), a hard-to-treat food allergy marked by chronic inflammation of the esophagus.

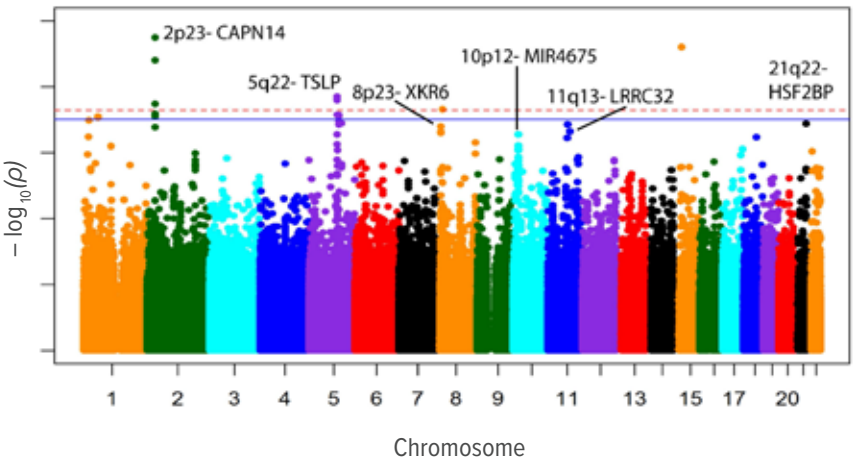
In effect, EoE turns out to develop from interplay of a patient's underlying genetic susceptibility to allergies and a tissue-specific process dictated by the molecular aspects of the CAPN14 gene, according to Marc Rothenberg, MD, PhD, Director of the Cincinnati Center for Eosinophilic Disorders and the Division of Allergy and Immunology. Rothenberg, and colleagues in the Divisions of Gastroenterology, Hepatology and Nutrition; Human Genetics; and the Center for the Genetics Autoimmune Etiology, probed millions of genetic variants in nearly 1,000 people with EoE and 9,000 people without EoE.

They found several genetic linkages, with the strongest associations being at the CAPN14 and TSLP loci. The study was published online July 13, 2014, in the journal *Nature Genetics.*

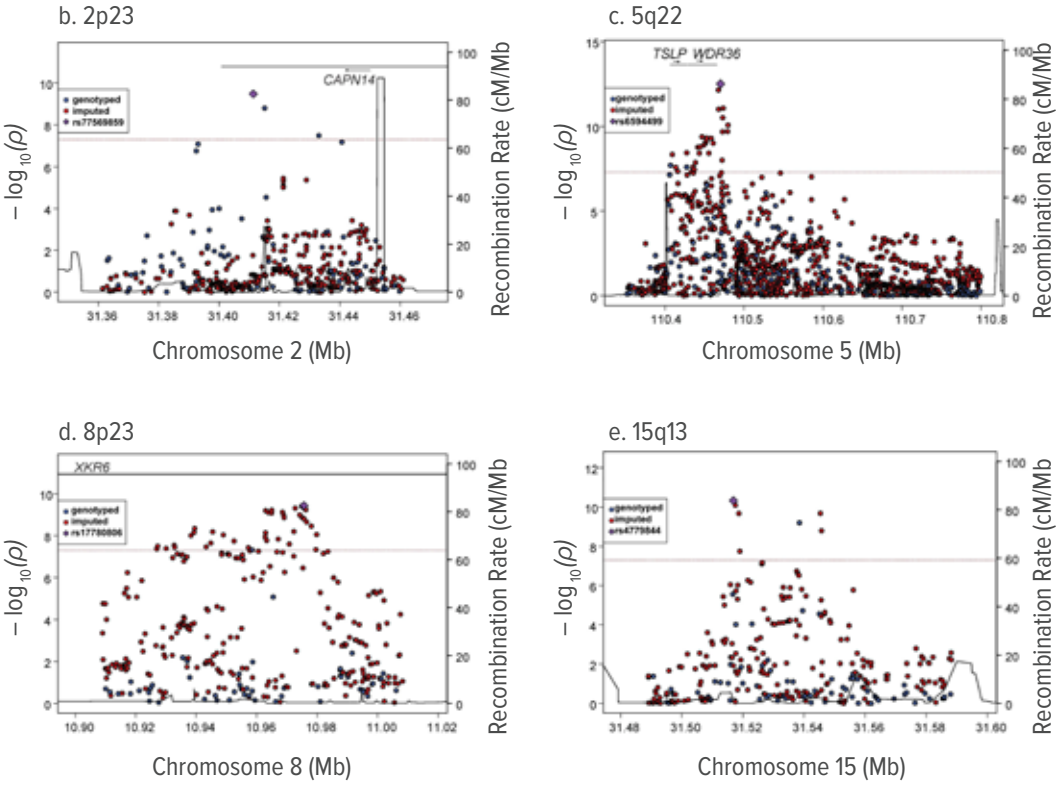
EoE is triggered by allergic sensitivity to certain foods and an accumulation of eosinophils, specialized immune cells, in the esophagus. Rothenberg and his team found that CAPN14, which encodes the enzyme calpain 14 in the esophagus, is dramatically upregulated when epithelial cells in the esophagus are exposed to IL-13, a known molecular activator of EoE. The CAPN14 gene is part of the esophageal disease process of EoE.

CAPN14's upregulation, Rothenberg's team noted, occurs in an epigenetic "hot spot" that encodes for an EoE-associated genetic variant that regulates the binding of transcription factors to the upstream region of the CAPN14 gene.

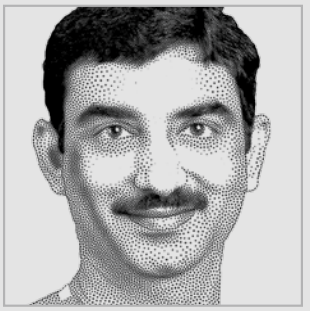
This new finding "is a breakthrough for this condition and gives us a new way to develop therapeutic strategies by modifying the expression of calpain 14 and its activity," Rothenberg says. "Our results are immediately applicable to EoE and have broader implications for understanding eosinophilic disorders, as well as allergies, in general."



This Manhattan plot (above) shows P values obtained from genome-wide association analysis of data from 736 subjects with EoE and 9,246 controls having 1,468,075 genetic variants. Genome-wide significance is indicated with the red dotted line, and suggestive significance marked with a solid blue line. Subsequent charts show genetic association of variants at the 2p23, 5q22, 8p23, and 15q13 loci with EoE risk. P values of the genetic association analysis of genotyped and imputed variants are plotted against the genomic positions of each genotyped (blue) and imputed (red) SNPs on the x axis on chromosomes 2, 5, 8, and 15. Genes in the region are shown above. The black lines indicate the recombination rates in cM per Mb using subjects of European ancestry from the 1,000 Genomes Project.



Genetic Variant Might Hold Key to Predicting Respiratory Depression and Personalizing Morphine Dosage



Senthilkumar Sadhasivam, MD, MPH

Vidya Chidambaran, MD

RESEARCH AND TRAINING DETAILS

Faculty	60
Joint Appointment Faculty	2
Research Fellows	10
Research Students	5
Support Personnel	30
Direct Annual Grant Support	$936,373
Direct Annual Industry Support	$227,279
Peer Reviewed Publications	61

Chidambaran V, Mavi J, Esslinger H, Pilipenko V, Martin LJ, Zhang K, Sadhasivam S. Association of OPRM1 A118G variant with risk of morphine-induced respiratory depression following spine fusion in adolescents. *Pharmacogenomics J.* 2015;15(3):255-262..

PUBLISHED ONLINE SEPT. 30, 2014
The Pharmacogenomics Journal

Better genetic-based predictors of morphine-induced respiratory depression could lead to more personalized pain-relief dosage for children recovering from surgery.

The key was found in μ1 opioid receptor genetic variant A118G, which decreases the binding potential of the μ-receptor in the brain, and therefore increases morphine requirement.

Postoperative respiratory depression is the most serious adverse effect of opioids. Researchers believe genetics account for up to 30 percent of the variability in respiratory depression.

The study, published online Sept. 30, 2014, in *The Pharmacogenomics Journal*, was led by Senthilkumar Sadhasivam, MD, MPH, and Vidya Chidambaran, MD, both of the Division of Anesthesia, and also involved researchers from the Division of Human Genetics. It was the first clinical study to show significant association of a functionally relevant polymorphism — A118G — of the opioid receptor gene in predicting MIRD susceptibility.

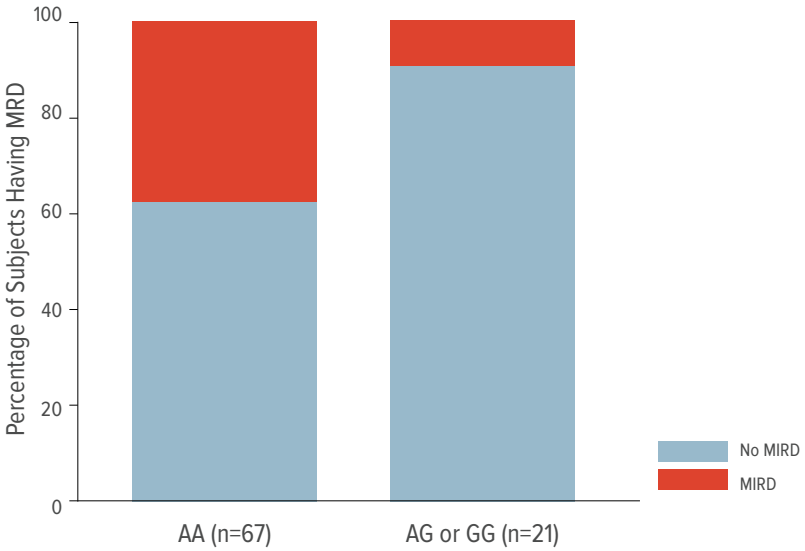
The team analyzed 88 cases of adolescents who had undergone spine fusion for scoliosis, and found that in the first 48 hours after surgery, MIRD in patients with AA genotype was significantly higher. The findings could have wide implications because the variant is present in an estimated 11-17 percent of the Caucasian population.

"This translates to immediate clinical relevance for a large part of the population," Chidambaran says, "as identifying genotype predicted risk of MIRD will facilitate safer individualized opioid dosing."

The team also has recently reported additional associations of respiratory depression in children with novel variants of the gene FAAH, published in January 2015, and with ABCB1, published in April 2015.

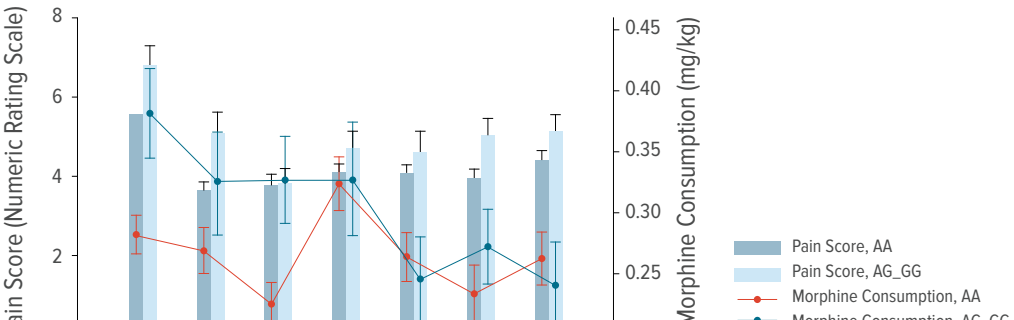
"These findings show that multiple genes play a role in opioids' clinical responses," Sadhasivam says. "As we identify more of these associations, the future of preoperative genetic risk signature stratification and personalizing postoperative pain management in children is promising."

MIRD IN PATIENTS WITH DIFFERENT GENOTYPES



This chart compares morphine-induced respiratory depression (MIRD) in the two genotype groups (AA and AG+GG) for the A118G polymorphism of the OPRM1 gene. Incidence was significantly higher in the AA subgroup (25/67 or 37 percent) compared with AG or GG subgroup (2/21 or 9 percent).

PAIN SCORES, MORPHINE CONSUMPTION POST-SURGERY



When looking across time, pain was highest two hours after surgery, and was consistently higher in individuals carrying a G allele (white bars). Differences in morphine consumption by genotype (blue and red lines) were greatest between two and 16 hours after surgery.

Genetic Biomarker Opens Doors to New Therapies for Hard-to-Treat Asthma

Gurjit Khurana Hershey, MD, PhD

PUBLISHED ONLINE APRIL 21, 2014
The Journal of Allergy and Clinical Immunology

Asthma specialists may one day be able to use a biomarker to more easily identify and treat children whose asthma attacks do not respond well to commonly prescribed corticosteroids.

The discovery of the biomarker VNN-1, reported April 21, 2014, in *The Journal of Allergy and Clinical Immunology*, provides a genetic basis for understanding why some children with asthma respond effectively to medicines that control underlying inflammation — and why other children do not. It also holds out hope that difficult-to-treat patients can be identified quickly, and that researchers can find better therapies for asthma, which affects seven million children in the U.S.

Gurjit Khurana Hershey, MD, PhD, Director of Asthma Research, and her team collected and tested cells from the nasal passages of 57 children whose emergency room visits for acute asthma required hospitalization. From a list of 20,000 gene candidates, doctors singled out and tested one gene, vanin-1 or VNN1, as the primary target for their study.

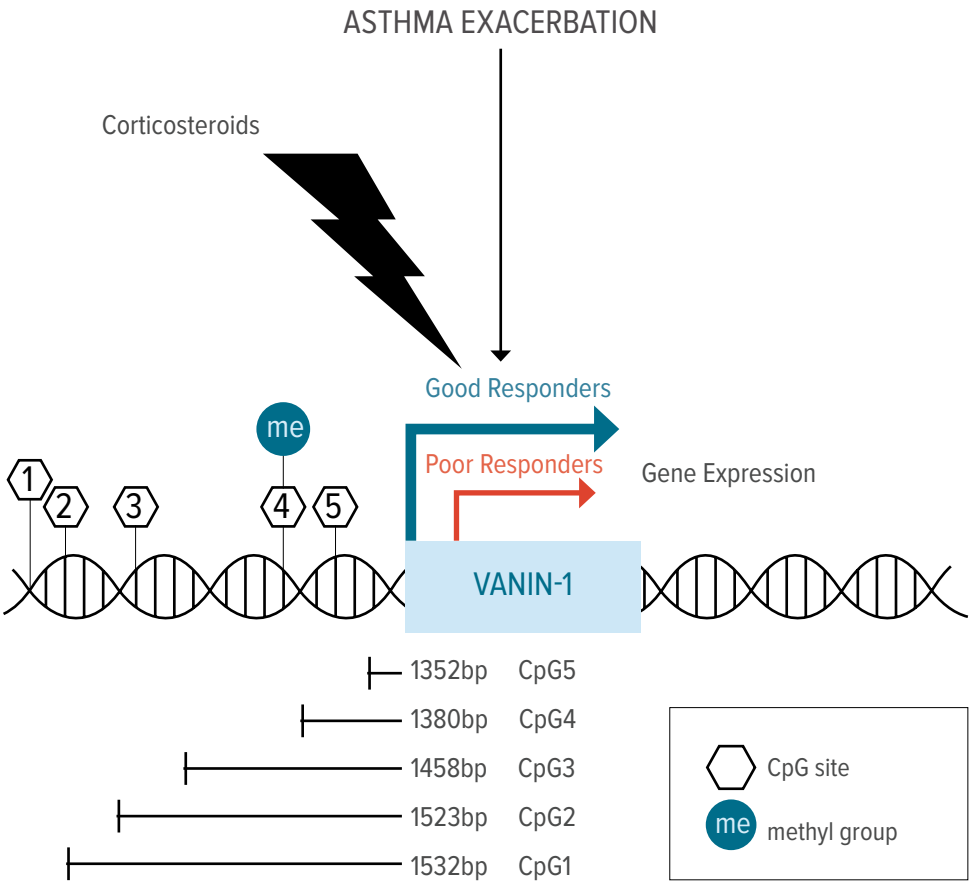
According to Hershey, expression of the VNN-1 gene helps discriminate between good and poor responders to corticosteroids.

Laboratory tests showed that VNN-1 expression is required for inhaled corticosteroids to provide relief during an asthma attack. Children whose asthma did not respond well to corticosteroids exhibited a biochemical variation of VNN-1 that hindered its expression. Subsequent tests on laboratory mouse models of asthma suggested that targeting the VNN-1 pathway therapeutically might be valuable for improving outcomes for difficult-to-treat patients.

RESEARCH AND TRAINING DETAILS

Faculty	8
Joint Appointment Faculty	1
Research Fellows	5
Research Students	18
Support Personnel	20
Direct Annual Grant Support	$1.9M
Peer Reviewed Publications	19

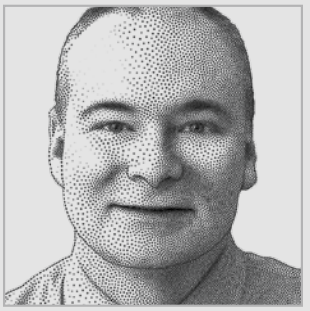
Xiao C, Biagini Myers JM, Ji H, Metz K, Martin LJ, Lindsey M, He H, Powers R, Ulm A, Ruff B, Ericksen MB, Somineni HK, Simmons J, Strait RT, Kercsmar CM, Khurana Hershey GK. Vanin-1 expression and methylation discriminate pediatric asthma corticosteroid treatment response. *J Allergy Clin Immunol.* 2015



In this proposed model, the VNN1 gene is modestly expressed at baseline and this level of expression is not altered in patients with stable or acute asthma. Corticosteroid treatment for an exacerbation induces DNA methylation at the CpG4 site of the VNN1 gene promoter, enhancing expression of the VNN1 gene. Enhanced VNN1 expression contributes to optimal response to corticosteroid treatment.

Asthma affects seven million children in the U.S.

Preschoolers With CF Benefit When Parents Learn to Optimally Address Behavioral and Nutritional Needs



Scott Powers, PhD, ABPP

RESEARCH AND TRAINING DETAILS

Faculty	50
Joint Appointment Faculty	2
Research Fellows	11
Research Students	63
Support Personnel	62
Direct Annual Grant Support	$7.2M
Direct Annual Industry Support	$19,250
Peer Reviewed Publications	114

Powers SW, Stark LJ, Chamberlin LA, Filigno SS, Sullivan SM, Lemanek KL, Butcher JL, Driscoll KA, Daines CL, Brody AS, Schindler T, Konstan MW, McCoy KS, Nasr SZ, Castile RG, Acton JD, Wooldridge JL, Ksenich RA, Szczesniak RD, Rausch JR, Stallings VA, Zemel BS, Clancy JP. Behavioral and nutritional treatment for preschool-aged children with cystic fibrosis: a randomized clinical trial. *JAMA Pediatr.* 2015;169(5):e150636.

PUBLISHED MAY 4, 2015
JAMA Pediatrics

Researchers in the Division of Behavioral Medicine and Clinical Psychology have discovered that in-person and telephone intervention programs designed for parents of preschool children with cystic fibrosis (CF) can help the children improve their eating habits and daily calorie intake — a major challenge in meeting the important nutritional needs and critical growth milestones known to improve their survival into adolescence.

Parents who participated in the behavior- and nutrition-focused sessions for six months learned about food records, mealtime behaviors, energy intake goals, snacking, directed praise of the child when eating, strategies for introducing new foods and other tactics to help their children meet their daily calorie needs and the specific enzyme/nutritional demands of CF, a chronic respiratory disease.

The children's' food intake increased by 485 calories a day and met 140 percent of the dietary recommendations for an active preschool child, according to the May 4, 2015, study in *JAMA Pediatrics*. Children whose parents participated in the program also experienced increases in scores for height milestones, but not weight, according to the multi-center study led by Scott Powers, PhD, ABPP. The study, involving parents of 78 children from seven CF centers across the United States (including Cincinnati Children's), compared results from the behavior-nutritional intervention program against a control group of parents who received basic education about their children's nutritional and enzyme needs, respiratory control, growth milestones and child safety.

"Our most important findings are that clinical trials can be successfully executed in this age range across multiple centers, and that intervention has an impact on change in growth in this age group," says Powers. "Our hope is that this type of evidence will become part of routine care and be incorporated into the new CF Foundation Preschool Care Guidelines to be used by CF centers in the U.S."

CHANGES IN ENERGY INTAKE, WEIGHT AND HEIGHT

	Mean (SD)							
	Behavioral & Nutritional Treatment (n = 36)			Education & Attention Control Treatment (n = 42)			Group Mean Difference in Change (95% CI)	P Value
Variable	Baseline	Post-treatment	Change	Baseline	Post-treatment	Change		
Energy intake, kcal/d[b]	1462 (330)	1947 (459)	485 (335)	1461 (332)	1529 (387)	58 (248)	431 (282 to 581)	< .001
Weight z score	-0.36 (0.75)	-0.24 (0.76)	0.12 (0.40)	0.51 (0.85)	-0.45 (0.77)	0.06 (0.32)	0.09 (-0.6 to 0.24)	.25
	Baseline	Follow-up	Change	Baseline	Follow-up	Change		
Height z score	-0.39 (0.85)	-0.30 (0.88)	0.09 (0.26)	-0.69 (0.82)	-0.71 (0.86)	-0.02 (0.32)	0.14 (0.001 to 0.27)	.049
Energy intake, kcal/d[b, c]	1462 (330)	1960 (440)	545 (504)	1461 (332)	1739 (416)	227 (429)	239 (33 to 444)	.02
Weight z score[c]	-0.36 (0.75)	-0.22 (0.83)	0.15 (0.48)	-0.51 (0.85)	-0.40 (0.96)	0.11 (0.62)	0.07 (-0.19 to 0.32)	.61

[a] All change variables are calculated as change from baseline. All group mean differences in change account for missing data using maximum likelihood estimation, when necessary, and are model based, which yields an adjusted difference, controlling for covariates. Least-square estimation was used when no missing data were present (this approach is equivalent to maximum likelihood for our models in this special case).

[b] At baseline, the mean (SD) energy intake met 110% (26%) of the estimated energy requirement benchmark (mean [SD], 106% [20%] for the behavioral and nutritional treatment group and 113% [30%] for the education and attention control treatment group); posttreatment mean (SD) energy intake met 129% (31%) of the estimated energy requirement benchmark overall (mean [SD], 142% [27%] for the behavioral and nutritional treatment group and 117% [30%] for the education and attention control group); follow-up mean (SD) energy intake met 139% (41%) of the estimated energy requirement benchmark overall (145% [42%] for the behavioral and nutritional treatment group and 134% [40%] for the education and attention control treatment group).

[c] Tests were post hoc exploratory comparisons.

Preschool children with cystic fibrosis experienced a mean increase in caloric intake of 485 calories per day after their parents participated in behavior- and nutrition-focused intervention sessions for six months and demonstrated greater change in height than the control group, according a study published May 4, 2015, in *JAMA Pediatrics.*

"Our hope is that this type of evidence will become part of routine care."

Quantitative Model Provides Big Clues Into How Tiny Embryos Develop

Jun Ma, PhD

PUBLISHED MARCH 26, 2015
Nature Communications

A biological-mathematical-genetic model called TEMS, or Tissue Expansion-Modulated Maternal Morphogen Scaling, is providing insights into one of nature's most intriguing genetic questions about scaling — the proportional growth of tissues, organs and structures from tiny ovaries, eggs and embryos.

The model provides new insights into the delicate connections between ovarian tissue and ensuing embryonic development, and it can be applied to future research into birth defects.

Developed by Biomedical Informatics scientist Jun Ma, PhD, and published March 26, 2015, in *Nature Communications*, the TEMS model was applied to fruit flies from development of the ovary through embryo. Ma's team focused on two developmental components: morphogens — proteins that form concentration gradients along the axis of an embryo and instruct the genes that control the proportional formation of body parts and organs; and ovary-active genes that produce messengers delivered to the egg for instructing the production of morphogen proteins in the embryo.

His team studied front-to-back proportional scaling in embryos before organs start to develop, and found that the size of embryos was influenced by the quantity of initial tissue in the female's ovary, particularly the size of the ovarian egg chamber and the expansion of the copy numbers of an ovary-active gene called bicoid.

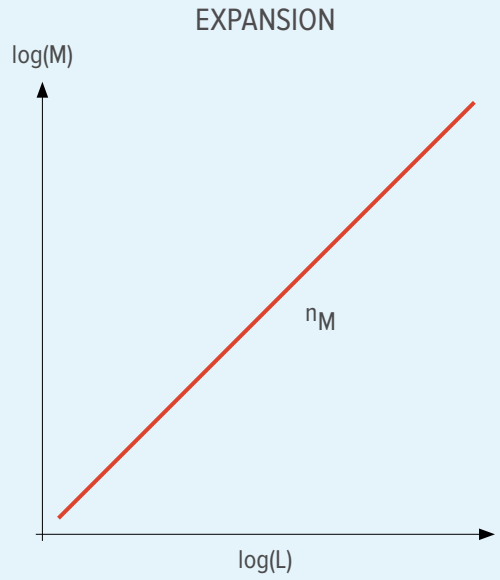
"The model provides a new perspective of embryonic development and contributes to our fundamental knowledge that may ultimately lead to an improved understanding of the basis of birth defects," says Ma.

Of particular interest for future research, according to Ma, is the similarity between the peak number of bicoid gene copies in a female fly's nurse cells and the peak number of cell nuclei in the offspring blastoderm, or early-stage embryo. Aided by TEMS, Ma and his team hope to better understand, quantify and predict how life forms grow and develop, and to explore the interplay between biology and evolution.

RESEARCH AND TRAINING DETAILS

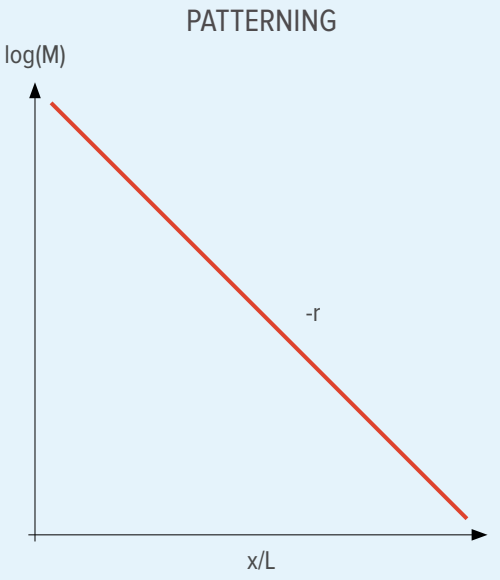
Faculty	13
Joint Appointment Faculty	11
Research Fellows	6
Research Students	19
Support Personnel	117
Direct Annual Grant Support	$2.9M
Peer Reviewed Publications	60

He F, Wei C, Wu H, Cheung D, Jiao R, Ma J. Fundamental origins and limits for scaling a maternal morphogen gradient. *Nat Commun*. 2015;6:6679.



Maternal tissue expansion

A biological-mathematical-genetic model developed at Cincinnati Children's called Tissue Expansion-Modulated Maternal Morphogen Scaling (TEMS) provides new insights into the delicate connections between ovarian tissue and ensuing embryonic development. This study shows that embryo size is influenced by the quantity of initial ovary tissue, particularly the size of the ovarian egg chamber and the expansion of the copy numbers of the ovary-active gene bicoid. The figures here depict the mathematical relationships governing maternal tissue expansion (left) and morphogen control of embryonic patterning (below).



Morphogen control
of embryonic patterning

"The model provides a new perspective of embryonic development and contributes to our fundamental knowledge that may ultimately lead to an improved understanding of the basis of birth defects."

Early Escape Option Helps Balance Needs of Patients, Investigators

Bin Huang, PhD

PUBLISHED JULY 2014
Contemporary Clinical Trials

Scientists designing crossover clinical trials, particularly those involving rare diseases, often must strike a delicate balance between maximizing the study's potential for discovery and minimizing patient exposure to ineffective treatment.

The Division of Biostatistics and Epidemiology partners with nearly all clinical and translational research programs at Cincinnati Children's to help them design studies and manage statistical analysis. Its faculty members also conduct direct studies to ensure and enhance the strength and innovation of research.

In a recent example of this work, a study published in July 2014 in *Contemporary Clinical Trials*, explored findings from previous work on treatments for the inflammatory disorder familial Mediterranean fever (FMF). The study's authors included first author Bin Huang, PhD, from Biostatistics and Epidemiology; senior author Philip Hashkes, MD, from the Shaare Zedek Medical Center in Israel; and colleagues from the Division of Rheumatology at Cincinnati Children's.

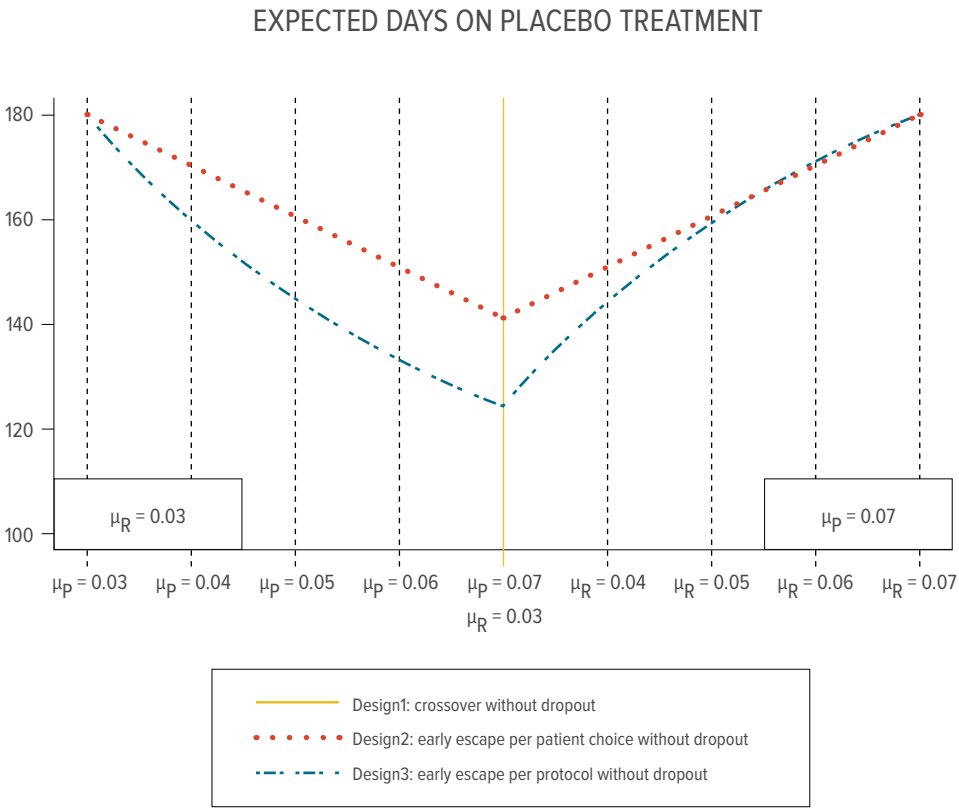
The team compared study power and dropout rates among three types of crossover trial designs: traditional without early escape, early escape as a patient choice, and early escape based on accepted protocol.

Both early-escape approaches were preferable to the traditional method. Researchers found that supporting exits based on patient choice reduced the dropout rate as much as 29 percent. However, the patient choice approach also increased patient exposure to the less effective treatment arm, which suggests that investigators must be mindful of the trade-offs between study design methods.

RESEARCH AND TRAINING DETAILS

Faculty	22
Joint Appointment Faculty	14
Research Fellows	1
Research Students	12
Support Personnel	56
Direct Annual Grant Support	$3.7M
Peer Reviewed Publications	138

Huang B, Giannini EH, Lovell DJ, Ding L, Liu Y., Hashkes PJ. Maximizing study power and minimizing patient exposure to ineffective treatment, *Contemporary Clinical Trials*, July 2014. V38(2): 204-212.



Expected time on placebo was calculated under the assumption of a Poisson distribution following the formula presented in Appendix A, with parameter settings similar to the familial Mediterranean fever (FMF) trial, i.e. a = 1, A = 5. Y axis shows the expected days on placebo; X axis corresponds to the rate of attack. The solid line corresponds to results of early escape per protocol; dashed line corresponds to the results of early escape per patient choice. Under the traditional crossover design without escape, the number of days on placebo treatment is 180 days.

Supporting exits based on patient choice reduced the dropout rate as much as 29 percent.

Algorithm Enables Prompt Response to High-Risk Cases of Transplant-Associated Thrombotic Microangiopathy (TMA)

Sonata Jodele, MD

The Cancer and Blood Diseases Institute (CBDI) includes the Divisions of Bone Marrow Transplant and Immune Deficiency, Experimental Hematology, Hematology, and Oncology

CANCER AND BLOOD DISEASES INSTITUTE RESEARCH AND TRAINING DETAILS

Faculty	85
Joint Appointment Faculty	26
Research Fellows	54
Research Students	23
Support Personnel	341
Direct Annual Grant Support	$16.6M
Direct Annual Industry Support	$1.1M
Peer Reviewed Publications	209

Jodele S, Davies SM, Lane A, Khoury J, Dandoy C, Goebel J, Myers K, Grimley M, Bleesing J, El-Bietar J, Wallace G, Chima RS, Paff Z, Laskin BL. Diagnostic and risk criteria for HSCT-associated thrombotic microangiopathy: a study in children and young adults. *Blood.* 2014;124(4):645-653.

PUBLISHED JULY 24, 2014
Blood

After children undergo hematopoietic cell transplantation (HSCT), one of the most severe complications they can develop is thrombotic microangiopathy (TMA). This condition can trigger a cascade of events leading to potentially fatal multi-organ injury.

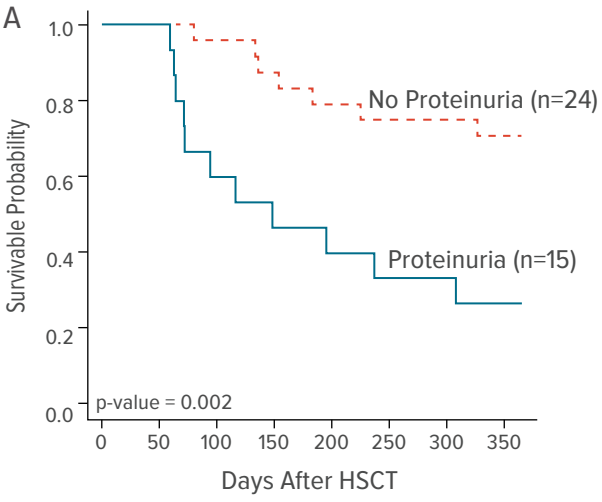
Prompt clinical intervention can save lives, but only if TMA is detected in its earliest stages. In an important paper published July 24, 2014, in the journal *Blood*, a research team led by Sonata Jodele, MD, Division of Bone Marrow Transplantation and Immune Deficiency, reports developing an algorithm that can provide the information clinicians need to act.

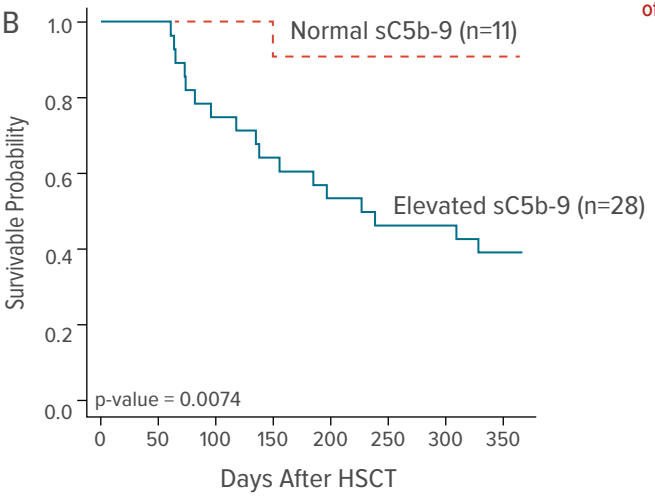
The researchers prospectively evaluated 100 HSCT recipients to track TMA incidence and outcomes. They found 39 children who met criteria for TMA. These children had a 43.6 percent non-relapse mortality rate at one year post-transplant, compared to 7.8 percent mortality among children who did not develop TMA.

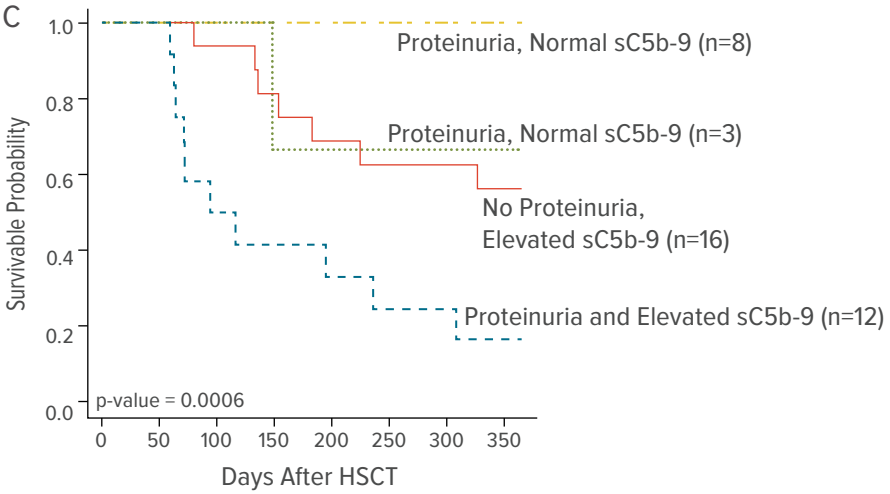
The team observed that those who died after TMA diagnosis had a greater degree of anemia, higher risk of proteinuria, and were more likely to have evidence of terminal complement activation. Elevated levels of sC5b-9 were present in nearly all subjects with TMA who died but in only about half of those who survived. In contrast, kidney dysfunction assessed by serum creatinine was a very late marker of TMA.

The paper details a series of daily, twice weekly and weekly tests that can detect early TMA markers. Specifically, proteinuria >30 mg/dL as measured by routine dipstick and hypertension >95th percentile were the earliest signs of TMA, along with elevated lactate dehydrogenase (LDH).

These data suggest that complement activation plays a significant role in the pathogenesis of severe TMA after HSCT. The team recommends that patients with proteinuria and evidence of complement activation should be considered for treatment with eculizumab, a humanized monoclonal antibody that functions as a terminal complement inhibitor.

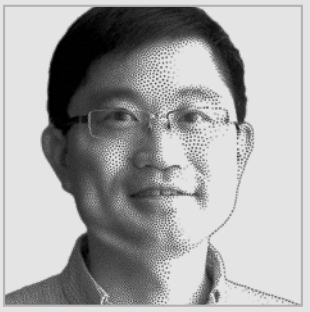






A new algorithm offers a possible tool for intervening early to treat children who develop a severe complication following hematopoietic cell transplantation. This figure displays Kaplan-Meier survival curves for 39 children with thrombotic microangiopathy (TMA) in (A) those with proteinuria of ≥30 mg/dL vs. no proteinuria, (B) those with elevated serum sC5b-9 concentration vs. normal, and (C) subjects with no proteinuria and normal sC5b-9, proteinuria ≥30 mg/dL and normal sC5b-9, no proteinuria, and elevated sC5b-9 and both proteinuria ≥30 mg/dL and elevated sC5b-9 at the time of TMA diagnosis.

Antidepressant Identified as Potential Brain Tumor Suppressor



Qing Richard Lu, PhD

CANCER AND BLOOD DISEASES INSTITUTE RESEARCH AND TRAINING DETAILS

Faculty	85
Joint Appointment Faculty	26
Research Fellows	54
Research Students	23
Support Personnel	341
Direct Annual Grant Support	$16.6M
Direct Annual Industry Support	$1.1M
Peer Reviewed Publications	209

He X, Zhang L, Chen Y, Remke M, Shih D, Lu F, Wang H, Deng Y, Yu Y, Xia Y, Wu X, Ramaswamy V, Hu T, Wang F, Zhou W, Burns DK, Kim SH, Kool M, Pfister SM, Weinstein LS, Pomeroy SL, Gilbertson RJ, Rubin JB, Hou Y, Wechsler-Reya R, Taylor MD, Lu QR. The G protein alpha subunit Galphas is a tumor suppressor in Sonic hedgehog-driven medulloblastoma. *Nat Med*. 2014;20(9):1035-1042.

PUBLISHED SEPTEMBER 2014
Nature Medicine

An international research team, led by Qing "Richard" Lu, PhD, scientific director of the Brain Tumor Center at Cincinnati Children's, has discovered a novel tumor suppressor gene that could help overcoming rapid drug resistance when treating pediatric brain cancer.

The latest findings specifically address aggressive sonic hedgehog (SHH)-driven medulloblastomas. However, the work may have wider impact. The team showed that Rolipram, a cellular cAMP-elevating agent and antidepressant approved for use in Europe and Japan, effectively inhibits tumor cell proliferation and progression in mice.

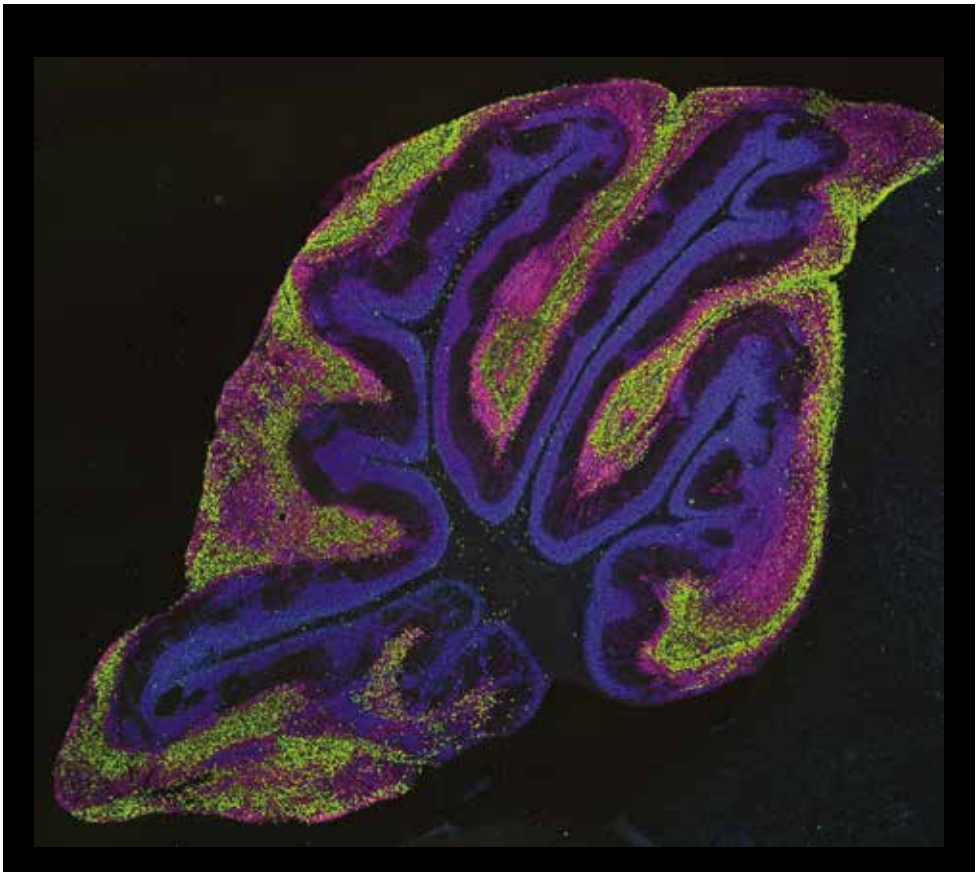
The findings were published in September 2014 in *Nature Medicine*. The study included collaborators from nine medical centers in four countries.

In healthy people, the GNAS gene encodes a Gs-alpha protein, which initiates a molecular signaling cascade that suppresses tumor growth. Mutations disrupting this pathway can lead to rapid cancer cell growth. Lu and colleagues discovered the gene's role while employing a genome-wide screen to analyze childhood brain tumor samples.

In a line of mice bred to lack the GNAS gene, medulloblastomas shrank when given Rolipram. The researchers believe the drug restores the Gs-alpha pathway's tumor suppressing power by elevating levels of the signaling molecule cAMP.

"Many chemotherapies become ineffective as soon as the surface receptors they target change, but this drug may help to get inside the cells by targeting a signaling juncture downstream to overcome the drug resistance," Lu says.

Rolipram is only one drug affecting one part of the Gs-alpha signaling pathway. Lu and colleagues are working to identify other genes and related markers along the pathway. It may be that other drugs acting at other points will prove to be even more effective.



This confocal microscope image of the mouse cerebellum from Gnas mutants is immunostained to show tumor cells (in purple), rapidly dividing tumor cells (in yellow) and granule neurons (in blue). A study published in *Nature Medicine* reveals that treatment with the anti-depressant Rolipram can suppress aggressive sonic hedgehog (SHH)-driven medulloblastomas.

Clot-Stabilizing Enzyme Heals Colitis Damage in Mice and Shows Potential Wider Applications

Joseph Palumbo, MD

CANCER AND BLOOD DISEASES INSTITUTE RESEARCH AND TRAINING DETAILS

Faculty	85
Joint Appointment Faculty	26
Research Fellows	54
Research Students	23
Support Personnel	341
Direct Annual Grant Support	$16.6M
Direct Annual Industry Support	$1.1M
Peer Reviewed Publications	209

Andersson C, Kvist PH, McElhinney K, Baylis R, Gram LK, Pelzer H, Lauritzen B, Holm TL, Hogan S, Wu D, Turpin B, Miller W, Palumbo JS. Factor XIII Transglutaminase Supports the Resolution of Mucosal Damage in Experimental Colitis. *PLoS One*. 2015;10(6):e0128113.

PUBLISHED JUNE 22, 2015
PLOS ONE

The thrombin-activated transglutaminase factor XIII (FXIII) plays an important supportive role in the repair of colitis-induced mucosal damage in mice, according to research led by Joseph Palumbo, MD, a scientist in the Cancer and Blood Diseases Institute.

FXIII is best known as the enzyme that stabilizes fibrin clots. However, new findings published June 22, 2015, in *PLOS ONE* demonstrate that FXIII also plays a larger-than-expected role in tissue regeneration.

"Until our published report, the only direct evidence for a contribution of FXIII to tissue remodeling was for incisional skin wounds," Palumbo says. "Our findings illustrate the potential to utilize FXIII to resolve a wider range of injuries."

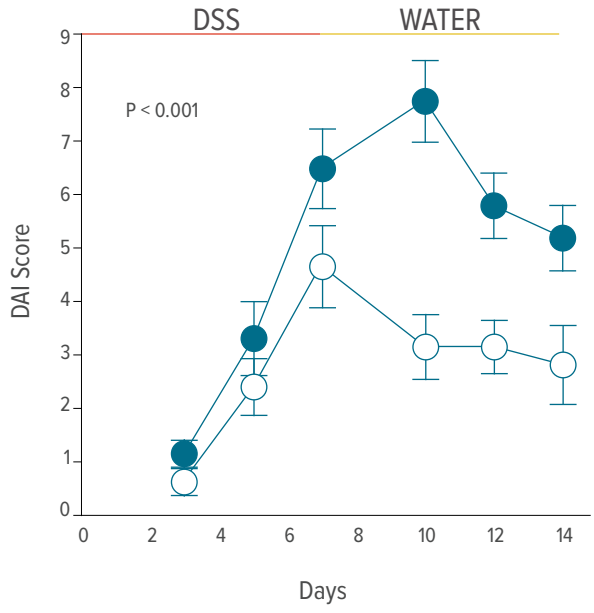
Palumbo, in collaboration with Novo Nordisk scientists Christina Andersson and Brian Lauritzen, evaluated how colitis-challenged mice responded when treated with recombinant human FXIII-A (rFXIII). They found that wildtype (WT) mice and mice genetically bred to lack the FXIII enzyme developed comparable mucosal damage when challenged with dextran sulfate sodium (DSS) to induce colitis symptoms. However, the FXIII-deficient mice failed to resolve the damage after DSS was withdrawn.

Treating mice with rFXIII significantly mitigated the clinical signs of colitis (e.g., weight loss, intestinal bleeding, diarrhea) while also largely resolving mucosal ulceration. Most strikingly, the benefit was not limited to FXIII-deficient animals. Control mice with normal FXIII gene expression also demonstrated a dramatic improvement in mucosal repair when treated with rFXIII following colitis challenge.

Further research is needed to determine the ultimate clinical utility of FXIII in inflammatory bowel disease (IBD). However, the impact of this work may extend beyond IBD.

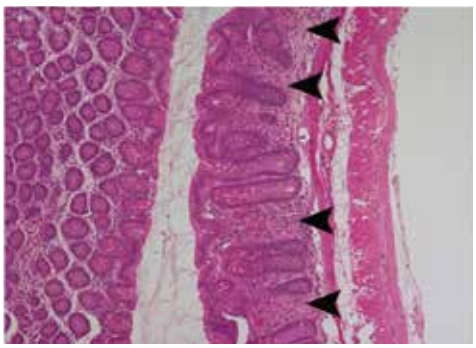
"For example, Matthew Flick in Experimental Hematology has published work detailing the contribution of FXIII to inflammatory arthritis pathogenesis, and Eric Mullins in Hematology has findings suggesting FXIII is linked to neuroinflammatory disease," Palumbo says. "Furthermore, FXIII may play a fundamental role in cardiac tissue repair, another area of intense interest for our group."

A



The transglutaminase factor XIII (FXIII) plays a significant role in mucosal tissue regeneration. Image (A) shows a comparison of Disease Activity Index (DAI), a semiquantitative score of colitis severity based on multiple clinical metrics, in mice challenged with dextran sodium sulfate (DSS) for seven days to induce colitis, then allowed to recover for seven days. Note that mice treated with rFXIII (open circles) showed dramatic improvement in DAI compared to vehicle-treated control mice (closed circles). Image (B) shows colon tissue harvested from a vehicle-treated control mouse at the end of the 14-day experiment. Large remaining areas of inflammatory crypt spacing (arrowheads) demonstrate incomplete mucosal healing. In contrast, image (C) shows that mice treated with rFXIII exhibit near-complete mucosal healing at this time point.

B



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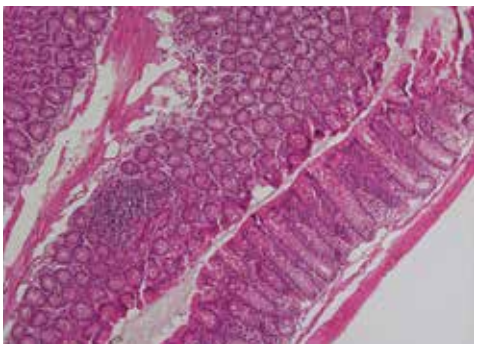


Novel Mapping Approach for DNA Sequence Binding Motifs Sharply Expands Library of Genetic Knowledge

Matthew Weirauch, PhD

RESEARCH AND TRAINING DETAILS

Faculty	11
Joint Appointment Faculty	3
Research Fellows	8
Research Students	12
Support Personnel	31
Direct Annual Grant Support	$2.3M
Direct Annual Industry Support	$196,802
Peer Reviewed Publications	57

Weirauch MT, Yang A, Albu M, Cote AG, Montenegro-Montero A, Drewe P, Najafabadi HS, Lambert SA, Mann I, Cook K, Zheng H, Goity A, van Bakel H, Lozano JC, Galli M, Lewsey MG, Huang E, Mukherjee T, Chen X, Reece-Hoyes JS, Govindarajan S, Shaulsky G, Walhout AJ, Bouget FY, Ratsch G, Larrondo LF, Ecker JR, Hughes TR. Determination and inference of eukaryotic transcription factor sequence specificity. *Cell*. 2014;158(6):1431-1443.

PUBLISHED SEPT. 11, 2014
Cell

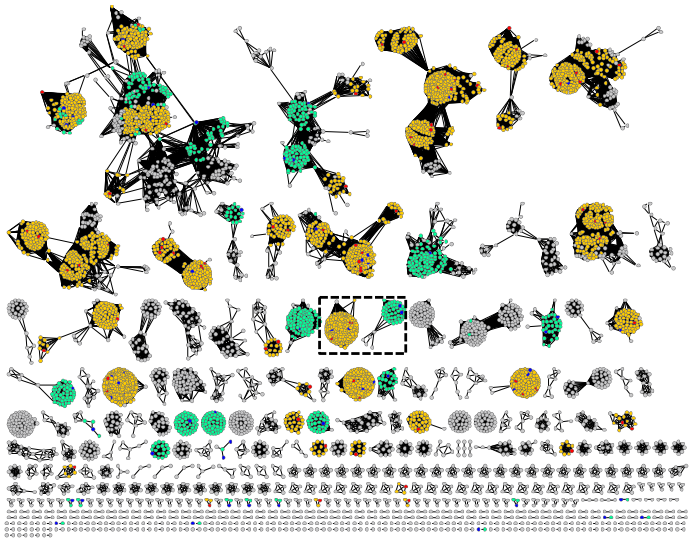
In a study with wide-ranging impact, researchers effectively increased the DNA sequence binding motifs that are known for eukaryotic transcription factors over 10-fold, including doubling knowledge for human transcription factors.

This new insight significantly improves predicting capacity for gene expression mechanisms for many disease-mechanism problems, and essentially all of eukaryotic biology.

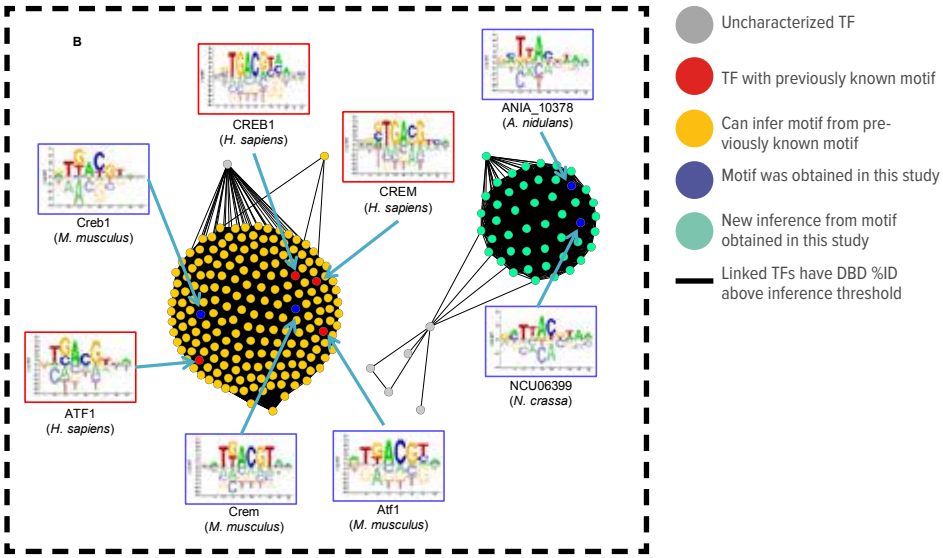
The study, led by Matthew Weirauch, PhD, a computational biologist in the Center for Autoimmune Genomics and Etiology, was published Sept. 11, 2014, in the journal *Cell*. The findings have enabled researchers who study any organism to begin to understand how genes are regulated on a global scale. For human disease, the study increases researchers' ability to understand the function of disease-associated genetic variants that fall in non-coding regions. It is estimated that approximately 90 percent of disease-associated variants are non-coding. In genomics, noncoding DNA sequences are components of an organism's DNA that do not encode protein sequences. "Doubling our knowledge of human DNA sequence binding motifs essentially doubles our chance of figuring out which proteins these variants might affect the binding of," Weirauch says.

The center's primary focus is the genesis of lupus and other immunological diseases, and to explore the mechanisms of disease through the complex interactions of genetics, the immune system and environmental factors such as stress, exercise and diet.

Two findings of the study surprised researchers. "First, that our scheme for mapping DNA sequence binding motifs across organisms based on protein similarity works for most protein families," says Weirauch. "Second, the fact that we increased knowledge of these motifs so substantially across all of eukaryotic life, from less than one percent to almost 40 percent of all proteins."



In a pictorial overview of transcription factors (TF) choosing strategy and motif inferences, this figure shows the network schematic depicting TFs (nodes), their related TFs (edges with nodes), and their motif status (node color.) This figure depicts all 3,715 TFs across 246 species that contain a single bZIP domain.



This figure is a close-up of the boxed region in the first figure. Here, motifs are shown for characterized TFs. Researchers noticed that motifs from the left group strongly resemble one another, as do motifs within the right group (as predicted by their DBD AA %ID). However, the motifs from the left and right groups are not related, as predicted by the fact that the DBD %ID of their TF members fall below the inference threshold for bZIPs. That is, there are no links between the two groups. Motifs with blue outlines were determined using PBMs; red outlined motifs are from the Transfac database. The findings illustrated here improve the understanding of gene expression mechanisms connected to disease.

Systems Pharmacology Approach Leads to Precision Drug Dosing Algorithm for Neonates, Infants and Children

Alexander Vinks, PharmD, PhD

RESEARCH AND TRAINING DETAILS

Faculty	3
Joint Appointment Faculty	3
Research Fellows	6
Research Students	3
Support Personnel	2
Direct Annual Industry Support	$356,997
Peer Reviewed Publications	13

Emoto C, Fukuda T, Johnson TN, Adams DM, Vinks AA. Development of a Pediatric Physiologically Based Pharmacokinetic Model for Sirolimus: Applying Principles of Growth and Maturation in Neonates and Infants. *CPT Pharmacometrics Syst Pharmacol.* 2015;4(2):127-134.

PUBLISHED ONLINE FEB. 4, 2015
CPT: Pharmacometrics and Systems Pharmacology

A predictive computer model developed at Cincinnati Children's is helping doctors fine-tune the doses of drugs they give to neonates, infants and children, based on specific measurements on how their bodies metabolize and respond to the drugs.

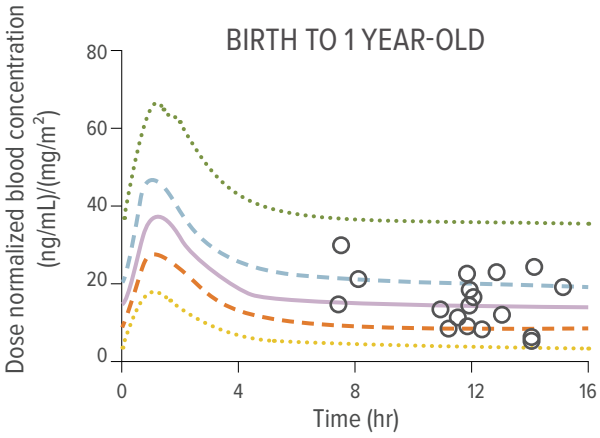
It is a far better system, says lead author Alexander Vinks, PharmD, PhD, Director of Clinical Pharmacology, than the traditional practice of dosing drugs for infants and neonates using a kilogram-to-milligram scale based on a child's weight — a scaled-down but inaccurate version of adult doses that are downsized and applied to tinier bodies.

"It's always been a tricky question, one that's very difficult to predict at any given age: how much of a drug to give to a two-week old infant vs. a year-and-a-half-old toddler," says Vinks. "The moment a child is born, all these physiological systems kick in, but we haven't known the pace and maturation levels of various systems until we looked at data from various studies."

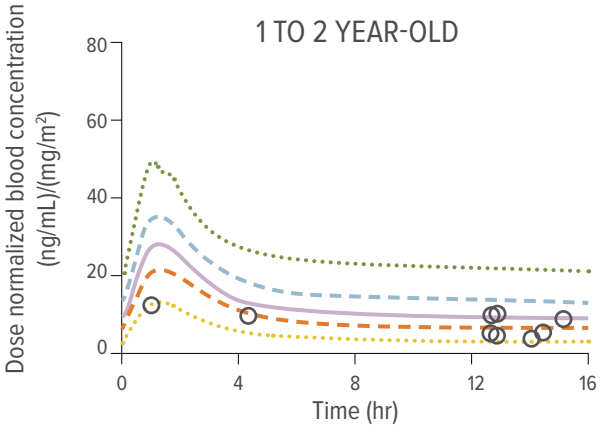
Vinks and colleagues participated in a concentration-controlled clinical trial of sirolimus, a drug increasingly used to treat vascular abnormalities but not extensively studied in children. Researchers started a dose and measured sirolimus levels. Next, enzyme measurements reflective of each patient's liver enzyme CYP3A activity, known to metabolize drugs, were combined with other data, including the child's age, weight, gender, ethnicity, height, sirolimus concentrations, dosing regimens and other medications. Data from three studies were combined to create an algorithm — still being refined — to pinpoint accurate drug dosing levels. Findings appeared Feb. 4, 2015, in *CPT: Pharmacometrics and Systems Pharmacology*.

"It's a very sophisticated way of using state-of-the-art techniques to tease out data for specific drug doses — a sophisticated way of personalized precision dosing," according to Vinks. His division is working on similar algorithims for methadone and morphine, and receiving inquiries from the around the world about pediatric doses for other drugs.

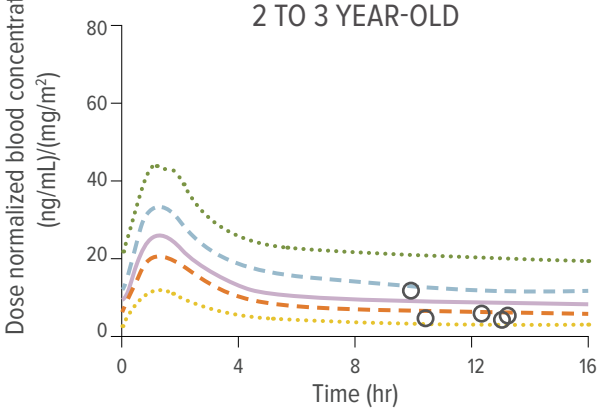
A



B



C



These graphs compare observed sirolimus concentrations with simulated concentration-time profiles using a pediatric physiologically based pharmacokinetic model based on healthy children aged birth to 1 year (a), 1-2 years (b), and 2-3 years (c). In the simulations, sirolimus was administrated orally at 1.0 mg/m2 twice a day for 30 days. Each bold line shows the median; dashed lines indicate 25 to 75 percentiles; dotted lines show 5 to 95 percentiles. The circles represent observed concentrations in each patient.

Genetic Expression Method Allows Doctors to Rapidly Identify Subclasses of Septic Shock

Hector Wong, MD

RESEARCH AND TRAINING DETAILS

Faculty	11
Research Students	3
Support Personnel	17
Direct Annual Grant Support	$1.3M
Peer Reviewed Publications	24

Wong HR, Cvijanovich NZ, Anas N, Allen GL, Thomas NJ, Bigham MT, Weiss SL, Fitzgerald J, Checchia PA, Meyer K, Shanley TP, Quasney M, Hall M, Gedeit R, Freishtat RJ, Nowak J, Shekhar RS, Gertz S, Dawson E, Howard K, Harmon K, Beckman E, Frank E, Lindsell CJ. Developing a clinically feasible personalized medicine approach to pediatric septic shock. *Am J Respir Crit Care Med.* 2015;191(3):309-315.

PUBLISHED FEB. 1, 2015
American Journal of Respiratory and Critical Care Medicine

For 20 years, scientific research into septic shock has tried to determine how best to identify, diagnose and treat the potentially life-threatening infection, which can quickly overwhelm the body's immune system. But researchers have been limited by the disease's non-specific spectrum of symptoms and treatment results that vary from patient to patient.

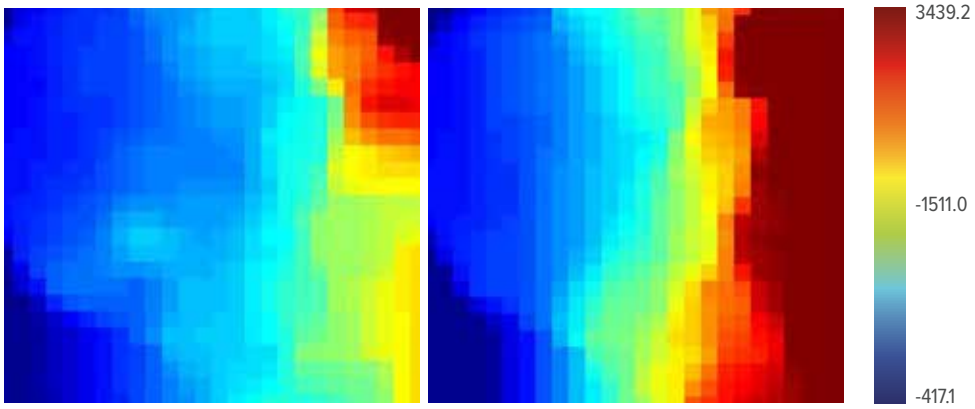
The editors of the *American Journal of Respiratory and Critical Care Medicine* describe a finding by Hector Wong, MD, Director of the Division of Critical Care Medicine, as a new approach that "might help shift this impasse for children with septic shock."

Published Feb. 1, 2015, the study reports success at identifying subclasses of septic shock in individual patients based on gene expression patterns linked to their immune system responses and glucocorticoid receptor signaling. The RNA-quantifying gene expression method also has the potential to rapidly generate clinical data, possibly within 8 to 10 hours. This could become a valuable advantage for a disease that can progress from diagnosis to death in a matter of days or hours. Septic shock has a mortality rate of 40-60 percent in adults and 25 percent in children.

Knowing a patient's specific disease subclass for septic shock can potentially aid therapeutic decisions. Corticosteroids — a standard protocol for septic shock treatment that works through the glucocorticoid receptor — can be life saving for many patients. However, this study shows that steroids are associated with a four-fold increase in mortality within one subclass of septic shock patients. Having a gene-based classification method for septic shock patients will help doctors quickly identify which patients should not receive steroid therapy, Wong says.

Information from gene expression also holds hope of more personalized medicine approaches for treating septic shock. Doctors may one day be able to use the patient's own adaptive immune responses to treat the disease, or to link symptom-specific drugs to the patient's symptom-based subclass in hopes of a greater chance of survival, Wong says.

A



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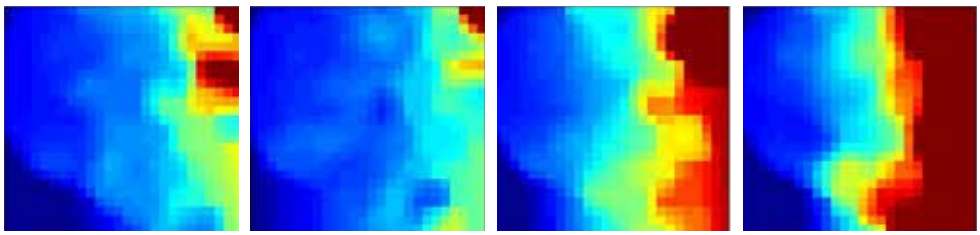


These composite gene expression mosaics show the mean expression values for 100 subclass-defining genes based on NanoString-derived expression data. Red intensity correlates with increased gene expression, and blue intensity correlates with decreased gene expression. Examples 1 and 2 were allocated to subclass A; examples 3 and 4 were allocated to subclass B. Compared to subclass B, those in subclass A had a higher mortality rate and a more complicated course, including higher median PRISM scores, lower total white blood cell and absolute neutrophil counts, and higher absolute lymphocyte counts.

This study shows that steroids are associated with a four-fold increase in mortality within one subclass of septic shock patients.

Children With Cleft Conditions Far More Likely to Have Transposed Teeth

Howard Saal, MD

Richard Campbell, DMD, MS

RESEARCH AND TRAINING DETAILS

Faculty	7
Joint Appointment Faculty	1
Support Personnel	3
Peer Reviewed Publications	4

Campbell RE, Levin L, Mauseth SE, Hu J, Zheng S, Wilson S, Saal H. Prevalence of transposed teeth as seen on panoramic radiographs in children with cleft lip and palate. Cleft Palate *Craniofac J.* 2014;51(4):e88-93

PUBLISHED JULY 2014
Cleft-Palate-Craniofacial Journal

Researchers using panoramic radiography to analyze the prevalence of transposed developing teeth in children found surprisingly large disparities between children who had a repaired cleft lip or palate and those who did not.

The July 2014 study, published in *Cleft Palate-Craniofacial Journal*, was the first designed solely to quantify how often these unerupted teeth are aligned incorrectly in the gums. The goal of the study is to help identify children who need early dental and orthodontic interventions.

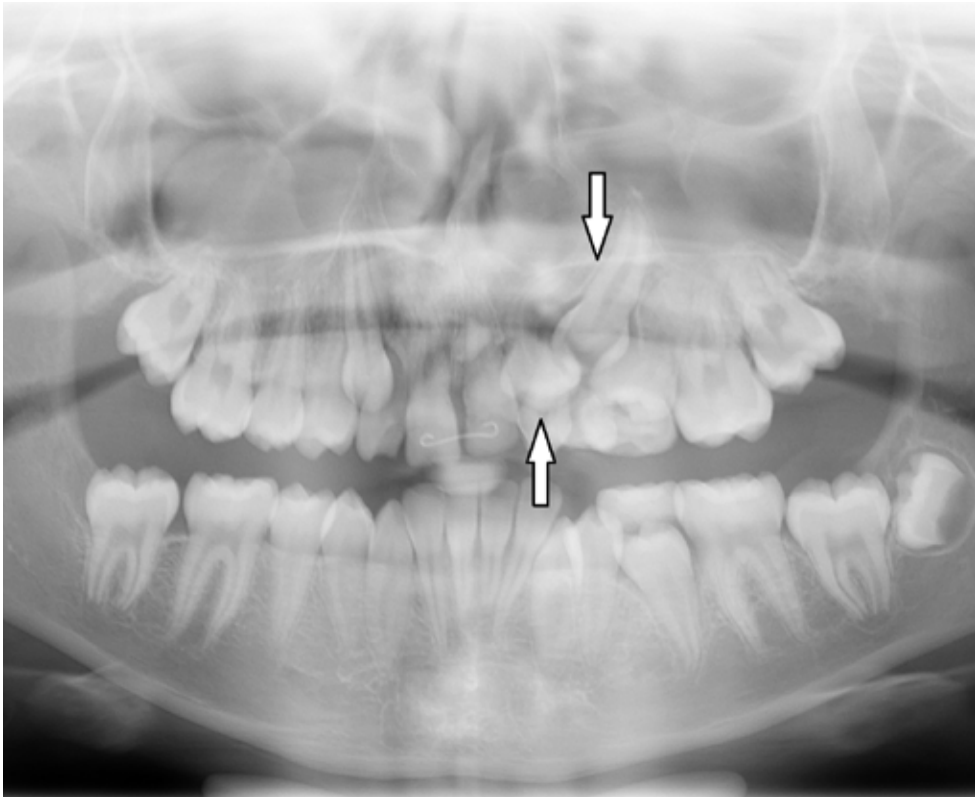
Researchers compared the images of 364 children who were born with a cleft lip or palate, and 364 kids who were not. Fifty-two children (14.3 percent) who were affected by a cleft condition also had transposed, missing or pegged teeth, compared to just one child (three-tenths of one percent) who did not. A tooth is considered transposed if it partially or fully occupies the space of an adjacent tooth.

The study was led by senior author Howard Saal, MD, Director of Clinical Genetics, and first author Richard Campbell, DMD, MS, Director of Orthodontics.

"Transposed teeth may be related to the overall smaller size of the upper jaw in children with clefts, which leads to more crowding, but that's speculative at this point," says Campbell. "Jaw size discrepancies are a fertile area for future research."

Cleft lip and palate result from *in utero* malformations in which tissue fails to fuse, or connect. Corrective surgery is typically done when a child is nine months to two years old.

Full panoramic radiograph images show all the developing teeth and jaws, unlike close-up bitewing radiographs used in cavity detection. Earlier studies referenced tooth transposition in children with clefts, but not the prevalence compared to kids who did not.



This panoramic radiograph illustrates the prevalence of transposed developing teeth in children with cleft conditions. Note the transposition of the maxillary left canine (down arrow) and first premolar (up arrow) in this patient with unilateral left cleft lip and palate. This image also shows an apparent lack of development of maxillary right, left, and mandibular right third molars and a midline supernumerary tooth that developed after alveolar bone grafting and initial incisor alignment. The patient has a bonded retainer on the maxillary central incisors.

This study is the first designed solely to quantify how often unerupted teeth are aligned incorrectly.

New Biologics Show Promise for Pediatric Psoriasis but Clinical Trials Needed First

Kara Shah, MD, PhD

Fernanda Bellodi Schmidt, MD

RESEARCH AND TRAINING DETAILS

Faculty	2
Research Fellows	1
Support Personnel	7
Direct Annual Industry Support	$24,603
Peer Reviewed Publications	4

Bellodi Schmidt F, Shah KN. Biologic response modifiers and pediatric psoriasis. *Pediatr Dermatol*. 2015;32(3):303-320.

PUBLISHED ONLINE FEB. 26, 2015
Pediatric Dermatology

Several biologic medications used to treat adult psoriasis appears to show promise for also treating pediatric psoriasis. However, clinical trials in children are needed to resolve concerns about increased risk of infections and malignancy.

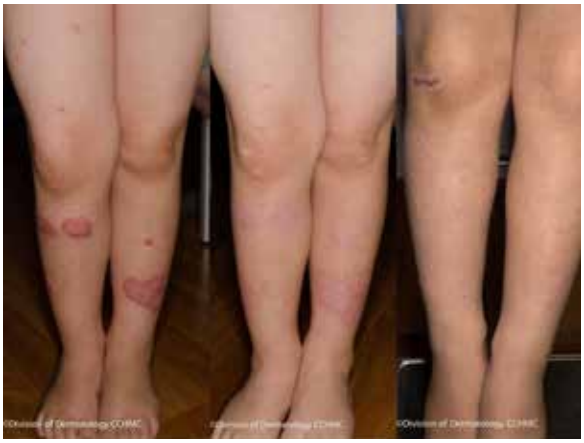
So states a review study published online Feb. 26, 2015, in the journal *Pediatric Dermatology* authored by Kara Shah, MD, PhD, Director of the Division of Dermatology, and Fernanda Bellodi Schmidt, MD, an assistant professor in the division.

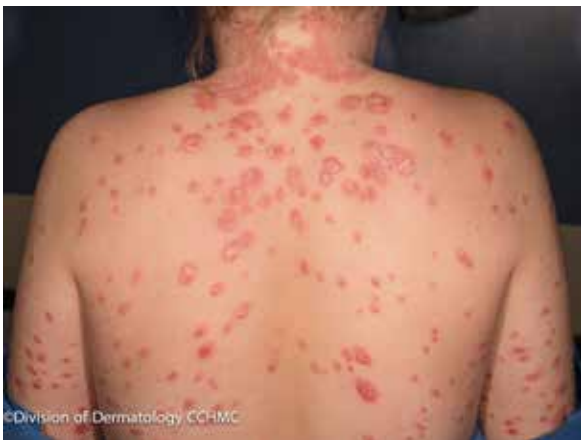
Currently, there are no FDA-approved medications to treat more severe cases of pediatric psoriasis. Shah and Bellodi Schmidt reviewed the current literature of the use of systemic medications to treat pediatric psoriasis, including case reports, case series, and a large clinical trial involving the use of etanercept, a biopharmaceutical effective in treating autoimmune diseases by acting as a tumor necrosis factor inhibitor. Clinical trials have demonstrated that etanercept and several other drugs in its class have shown safety and effectiveness in treating other pediatric inflammatory diseases such as Crohn's disease and juvenile arthritis. Only etanercept has been formally studied in pediatric psoriasis, and currently only the European Union has approved its use for the treatment of psoriasis in children.

"Pediatric dermatologists strongly support clinical trials evaluating the comparative efficacy and risks of medications used for the treatment of psoriasis in children, in particular systemic medications," Shah says.

Scientific concerns about this class of biologic response modifiers, which also includes adalimumab and infliximab, as well other biologic response modifiers such as ustekinumab, center on increased susceptibility to infection and increased risk of certain malignancies, particularly lymphoreticular malignancy.

"These risks appear low," says Shah, "and must be weighed against the concerns inherent to a chronic disease such as psoriasis, especially when severe enough to warrant systemic therapy."

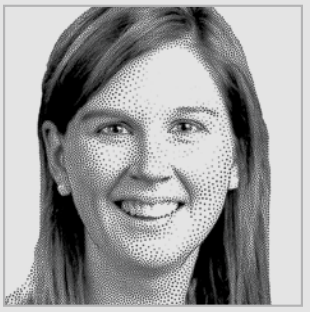




Psoriasis, a chronic skin condition, varies in severity but pediatric dermatologists have long been challenged by the lack of biologic medications approved specifically for severe cases in children. Psoriasis changes the life cycle of skin cells, prompting them to build up rapidly on the skin, forming thick scales. The resulting itchy, dry, red patches can be painful. In this study, researchers analyzed previous reports, case series and clinical-trial data that might one day lead to FDA approval of a drug that resolves concerns about infection-risk and malignancy. The top image shows three different patients with, from left to right, a progression of improvement.

Only etanercept has been formally studied in children.

More Autistic Teens Need Early Intervention to Learn the Life Skills Needed for Adulthood



Amie Duncan, PhD

RESEARCH AND TRAINING DETAILS

Faculty	18
Joint Appointment Faculty	1
Support Personnel	73
Direct Annual Grant Support	$1.9M
Peer Reviewed Publications	14

Duncan AW, Bishop SL. Understanding the gap between cognitive abilities and daily living skills in adolescents with autism spectrum disorders with average intelligence. *Autism*. 2015;19(1):64-72.

PUBLISHED DEC. 15, 2014
Autism

As more children with autism spectrum disorder (ASD) make the transition to adulthood, researchers in the Division of Developmental and Behavior Pediatrics are finding that many need early intervention strategies to facilitate a successful transition.

Researchers Amie Duncan, PhD, and Somer Bishop, PhD, studied the daily living skills (DLS) of 417 autistic teens ages 10-17 with average intelligence (IQ of 85 or higher). Daily living skills include activities such as taking a shower, getting dressed, cooking, doing laundry, managing finances, and navigating the community. The researchers were surprised to find that more than half of these high-functioning autistic teens exhibited DLS abilities that were "significantly below" expectations based on their intellectual abilities.

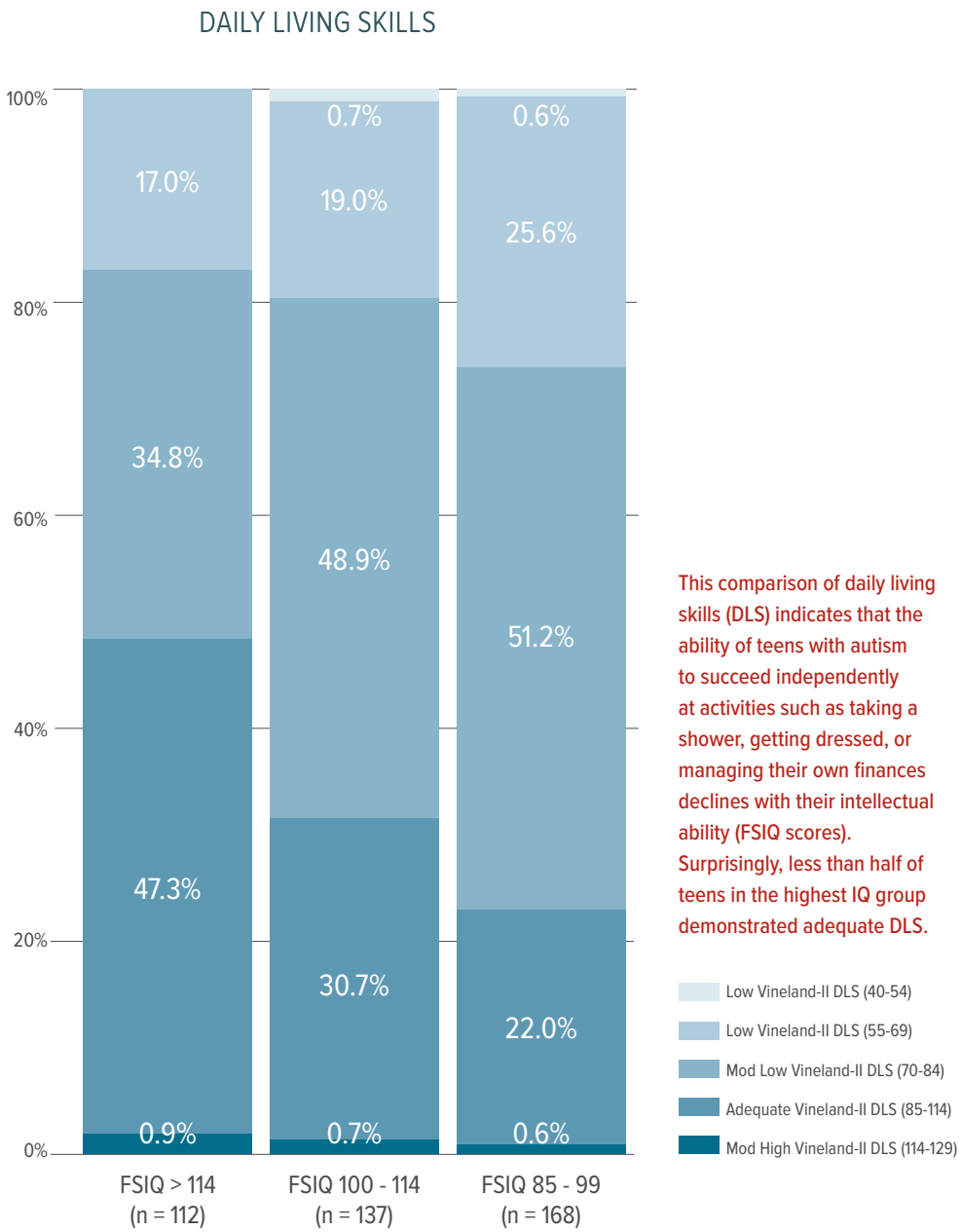
"There is clearly a need to address the substantial gap between cognitive ability and actual performance in activities of daily living," says Duncan, whose study was published Dec. 15, 2014, in *Autism*. "Addressing these skills prior to the transition to adulthood is crucial if we expect young adults to have the necessary skills to live independently."

Interestingly, being older and having more social-communication impairments accounted for only 10 percent of the DLS deficit — a finding that raises hope that adolescents with high functioning ASD have the potential to acquire age-appropriate life skills regardless of the severity of their autism symptoms.

Duncan and Bishop theorize that other factors are involved in autistic teens' abilities to acquire daily living skills; factors that include executive functioning or language capabilities, the number of siblings in the family, the emotional well-being of caregivers, socioeconomic status, race, availability of community and school support services, and involvement in extracurricular activities.

Interventions that support the development of critical daily living skills may increase the likelihood that individuals with ASD can achieve positive outcomes in postsecondary education, employment, and independent living.

"There is clearly a need to address the substantial gap between cognitive ability and actual performance in activities of daily living."



This comparison of daily living skills (DLS) indicates that the ability of teens with autism to succeed independently at activities such as taking a shower, getting dressed, or managing their own finances declines with their intellectual ability (FSIQ scores). Surprisingly, less than half of teens in the highest IQ group demonstrated adequate DLS.

Breakthrough Stomach Organoids Open Doors for New Insights into Ulcers, Cancer, Diabetes and Other Diseases

James Wells, PhD

RESEARCH AND TRAINING DETAILS

Faculty	23
Joint Appointment Faculty	25
Research Fellows	70
Research Students	64
Support Personnel	53
Direct Annual Grant Support	$6.2M
Peer Reviewed Publications	46

McCracken KW, Cata EM, Crawford CM, Sinagoga KL, Schumacher M, Rockich BE, Tsai YH, Mayhew CN, Spence JR, Zavros Y, Wells JM. Modelling human development and disease in pluripotent stem-cell-derived gastric organoids. *Nature*. 2014;516(7531):400-404.

PUBLISHED ONLINE OCT. 29, 2014
Nature

In 1984, Australian physician Barry Marshall resorted to drinking a petri dish brimming with *H. pylori* bacteria and then treated himself with antibiotics to prove that ulcers were caused by an infection, not by stress, spicy foods or stomach acid.

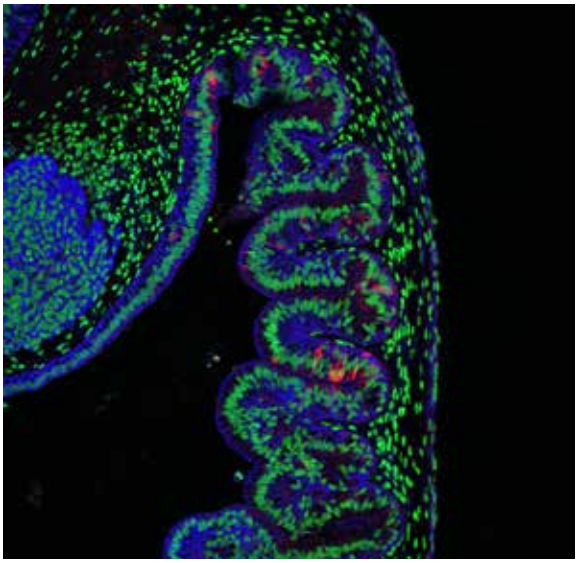
Thirty years later, James Wells, PhD, and fellow researchers with the Divisions of Developmental Biology and Endocrinology are using human pluripotent stem cells as building blocks to create functional, three-dimensional, architecturally complex stomach tissues in the laboratory. Their anatomical breakthrough will enable researchers to study stomach development and a wide range of diseases including peptic ulcer disease, cancer and diabetes — without resorting to Marshall's drastic solution.

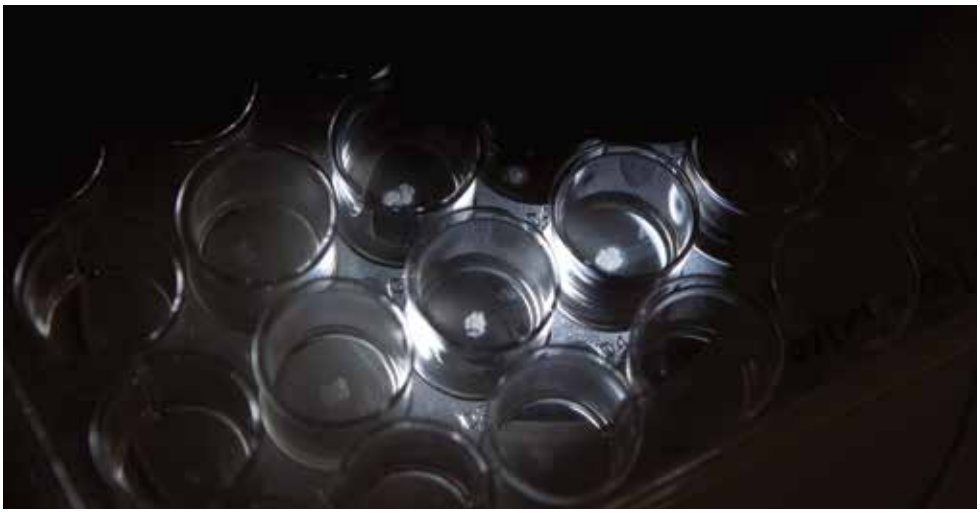
Until Wells' team's discovery, experimental models of human stomach tissue did not exist, and mouse stomachs and other animal tissues have not been ideal models for studying stomach diseases in humans.

In a study published online Oct. 29, 2014, in *Nature*, Wells described how his team performed a series of manipulations of the growth environment to guide human pluripotent stem cells (hPSCs) — stem cells that can grow into any type of tissue — into forming tiny, pea-sized human stomachs, dubbed "human gastric organoids" (hGOs).

In collaboration with colleague Yana Zavros, PhD, at the University of Cincinnati, Wells demonstrated the hGOs can be used to study how the bacteria *H. pylori* causes peptic ulcers and stomach cancer, as the team was able to observe cellular and tissue changes associated with the bacterial infection.

The team's accomplishment represents the first time that researchers have produced a 3-D model of the human stomach. The team plans to use a similar approach to develop other "mini-organs," including the lungs and esophagus. It also creates possibilities for studying new drugs, building tissue models of stomach cancer and investigating the underpinnings of obesity-related diabetes.

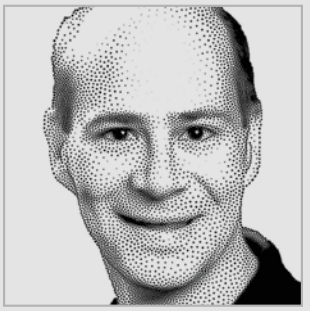




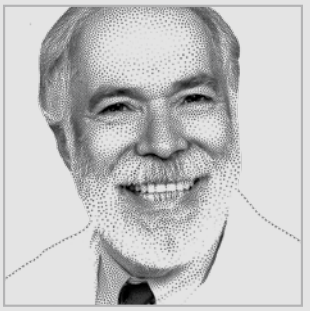
Researchers plan to use a similar approach to develop other "mini-organs."

Drs. James Wells, Yana Zavros, and Kyle McCracken received national attention for their work using differentiation of human pluripotent stem cells to generate 3-D human gastric tissue. The confocal microscope image (top) shows a functioning cross section of the lining of the stomach organoid. The next image shows the organoids growing *in vitro* in the Wells laboratory.

Some Antibodies Play 'Back-Up Defense' Against Kidney Disease in Mice



Richard Strait, MD



Fred Finkelman, MD

RESEARCH AND TRAINING DETAILS

Faculty	42
Joint Appointment Faculty	3
Research Students	6
Support Personnel	47
Direct Annual Grant Support	$1.6M
Peer Reviewed Publications	77

Strait RT, Posgai MT, Mahler A, Barasa N, Jacob CO, Kohl J, Ehlers M, Stringer K, Shanmukhappa SK, Witte D, Hossain MM, Khodoun M, Herr AB, Finkelman FD. IgG1 protects against renal disease in a mouse model of cryoglobulinaemia. *Nature.* 2015;517(7535):501-504

PUBLISHED ONLINE NOV. 2, 2014
Nature

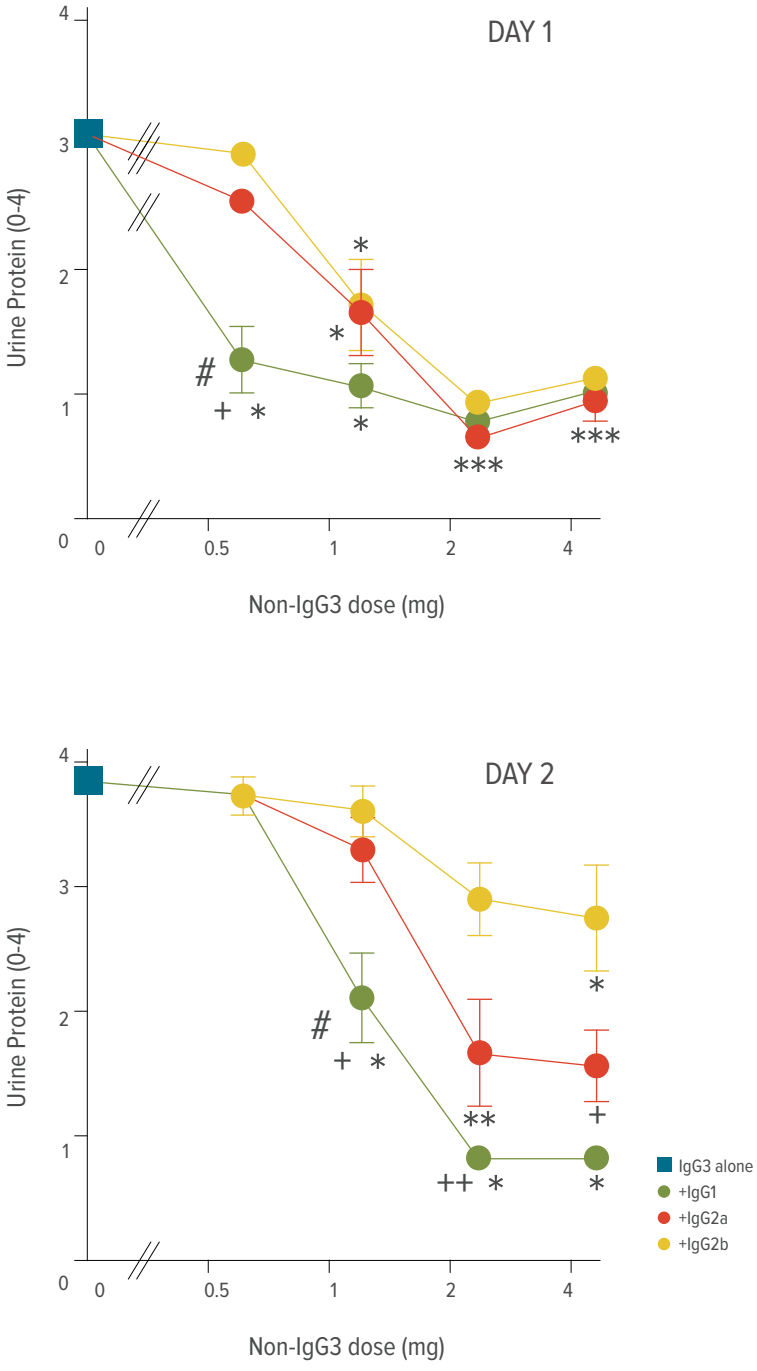
Immune system researchers have long known that some antibodies are highly capable of identifying and disabling foreign substances inside the body — called antigens — by clumping them together and removing them from the body by activating complement and binding to antibody surface receptors, called FcgRs, on cells.

In a Nov. 2, 2014, study published online in *Nature*, researchers led by Richard Strait, MD, have discovered that certain types of antibodies — even though they do not activate, complement or interact strongly with activating antibody receptors — still play critical roles in clearing these antigens from the body and preventing disease.

Strait's study, co-published with several other investigators including senior author and his mentor, Fred Finkelman, MD, Division of Immunobiology, looked specifically at kidney disease associated with the interaction of antibodies with antigen in mice. In normal healthy mice, antigen exposure did not cause disease. However, in mice genetically deficient in IgG1, the most abundant subtype of antibody and roughly equivalent to human IgG4, antigen exposure resulted in developing a fatal kidney disease.

The fatal kidney disease arose secondary to the occurrence of large and numerous cryoglobulin complexes made from the interaction between the antigen and the antibody subtype IgG3. These complexes proceeded to obstruct blood flow to the kidney, eventually causing organ failure and death.

Cryoglobulins also are responsible for kidney damage and other tissue injury in hepatitis C and other diseases. Providing IgG1 back to IgG1-deficient mice prevented cryoglobulin development and saved the mice. Of extreme interest is that the IgG1 performed this disease prevention independent of the usual involvement of the complement system and the antibody receptors.



This figure demonstrates *in vivo* how the addition of antigen-specific IgG1, IgG2a or IgG2b intravenously in increased amounts differentially prevents kidney disease (as measured by proteinuria) invoked by antigen plus antigen-specific IgG3 immune complex precipitation in the capillaries of the glomeruli.

Whole Exome Sequencing Uncovers Defective Gene Linked to Severe Growth and Metabolic Disorder

Andrew Dauber, MD, MSc

RESEARCH AND TRAINING DETAILS

Faculty	17
Joint Appointment Faculty	3
Support Personnel	27
Direct Annual Grant Support	$1.4M
Direct Annual Industry Support	$113,816
Peer Reviewed Publications	37

de Bruin C, Mericq V, Andrew SF, van Duyvenvoorde HA, Verkaik NS, Losekoot M, Porollo A, Garcia H, Kuang Y, Hanson D, Clayton P, van Gent DC, Wit JM, Hwa V, Dauber A. An XRCC4 splice mutation associated with severe short stature, gonadal failure, and early-onset metabolic syndrome. *J Clin Endocrinol Metab.* 2015;100(5):E789-798.

PUBLISHED MARCH 5, 2015
The Journal of Clinical Endocrinology & Metabolism

An emerging technology called whole exome sequencing — which enables researchers to sequence all 20,000 of the body's genes at once rather than one gene at a time — has helped identify a defective gene that causes a rare condition marked by a spectrum of growth and metabolic disorders in children.

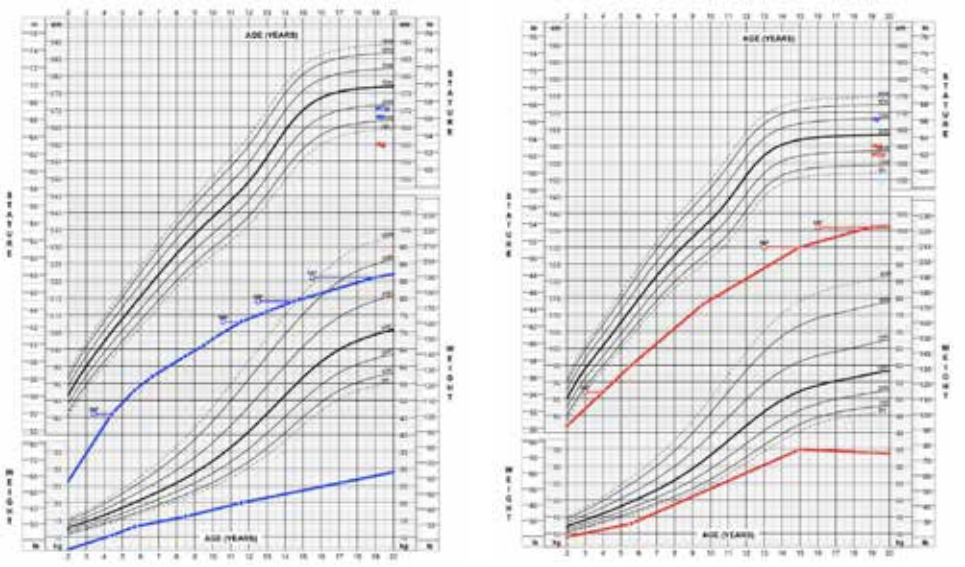
The defective XRCC4 gene, identified by Andrew Dauber, MD, MMSc, co-leader of the newly formed Cincinnati Center for Growth Disorders, includes a splice mutation that deletes 23 amino acids from the gene, interfering with the body's ability to repair DNA damage.

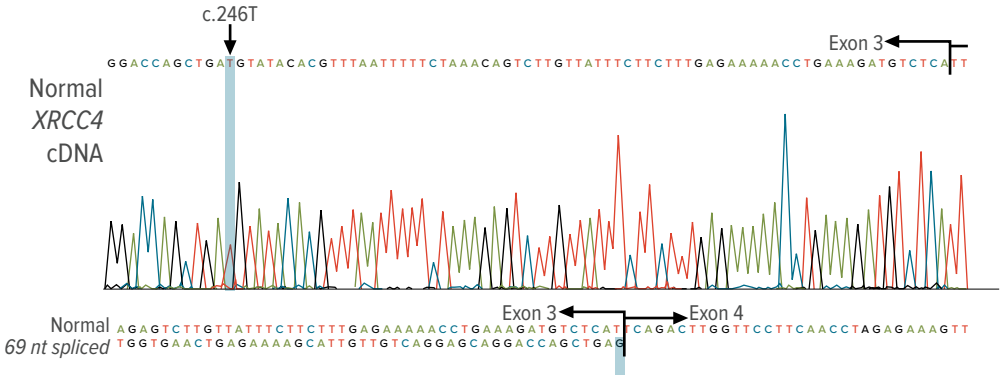
XRCC4 was discovered by examining DNA from a Chilean brother and sister, both of whom were diagnosed with severe short statue, gonadal failure and early-onset metabolic syndromes that resulted in a gastrointestinal tumor in the sister, diabetes and other multi-system complications. The 39-year-old brother is still alive; the sister died at 36. Findings based on their tissues were published online March 5, 2015, in *The Journal of Clinical Endocrinology & Metabolism*.

"The body is constantly repairing damage that occurs to DNA, and this gene is part of the DNA damage repair process," says Dauber. "We were able to show in skin cells from one of the patients that the cells were not able to execute the DNA damage repair process correctly."

The finding creates deeper understanding of a spectrum of complications associated with the defective gene, and creates direct links among patient genome sequencing, translational biology in the lab, patient diagnosis and genetic counseling, he says.

"Our patients are among the oldest in the world to be identified, and because of this new understanding, the hope is that we'll be better able to counsel them and their families about a variety of other issues that they might face, including predisposition to tumors, insulin resistance and other complications."





Growth charts (top) of two siblings from a rural family in Chile exhibit a novel syndrome consisting of severe short stature, microcephaly, hypergonadotropic hypogonadism, early-onset metabolic syndrome, and possible increased tumor susceptibility. Combined microarray analysis and whole exome sequencing detected an underlying XRCC4 mutation, a gene involved in the DNA damage repair process. The next figure shows Sanger sequencing of XRCC4 cDNA with the nucleotide c.246T highlighted demonstrating that the mutation results in a novel splice site causing deletion of 69 nucleotides.

"The body is constantly repairing damage that occurs to DNA, and this gene is part of the DNA damage repair process."

Home-Visiting Program Links Response to Maternal Depression Treatment and Histories of Abuse

Robert Ammerman, PhD, ABPP

Judith Van Ginkel, PhD

RESEARCH AND TRAINING DETAILS

Faculty	1
Joint Appointment Faculty	1
Research Fellows	2
Direct Annual Grant Support	$1.1M
Peer Reviewed Publications	3

Ammerman RT, Peugh JL, Teeters AR, Putnam FW, Van Ginkel JB. Child Maltreatment History and Response to CBT Treatment in Depressed Mothers Participating in Home Visiting. *J Interpers Violence*. 2014

PUBLISHED ONLINE NOV. 13, 2014
Journal of Interpersonal Violence

At-risk mothers who participate in home visiting programs often experience high rates of depression, and a new study by the Every Child Succeeds (ECS) program finds that the mothers' own histories of underlying physical and emotional abuse must be addressed in order for cognitive behavioral therapy (CBT) to be optimally effective.

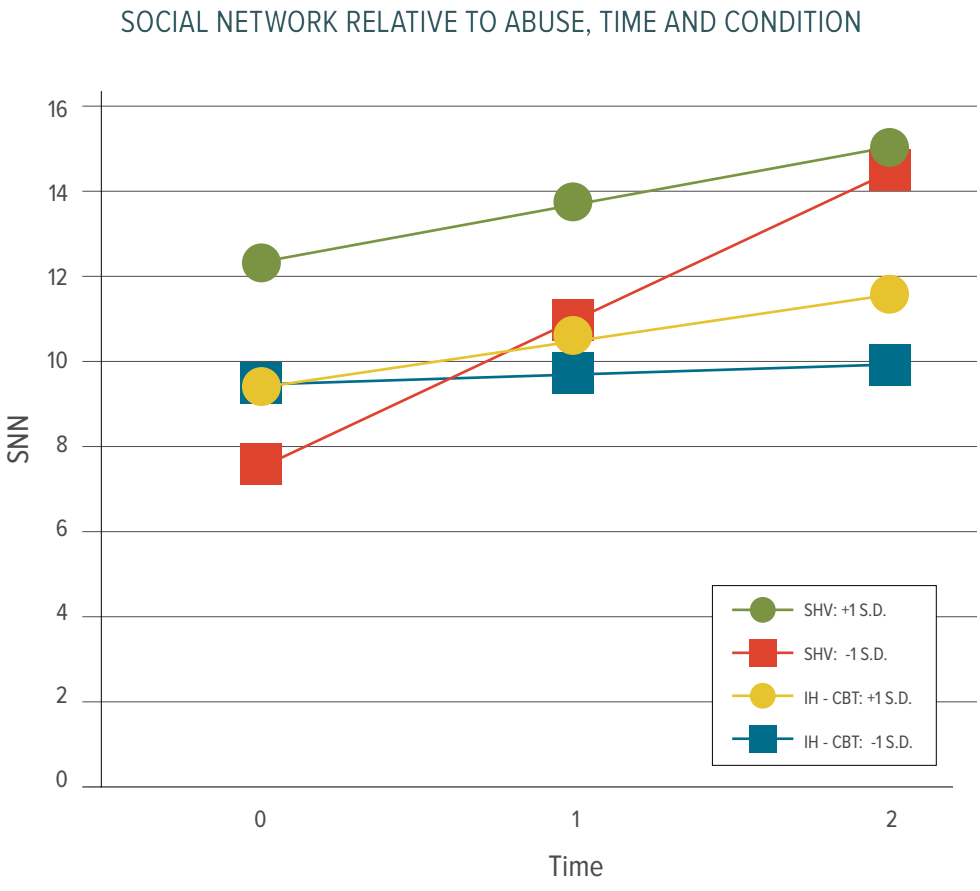
Robert Ammerman, PhD, ABBP, and colleagues studied the long-term progress of 93 post-partum mothers half of whom received 15 in-home visits from a licensed CBT therapist to treat depression. Before the study, mothers were screened for childhood histories of emotional or physical abuse.

Although women with histories of physical abuse responded more positively to CBT compared to those who did not receive therapy, those with the most severe histories of childhood abuse had comparatively lower CBT outcomes than those with less-severe abusive experiences.

At-risk depressed mothers with histories of emotional abuse, likewise, were able to develop stronger social networks as a result of CBT. Study findings were detailed online Nov. 13, 2014, in the *Journal of Interpersonal Violence*.

"Exposure to high levels of trauma changes the way the brain works, how you react to stress and how you control your emotions," says Ammerman, Scientific Director of ECS. "We are going to have to augment our depression treatment to address more directly any trauma experiences in order to help these women do even better in their lives."

Colleague and co-author Judith Van Ginkel, PhD, President of ECS, says the study's findings indicate that public health funds spent on in-home visits for at-risk mothers also need to address underlying, abuse-related trauma in order for depression treatments to be most effective.



Public health funds spent on in-home visits for at-risk mothers also need to address underlying, abuse-related trauma for depression treatments to be most effective.

This graph shows the three-way interaction between emotional abuse, time, and condition and size of social network. Mothers with more extensive experiences of emotional abuse in childhood showed an increase in size of social network when receiving IH-CBT treatment, but not when in the no-treatment control condition.

Novel Genetic, Bacterial Signature for IBD Suggests a Target for Developing New Therapies

Lee Denson, MD

RESEARCH AND TRAINING DETAILS

Faculty	37
Joint Appointment Faculty	2
Research Fellows	9
Research Students	9
Support Personnel	45
Direct Annual Grant Support	$6M
Direct Annual Industry Support	$558,000
Peer Reviewed Publications	120

Haberman Y, Tickle TL, Dexheimer PJ, Kim MO, Tang D, Karns R, Baldassano RN, Noe JD, Rosh J, Markowitz J, Heyman MB, Griffiths AM, Crandall WV, Mack DR, Baker SS, Huttenhower C, Keljo DJ, Hyams JS, Kugathasan S, Walters TD, Aronow B, Xavier RJ, Gevers D, Denson LA. Pediatric Crohn disease patients exhibit specific ileal transcriptome and microbiome signature. *J Clin Invest.* 2014;124(8):3617-3633.

PUBLISHED ONLINE JULY 8, 2014
Journal of Clinical Investigation

Gastroenterologists have known that several genes and types of bacteria are associated with inflammatory bowel disease (IBD) in children, including Crohn's disease and ulcerative colitis. And while more than 160 areas of the genome have been identified as containing risk factors for IBD, no definite cause has been found.

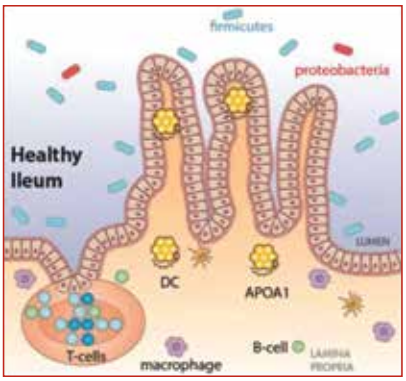
Lee Denson, MD, Director of the Inflammatory Bowel Disease Center, and his team have narrowed the focus to the ileum as the primary inductive site for IBD, and they have identified specific bacteria activated by ileal cells, depending on the IBD diagnosis. Their findings appeared July 8, 2014, in the *Journal of Clinical Investigation*.

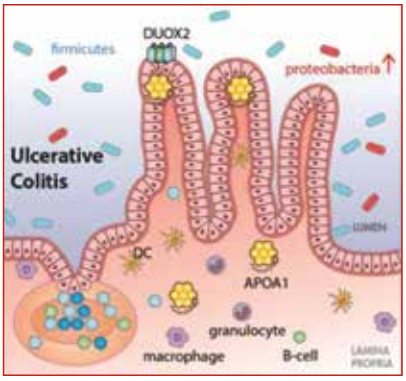
By comparing ileal tissues from children with IBD and healthy tissues, they found that Crohn's disease and ulcerative colitis patients had higher levels of Proteobacteria and an increase in the activity of the DUOX2 gene. Some patients with Crohn's disease also had lower levels of Firmicutes bacteria and lower activity of the APOA1 gene.

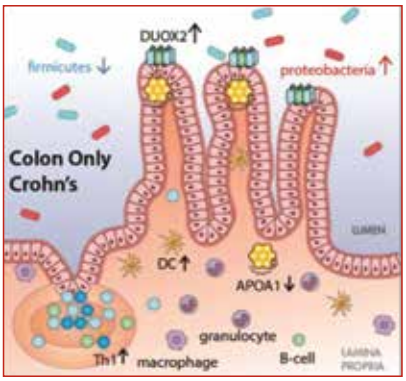
More than 80,000 children in the U.S. have been diagnosed with IBD, and the number is climbing. By identifying a microbial and gene expression "signature" for the disease, researchers are now better positioned to understand IBD, diagnose it more accurately and develop targeted therapies, Denson says.

Of special interest, he notes, is new knowledge about the role of the APOA1 gene, which is linked to changes in about 500 other genes. Patients with the APOA1 profile, regardless of the type of therapy or length of treatment, tend to have less successful outcomes.

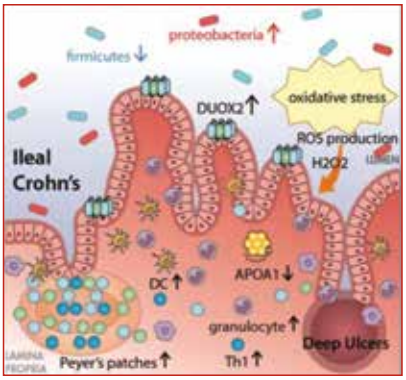
"By characterizing this profile, we have potentially identified a new pathway to target to benefit kids who have not done well with other types of treatments," Denson says.







These illustrations show the progressive induction of an ileal DUOX2 host gene coexpression signature in association with expansion of Proteobacteria taxa across multiple forms of inflammatory bowel disease. The greatest change was detected in those with ileal Crohn's disease with deep ulcers (bottom). These findings emphasize the central role of the ileum in the pathogenesis of Crohn's disease. Maximal alteration of microbial shifts was associated with the most severe tissue injury.



More than 80,000 children in the U.S. have been diagnosed with IBD, and the number is climbing.

Link Between Housing Code Violations and Asthma Morbidity Informs Better Care, Improved Outcomes

Andrew Beck, MD, MPH

RESEARCH AND TRAINING DETAILS

Faculty	27
Joint Appointment Faculty	6
Research Fellows	3
Support Personnel	35
Direct Annual Grant Support	$2.5M
Direct Annual Industry Support	$96,188
Peer Reviewed Publications	42

Beck AF, Huang B, Chundur R, Kahn RS. Housing code violation density associated with emergency department and hospital use by children with asthma. *Health Aff* (Millwood). 2014;33(11):1993-2002.

PUBLISHED NOVEMBER 2014
Health Affairs

Community housing code enforcement agencies collect and own critical data about the presence of mold or cockroaches that can help pediatricians identify clusters of asthma-related morbidity among children and better target care for children with asthma.

The critical role played by housing agency data is identified in a November 2014 *Health Affairs* study by Andrew Beck, MD, MPH, of the Division of General and Community Pediatrics, and the Virginia-based Project HOPE: The People-to-People Health Foundation, Inc.

Geo-mapping tools helped Beck and his team identify a link between the density of housing code violations — reports that logged the presence of mold or cockroaches, both of which are known asthma triggers — and asthma morbidity, based on asthma-related emergency department visits and hospitalizations.

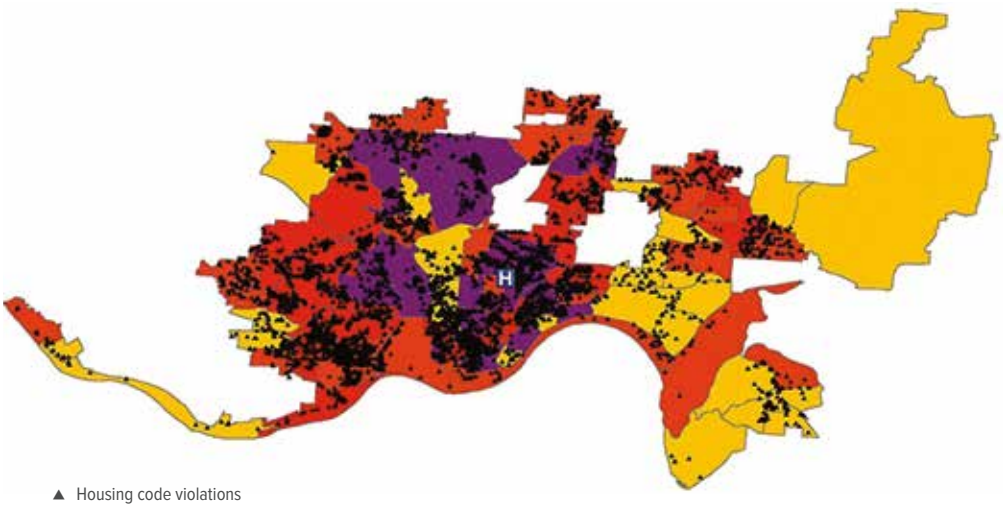
Independent of poverty, code violation density explained 22 percent of the variation in asthma utilization rates across the areas studied. Children who had been hospitalized for asthma had an 84 percent increased chance of returning to the emergency department or being rehospitalized within 12 months if they lived in areas with the highest rates of housing code violations, compared to children living in low-density areas for violations.

Beck's study involved 4,355 children, ages 1-16, who were hospitalized or received emergency room treatment for asthma at Cincinnati Children's over nearly four years.

"Integrating housing and health data could highlight at-risk areas and patients for targeted interventions," says Beck.

Housing data also could be used to study health disparities that occur in small geographic areas, identify medically at-risk areas for programs to improve housing conditions, incorporate into patients' electronic health records for improved clinical care, or inform health systems' strategies in support of preventive medicine and accountable care.

ASTHMA UTILIZATION AND HOUSING CODE VIOLATIONS



*Calculated from 8,736 emergency department visits and hospitalizations in 113 Greater Cincinnati census tracts between 2009-2012

This map shows an association between high rates of asthma-related Emergency Department visits and hospital admissions and the location and density of housing code violations within Greater Cincinnati census tracts. The "low" asthma utilization rate category represents census tracts with fewer than 21.3 utilizations per 1,000 children per year; "low medium" reflects 21.3-33.0; "high medium" is 33.0-47.5; and "high" areas include more than 47.5 utilizations per 1,000 children per year.

Children who had been hospitalized for asthma had an 84 percent increased chance of returning to the emergency department or being rehospitalized within 12 months if they lived in areas with the highest rates of housing code violations.

Efficient Transplacental Antibody Transfer From Mother to Fetus Crucial to Infant Health

Mark Steinhoff, MD

RESEARCH AND TRAINING DETAILS

Faculty	4
Joint Appointment Faculty	4
Support Personnel	11
Direct Annual Grant Support	$3.7M
Direct Annual Industry Support	$280,346
Peer Reviewed Publications	9

Chu HY, Steinhoff MC, Magaret A, Zaman K, Roy E, Langdon G, Formica MA, Walsh EE, Englund JA. *J Infect Dis.* 2014 Nov 15;210(10):1582-9. doi: 10.1093/infdis/jiu316. Epub 2014 Jun 5.

PUBLISHED NOV. 15, 2014
The Journal of Infectious Diseases

An international collaboration led by Mark Steinhoff, MD, Director of the Division of Global Child Health, is advancing scientific understanding of how to protect infants from respiratory syncytial virus (RSV), the most important viral cause of infant pneumonia, though there is limited information from tropical regions.

The study, published Nov. 15, 2014, in *The Journal of Infectious Diseases*, is crucial research because, globally, pneumonia is the leading cause of childhood mortality.

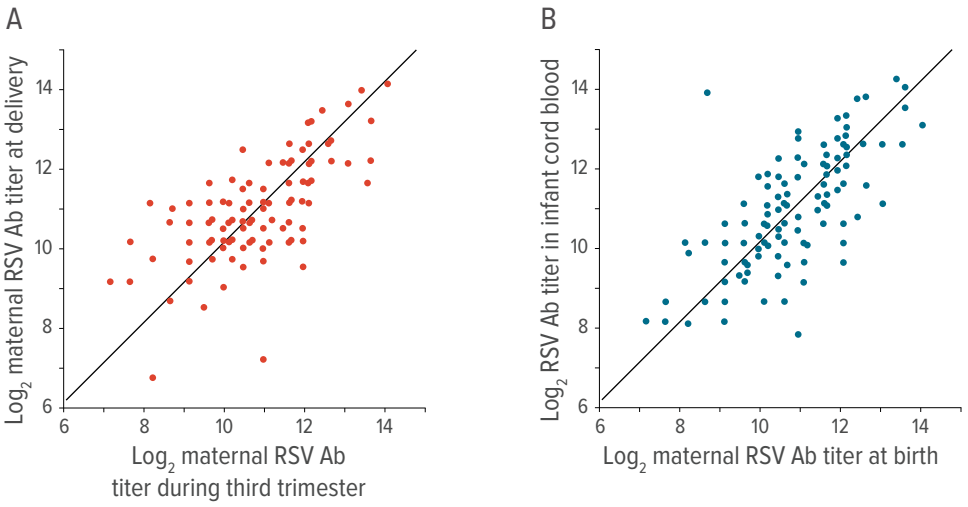
The research team, which included colleagues in Seattle, WA, Rochester, NY, and Dhaka, Bangladesh, examined the role of maternal serum antibody in protecting infants from RSV. The team found that efficient transplacental transfer of RSV-specific antibody from mother to the fetus was documented in mother-infant pairs in Asia, and that higher cord-blood antibody titers were associated with infant protection from serologic RSV infection.

Findings were based on serial serum samples collected from mother-infant pairs in Bangladesh, from the third trimester of pregnancy to 72 weeks postpartum. They tested these using an RSV antibody microneutralization assay, and defined serologic infection as a four-fold increase in antibody titer (the highest dilution factor at which a positive reading is yielded). Maternal antibody half-life was calculated using infant antibody titers from birth to 20 weeks.

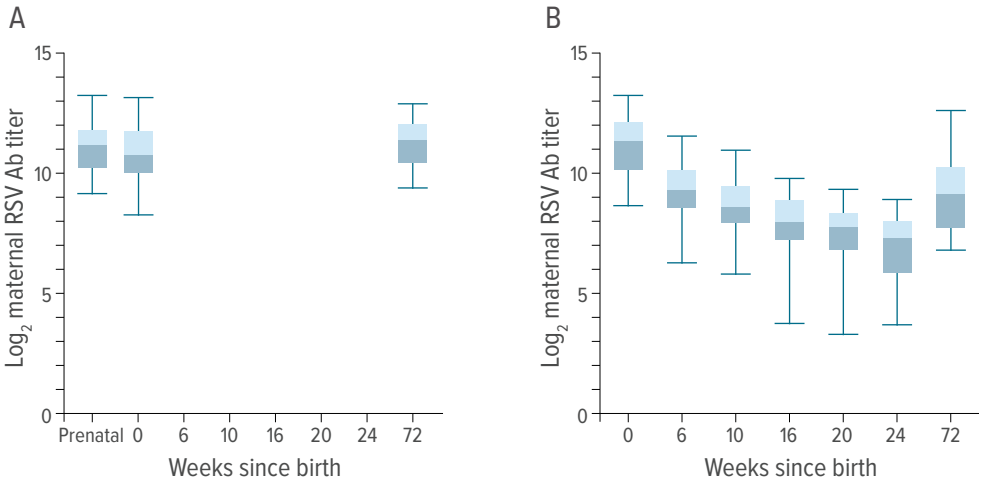
Researchers found that the ratio of infant cord blood to maternal serum RSV antibody titers in 149 mother-infant pairs was 1.01, and that there was a clear association between higher cord blood RSV antibody titers and lower risk of infant serologic infection.

In addition to this major initiative in battling pneumonia in Bangladesh, Global Child Health also works to assess the disease risks and increase the availability of childhood vaccines for pneumococcal and influenza viruses in other countries with limited resources, including India, Sri Lanka, Indonesia and Nepal.

COMPARISON OF LOG$_2$ MATERNAL RESPIRATORY SYNCYTIAL VIRUS (RSV) ANTIBODY (AB) TITERS IN THE THIRD TRIMESTER AND LOG2 MATERNAL RSV AB TITERS AT BIRTH (R=0.68).

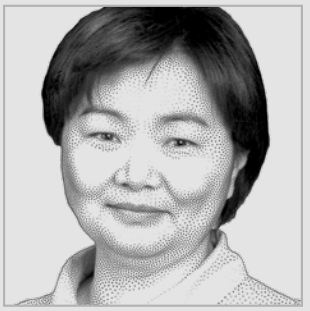


MATERNAL LOG$_2$ RESPIRATORY SYNCYTIAL VIRUS (RSV) ANTIBODY (AB) TITERS IN THE THIRD TRIMESTER, AT BIRTH, AND AT 72 WEEKS POSTPARTUM.



In a study examining the role of transplacental antibody transfer in infant health, these titer testing images show how mean maternal Ab titers in the third trimester were correlated with titers at birth and week 72 of the postpartum period (R = 0.68 and R = 0.47, respectively). The top charts compare the mother's antibody titer to respiratory syncytial virus (RSV) in the third trimester (left) and at birth. The bottom charts show the levels at birth (left) and then 72 weeks later. The findings show a clear association between higher cord blood RSV antibody titers and lower risk of infant infection.

MYPN Mutations Affect Medication Effectiveness for Children with Restrictive Cardiomyopathy



Enkhsaikhan Purevjav, MD, PhD

The Heart Institute includes the Divisions of Cardiology, Cardiothoracic Surgery, and Molecular Cardiovascular Biology

HEART INSTITUTE
RESEARCH AND TRAINING DETAILS

Faculty	67
Joint Appointment Faculty	2
Research Fellows	33
Research Students	24
Support Personnel	146
Direct Annual Grant Support	$11.3M
Direct Annual Industry Support	$227,476
Peer Reviewed Publications	194

Huby AC, Mendsaikhan U, Takagi K, Martherus R, Wansapura J, Gong N, Osinska H, James JF, Kramer K, Saito K, Robbins J, Khuchua Z, Towbin JA, Purevjav E. Disturbance in Z-disk mechanosensitive proteins induced by a persistent mutant myopalladin causes familial restrictive cardiomyopathy. *J Am Coll Cardiol*. 2014;64(25):2765-2776.

PUBLISHED DEC. 30, 2014
Journal of the American College of Cardiology

Particularly in children, restrictive cardiomyopathy (RCM) has the poorest prognosis among heart muscle diseases. The condition can lead to heart failure, arrhythmias and sudden cardiac death, with a 5-year mortality rate exceeding 70 percent.

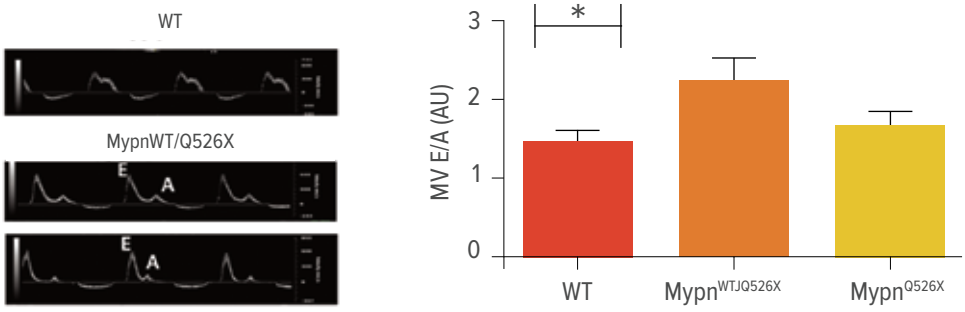
To date, no medications have proven effective against RCM, which has left heart transplantation as the sole definitive treatment option. Now, fresh clues for finding new therapeutic targets are emerging thanks to a study led by Enkhsaikhan Purevjav, MD, PhD, a former researcher with the Heart Institute at Cincinnati Children's who recently moved to the University of Tennessee Health Science Center.

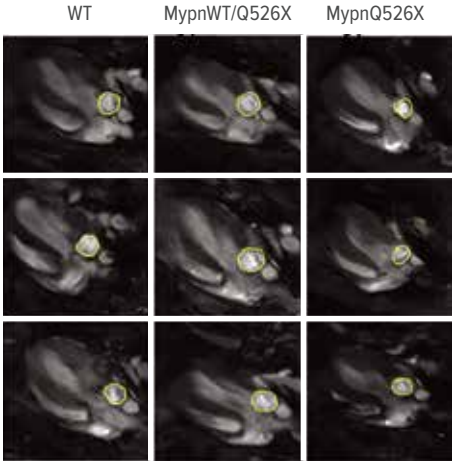
Purevjav and former research fellow Anne-Cecile Huby, PhD, report that mutations in the myopalladin (MYPN) gene cause diverse cardiomyopathic phenotypes within a critical "final common pathway." These mutations could explain why ACE inhibitors, beta-blockers and angiotensin receptor blockers are ineffective in patients with RCM. Their findings were published Dec. 30, 2014, in the *Journal of the American College of Cardiology*.

The study involved developing "knock-in" mice to carry mutations of the murine Mypn gene that would be homologous to the human MYPN-Q529X mutation. At six weeks, signs of restrictive physiology (RP) were detected in the mice carrying the mutation. At 12 weeks, the mice showed signs of impaired diastolic filling of the left ventricle, decreased T-wave duration, and other RCM symptoms.

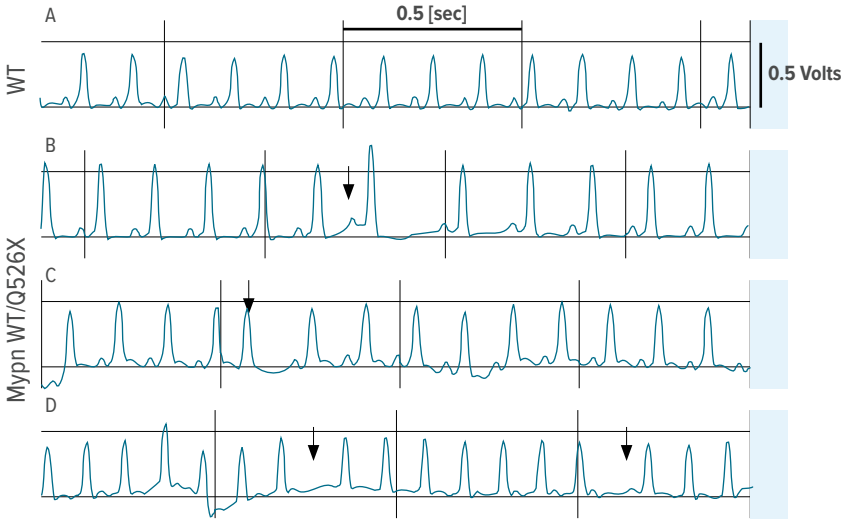
"From these data, we hypothesize that the RCM phenotype results from persistence of dysfunctional truncated MypnQ526X protein and consequent multiple pathological 'hits,' " Purevjav says.

MYPN is one of several genes that appear to be involved in RCM. These findings suggest that further studies on time-dependent expression changes in CARP, MLP, DES, and ERK1/2 proteins in patients with RCM may provide useful information for discovering diagnostic and therapeutic targets.





M-mode images of mitral valve (MV) movement (top) indicate an increase of early and late diastolic velocities (E/A) ratios in myopalladin (Mypn) WT/Q526X mice compared with wild-type (WT) mice or homozygotes, which became significant in 12-week-old heterozygote mutants compared with WT and homozygote mice. Cardiac magnetic resonance images (left) demonstrate enlarged left atria in MypnWT/Q526X mice (middle columns) compared with WT (left columns) and MypnQ526X (right columns) mice.



Electrocardiography in WT (A) and mutant (B-D) mice. MypnWT/Q526X mice display arrhythmias (arrows), including (B) premature atrial contractions, (C) premature ventricular contractions, and secondary atrioventricular block (D; 1:7 Wenkebach).

Shared Best Practices Can Reduce Mortality Risk in Complex Cardiac Surgery for Children With Heterotaxy Syndrome

David Morales, MD

HEART INSTITUTE
RESEARCH AND TRAINING DETAILS

Faculty	67
Joint Appointment Faculty	2
Research Fellows	33
Research Students	24
Support Personnel	146
Direct Annual Grant Support	$11.3M
Direct Annual Industry Support	$227,476
Peer Reviewed Publications	194

Khan MS, Bryant R, 3rd, Kim SH, Hill KD, Jacobs JP, Jacobs ML, Pasquali SK, Morales DL. Contemporary Outcomes of Surgical Repair of Total Anomalous Pulmonary Venous Connection in Patients With Heterotaxy Syndrome. Ann *Thorac Surg*. 2015;99(6):2134-2140.

PUBLISHED APRIL 23, 2015
The Annals of Thoracic Surgery

Even with the many advances in surgical repair of complex congenital heart malformations, total anomalous pulmonary venous connection (TAPVC) repair in patients with heterotaxy syndrome carries a high mortality risk, particularly with functionally univentricular physiology.

Some of the most complex heart defects occur in children born with heterotaxy syndrome, in which abdominal organs form on the opposite side of the body. The cardiac lesions that result, which are almost always multiple, can vary widely in severity and potential outcome. An analysis led by David Morales, MD, and colleagues, published April 23, 2015, in the *Annals of Thoracic Surgery* is the first to provide national-level data on the mortality risks that certain patient populations face when receiving TAPVC repairs.

The study examined 261 operations for TAPVC repair in 258 patients from 65 medical centers. Overall, mortality was 38 percent. Heterotaxy patients undergoing TAPVC repair and requiring postcardiotomy ECMO had still higher operative mortality rates.

The findings suggest that early outcomes of TAPVC repair are significantly worse when patients with heterotaxy are in high-risk subgroups such as those who have functionally univentricular physiology.

"It is clear that TAPVC heterotaxy patients are very rare and present with extremely complicated anatomy and physiology that varies with patients, and therefore their care has to be individualized," Morales says. "However, there are centers that have achieved higher levels of success treating these patients such as ours. The key next steps will be to understand and share best patient management practices, so that through education they can be spread throughout the country to improve outcomes for all of these children."

OPERATIVE MORTALITY AMONG HIGH-RISK SUBGROUPS IN TOTAL ANOMALOUS PULMONARY VENOUS CONNECTION HETEROTAXY PATIENTS

Subgroups	Mortality With Risk Factor, n (%)	Mortality Without Risk Factor, n (%)	P Value a	Single Ventricle Mortality, n (%)	Non Single Ventricle Mortality, n (%)	P Value b
Systemic-to-pulmonary artery shunt	38/84 (45)	62/177 (35)	0.134	33/71 (46)	5/13 (38)	0.764
Pulmonary atresia	26/64 (41)	74/197 (38)	0.660	24/51 (47)	2/13 (15)	0.057
Age at surgery of ≤48 hours	20/54 (37)	80/207 (39)	0.876	17/38 (45)	3/16 (19)	0.122
Weight at surgery <2.5 kg	13/31 (42)	87/230 (38)	0.696	10/21 (48)	3/10 (30)	0.452
Infracardiac TAPVC type	24/59 (41)	76/202 (38)	0.761	20/44 (45)	4/15 (27)	0.238
Postcardiotomy ECMO	13/20 (65)	87/241 (36)	0.015	10/13 (77)	3/7 (43)	0.174
Concomitant Norwood/DKS procedure	3/3 (100)	97/258 (38)	0.055	3/3 (100)	…	…

DKS = Damus-Kaye-Stansel; ECMO = extracorporeal membrane oxygenation; TAPVC = total anomalous pulmonary venous connection.

a — Comparison between patients with and without particular risk factor.

b — Comparison between single ventricle and non-single ventricle groups.

Early outcomes of TAPVC repair are significantly worse when patients with heterotaxy are in high-risk subgroups, such as those who have functionally univentricular physiology. This table details outcomes from 261 operations for TAPVC repair involving 258 patients at 65 medical centers.

Myomaker Protein Proves Essential for Muscle Regeneration

Douglas Millay, PhD

HEART INSTITUTE
RESEARCH AND TRAINING DETAILS

Faculty	67
Joint Appointment Faculty	2
Research Fellows	33
Research Students	24
Support Personnel	146
Direct Annual Grant Support	$11.3M
Direct Annual Industry Support	$227,476
Peer Reviewed Publications	194

Millay DP, Sutherland LB, Bassel-Duby R, Olson EN. Myomaker is essential for muscle regeneration. *Genes Dev.* 2014;28(15):1641-1646.

PUBLISHED ONLINE JULY 13, 2014
Genes and Development

A new scientist at Cincinnati Children's is beginning to unlock the genetic secrets of muscle cell fusion, an advance that could have extensive implications for health.

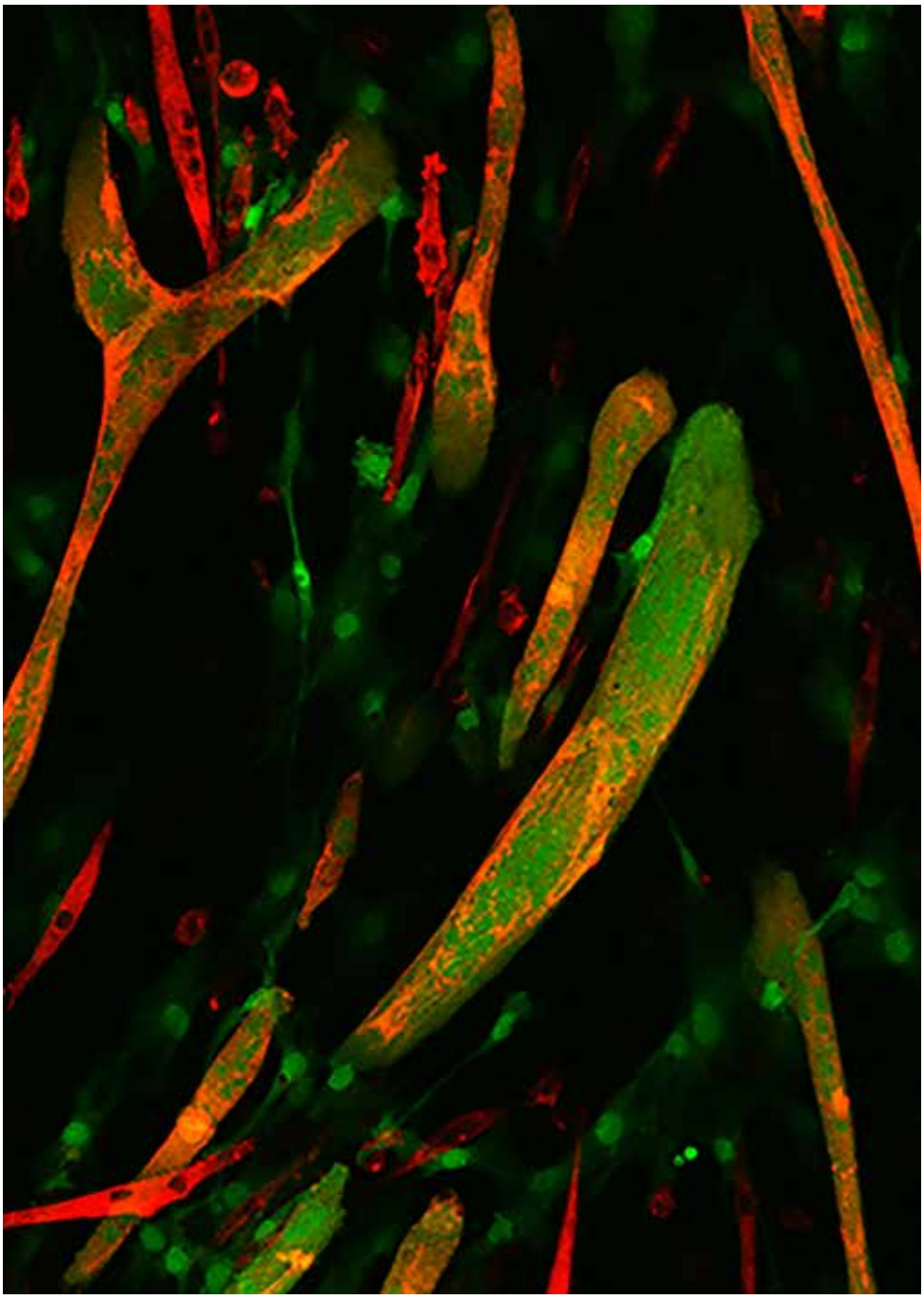
While working as a post-doctoral fellow at the University of Texas Southwestern in Dallas, Doug Millay, PhD, and his mentor found the only muscle-specific protein known to be essential for fusion of embryonic and adult myoblasts. In a paper published in 2013 in *Nature*, they dubbed the gene "myomaker." Then in a more recent paper in *Genes and Development*, the team showed that myomaker also is necessary for normal adult muscle cell regeneration.

Their work shows that myogenic basic helix-loop-helix (bHLH) transcription factors induce myomaker expression in satellite cells during acute and chronic muscle regeneration. Moreover, genetic deletion of myomaker in adult satellite cells completely abolishes muscle regeneration, resulting in severe muscle destruction after injury.

The ability of myomaker to promote fusion to adult muscle fibers and muscle regeneration suggests opportunities to enhance muscle repair through myomaker-directed cell-cell fusion. In mice, the myomaker gene can be expressed in non-muscle cells, which then allows these cells to fuse to skeletal muscle. This implies that myomaker might be useful as a delivery vehicle for future therapies to address muscle loss in Duchenne muscular dystrophy, cancer, AIDS, and COPD.

"In all of these conditions, restoring muscle cell growth may likely do even more than impact quality of life," Millay says. "It could also slow the progression of the disease itself."

Millay is one of two scientists at Cincinnati Children's to be named Pew Scholars in 2015. He is continuing his work to more fully understand the machinery of muscle cell development.



This confocal microscopic image shows that expression of the myomaker gene in fibroblasts (green) induces fusion with myoblasts (red) resulting in yellow/orange chimeric myotubes. Recent research shows that myomaker is necessary to promote fusion in skeletal muscle cells during prenatal development and later during the muscle repair process. In mice, myomaker also can be expressed in non-muscle cells, which implies that myomaker could serve as a delivery vehicle for future therapies to address muscle loss in conditions such as Duchenne muscular dystrophy and cancer.

Family-Activated Medical Emergency Team Model Demonstrates Wisdom of Loved Ones

Patrick Brady, MD, MSc

PUBLISHED DEC. 14, 2014
BMJ Quality and Safety

Every children's hospital could benefit from a family-activated Medical Emergency Team, (MET) based on results of a seven-year study showing that parents and family members of hospitalized children are capable of identifying complications and medical emergencies that require immediate intervention.

The 4.5-year study of the MET program at Cincinnati Children's found that parents did not overuse a system that empowers them to notify an on-site, multi-disciplinary team if they feel their child's condition is worsening (high fever, breathing difficulties, worsening abdominal pain) or if they feel hospital staff are not responding to their concerns. Parents made an average of 1.2 calls a month (2.9 percent of the total) to the hospital's MET team; 24 percent of which resulted in children being moved to the intensive care unit (ICU). Staff-initiated calls (97 percent of the total) to the MET resulted in 60 percent of children being transferred to ICU.

The study, published Dec. 14, 2014, in *BMJ Quality and Safety*, is accompanied by an editorial by a British father whose son's infection-caused death shortly after birth was linked to the UK hospital staff's inattentiveness to the parents' concerns. He advocates the formation of MET teams in pediatric hospitals worldwide because of the study's positive findings.

Cincinnati Children's adopted the MET program in 2007, supported by in-room posters that inform parents when, why and how to active the team.

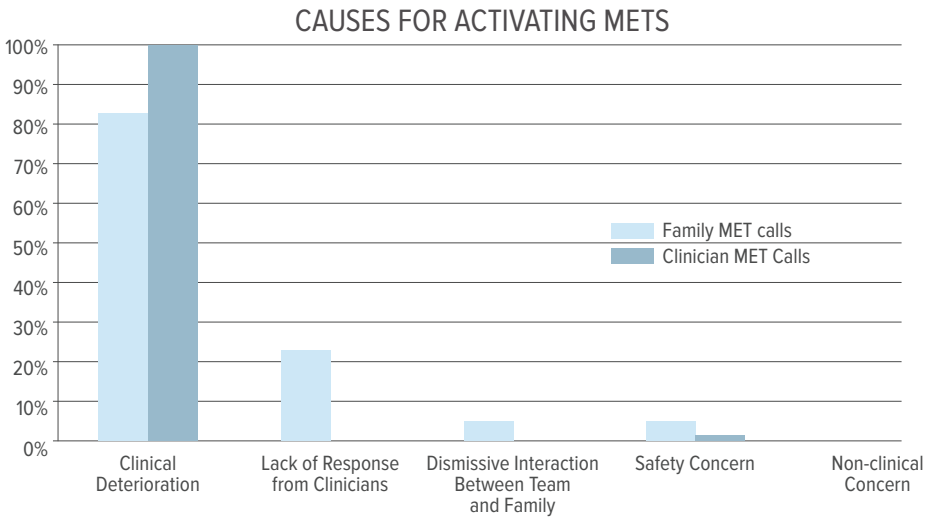
Lead author Patrick Brady, MD, MSc, attending physician with the Division of Hospital Medicine, says the study clearly shows some patient needs would have been missed without family-initiated alerts. He urges hospitals to devise their own MET strategies to leverage family expertise.

"Given the growing evidence of modest cost and potential benefits, we advocate for testing and adaptation of family-activated METs in all contexts," Brady says.

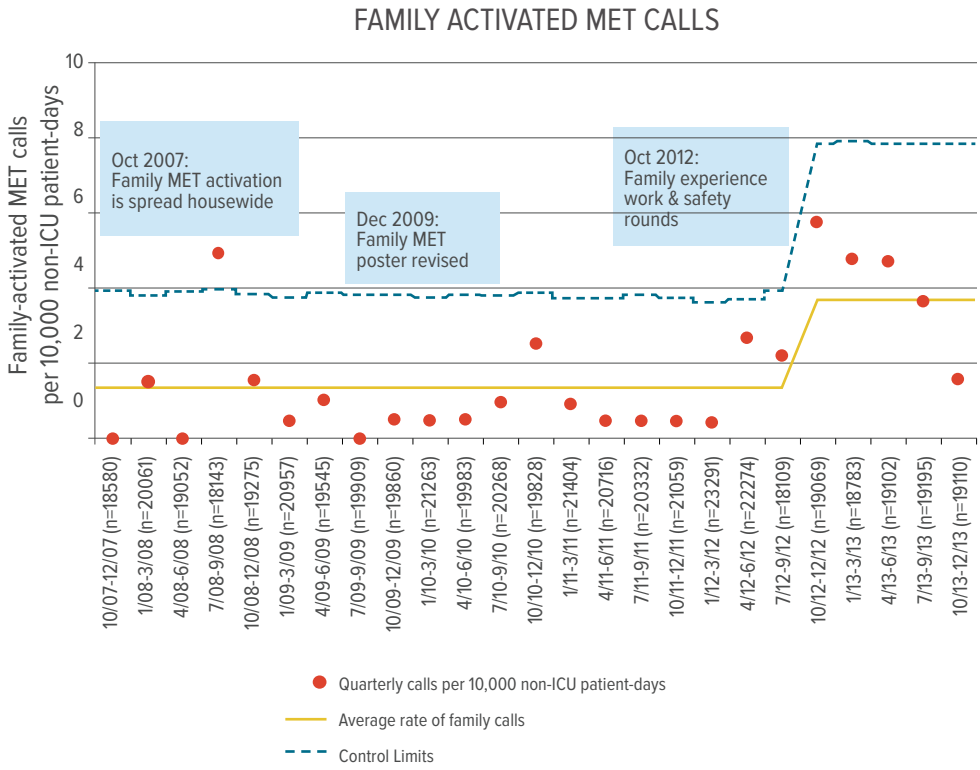
RESEARCH AND TRAINING DETAILS

Faculty	31
Joint Appointment Faculty	9
Support Personnel	14
Direct Annual Grant Support	$742,464
Direct Annual Industry Support	$14,017
Peer Reviewed Publications	122

Brady PW, Zix J, Brilli R, Wheeler DS, Griffith K, Giaccone MJ, Dressman K, Kotagal U, Muething S, Tegtmeyer K. Developing and evaluating the success of a family activated medical emergency team: a quality improvement report. *BMJ Qual Saf*. 2015;24(3):203-211.

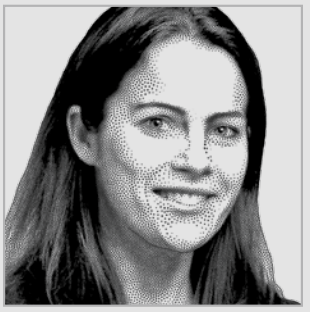


This chart shows the reasons why medical emergency teams (METs) were activated. While clinicians always activated METs to respond to clinical deterioration, families activated METs for multiple reasons, including lack of response from clinicians (23% of calls) and dismissive interactions between care teams and families (5% of calls).



This chart shows family-activated MET calls over the study period. The changes shown in 2012 reflect increased safety rounds conducted by unit leaders, the addition of a family advocate to daily huddles, and improved detection and mitigation of threats to family experience.

Dysfunctional Ribosome Gene Linked to Rare Craniofacial and Limb Abnormalities



K. Nicole Weaver, MD

RESEARCH AND TRAINING DETAILS

Faculty	27
Joint Appointment Faculty	5
Research Fellows	4
Research Students	3
Support Personnel	135
Direct Annual Grant Support	$2.9M
Direct Annual Industry Support	$544,845
Peer Reviewed Publications	72

Weaver KN, Watt KE, Hufnagel RB, Navajas Acedo J, Linscott LL, Sund KL, Bender PL, Konig R, Lourenco CM, Hehr U, Hopkin RJ, Lohmann DR, Trainor PA, Wieczorek D, Saal HM. Acrofacial Dysostosis, Cincinnati Type, a Mandibulofacial Dysostosis Syndrome with Limb Anomalies, Is Caused by POLR1A Dysfunction. *Am J Hum Genet.* 2015;96(5):765-774.

PUBLISHED APRIL 23, 2015
American Journal of Human Genetics

A Cincinnati geneticist's exploration of rare cranioskeletal malformations and abnormal limbs in three patients worldwide has led to the discovery of a dysfunctional gene as the culprit and a name for the syndrome — acrofacial dysostosis, Cincinnati Type.

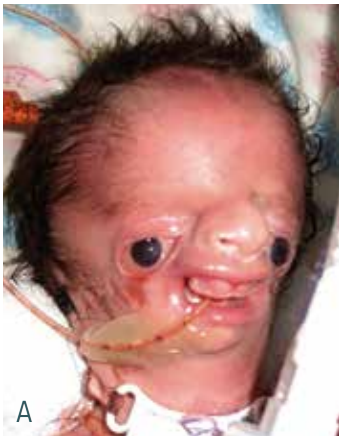
K. Nicole Weaver, MD, a geneticist with the Division of Human Genetics, said the severity of a Cincinnati patient's craniofacial abnormalities and discovery of a suspicious gene led her on a worldwide search for answers for the child's family. A German colleague scoured a large database of patients with undiagnosed craniofacial anomalies and identified two additional patients with a defective copy of the same gene, POLR1A, which is involved in ribosome biogenesis. Ribosomes play an essential role in the process of synthesizing proteins. A Missouri genetics colleague studied zebrafish with absent POLR1A expression, and the fish developed skull, facial, jaw and limb abnormalities similar to those in the children.

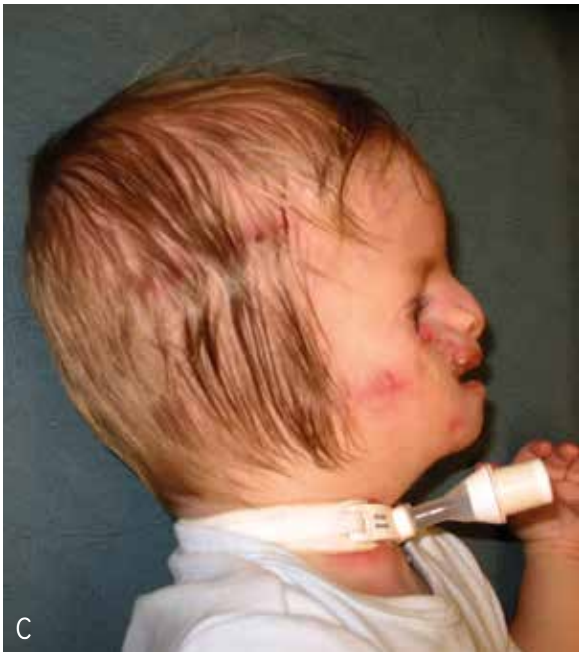
Discovering similar cranioskeletal abnormalities in zebrafish lacking expression of POLR1A provided "pretty strong evidence that dysfunction of this gene could cause these problems in a human," says Weaver, whose findings were published online April 23, 2015, in the *American Journal of Human Genetics*.

The defective POLR1A gene, the team found, resulted in a deficiency of neural-crest-derived skeletal precursor cells that led to the craniofacial anomalies.

"It's unclear why the dysfunction of this ribosome gene gene affects only certain parts of the body," she says. Follow-up research will try to reproduce the anomalies in mice as a way to learn more about the role of ribosome malfunction in human development.

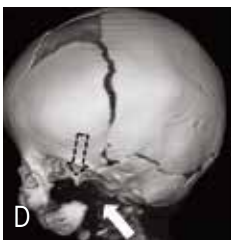
"For this patient, it was really important to be able to tell the family why this abnormality happened, that it wasn't inherited and that it likely would not happen again in another child," Weaver says. "And the patient is doing really, really well."

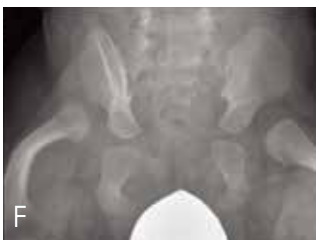




Individuals with acrofacial dysostosis, Cincinnati type, each have a heterozygous mutation in POLR1A, which encodes a core component of RNA polymerase 1. These images of an affected newborn show: (A) extensive craniofacial malformations at birth; (B and C) images taken at age 18 months after multiple reconstructive surgeries; (D) severe maxillary and zygomatic hypoplasia (black open-dashed arrow) and severe micrognathia and retrognathia (white block arrow); (E) severe microtia with absent pinnae (white arrows), external auditory atresia (white open-dashed arrows), and severe middle-ear hypoplasia and ossicular dysplasia (black open arrows); and (F) bilateral hip dysplasia and anterior bowing deformity of the femurs.

"It was really important to be able to tell the family why this abnormality happened, that it wasn't inherited and that it likely would not happen again in another child."





Targeting MicroRNA Emerges as Potential Weapon Against Acute Myelogenous Leukemia

H. Leighton Grimes, PhD

RESEARCH AND TRAINING DETAILS

Faculty	19
Research Fellows	18
Research Students	19
Support Personnel	29
Direct Annual Grant Support	$3.8M
Direct Annual Industry Support	$78,897
Peer Reviewed Publications	49

Velu CS, Chaubey A, Phelan JD, Horman SR, Wunderlich M, Guzman ML, Jegga AG, Zeleznik-le NJ, Chen J, Mulloy JC, Cancelas JA, Jordan CT, Aronow BJ, Marcucci G, Bhat B, Gebelein B, Grimes HL. Therapeutic antagonists of microRNAs deplete leukemia-initiating cell activity. *J Clin Invest.* 2014 Jan 2;124(1):222-36.

PUBLISHED JAN. 2, 2014
Journal of Clinical Investigation

When acute myelogenous leukemia (AML) strikes, five-year survival rates vary dramatically (15 percent-70 percent) depending upon the AML subtype. Children diagnosed with AMLs with 11q23 translocations have experienced especially poor outcomes.

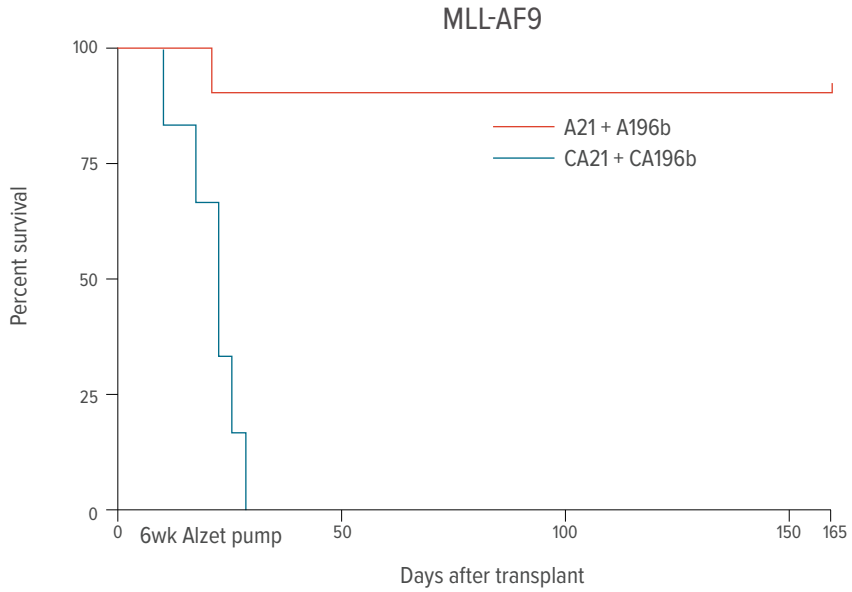
However, a new approach based on silencing targeted microRNAs that was developed by a Cincinnati Children's research team led by H. Leighton Grimes, PhD, has shown intriguing early success in mouse models. MicroRNAs have long been thought to play an important role in oncogenesis, but so far, converting this concept into therapeutics has been slow. Grimes and colleagues found one potential solution by exploiting an ancient competition between GFI1 and HOX transcription factors, which both act as leukemia-initiating "gatekeeper" pathways.

As the team studied the competing transcription factors, they found two microRNA that appeared to play important roles in AML relapse. The team went on to test antagomir treatment as a tool to silence the targeted microRNA.

"Therapeutic inhibition of microRNA-21 and microRNA-196b inhibited *in vitro* leukemic colony forming activity and depleted *in vivo* leukemia-initiating cell activity of HOX-based leukemias, which led to leukemia-free survival in a murine AML model and delayed disease onset in xenograft models," Grimes and co-authors wrote.

The study establishes microRNA as functional effectors of endogenous HOXA9 and HOX-based leukemia oncoproteins. At one level, this means the research world now has a mouse model that can serve effectively as an *in vivo* platform to test RNA-based cancer therapies. At another level, it means children who develop notoriously hard-to-treat forms of AML may have increased hope for longer-term survival.

"Overall, our studies provide a strong rationale to develop microRNA antagonists for clinical use in AML," Grimes says.



This figure shows the Kaplan-Meier survival curve of partially conditioned mice transplanted with one million leukemic splenocytes. Four days later, six-week osmotic pumps containing active A21+A196b or control CA21+CA196b anti-microRNA therapy were implanted. Treated mice were analyzed by flow cytometry for CD45.1 versus CD45.2 to identify leukemic cells at time of death (CA21+CA196b) or at the termination of the experiment at 165 days (A21+A196b).

At one level, this means the research world now has a mouse model that can serve effectively as an *in vivo* platform to test RNA-based cancer therapies. At another level, it means children who develop notoriously hard-to-treat forms of AML may have increased hope for longer-term survival.

Mother's Own Immune System May Cause Pregnancy Complications

Sing Sing Way, MD, PhD

Vandana Chaturvedi, PhD

RESEARCH AND TRAINING DETAILS

Faculty	19
Joint Appointment Faculty	6
Research Fellows	2
Research Students	4
Support Personnel	82
Direct Annual Grant Support	$8.9M
Direct Annual Industry Support	$1,748,213
Peer Reviewed Publications	59

Chaturvedi V, Ertelt JM, Jiang TT, Kinder JM, Xin L, Owens KJ, Jones HN, Way SS. CXCR3 blockade protects against Listeria monocytogenes infection-induced fetal wastage. *J Clin Invest.* 2015;125(4):1713-1725.

PUBLISHED MARCH 9, 2015
The Journal of Clinical Investigation

Redirecting an expectant mother's immune cells to prevent them from attacking the fetus might reduce complications such as stillbirth and prematurity, according to a study that identifies a cell traffic pathway that plays a critical role in the process.

The causes of premature birth and many other pregnancy complications are not completely known, but maternal infection is an important contributor, says senior author Sing Sing Way, MD, PhD, Division of Infectious Diseases. When infection strikes, maternal immune cells can "overreact" and attack the placenta as if it were a foreign invader.

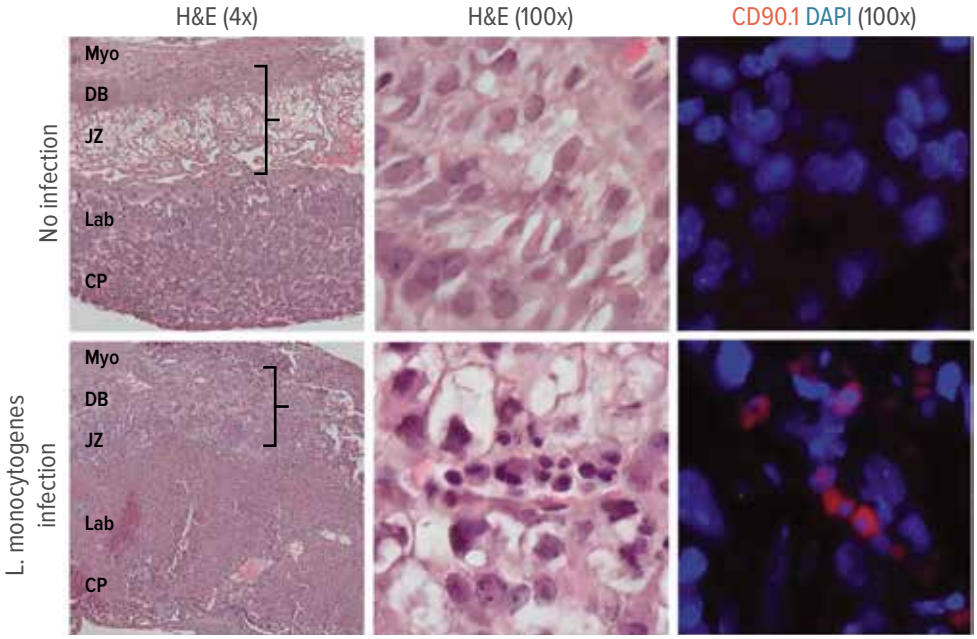
Way's study, published March 9, 2015, in *The Journal of Clinical Investigation*, identifies a pathway that could reduce these harmful overreactions.

"It might seem counterintuitive to prevent maternal immune cells from optimally penetrating into tissues," says Way. "But restricting harmful immune cells' access to developmentally delicate placental tissue represents a highly innovative therapeutic strategy."

The research team, led by Way and first author Vandana Chaturvedi, PhD, infected pregnant mice with *Listeria monocytogenes*, a bacterium commonly found in food supplies that causes invasive infection in pregnant women. It is often fatal to the fetus. Mice and human share this susceptibility.

The researchers found that specialized subsets of first-responder immune cells — neutrophils and macrophages — rapidly infiltrate the placenta, producing high levels of a chemoattractant protein, CXCL9, which attracts harmful maternal T cells to attack genetically foreign placental-fetal tissue.

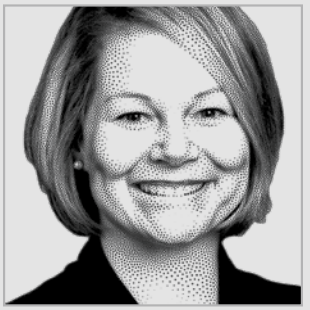
The finding is significant because placental cells are not programmed to express chemoattractant proteins like CXCL9. The team found neutralizing CXCL9 activity by blocking its receptor on T cells efficiently protects against fetal injury after prenatal *Listeria* infections.



Histological analysis of the placentas recovered from female mice during allogeneic pregnancies sired by ovalbumin expressing transgenic male mice showing no infection control compared with *L. monocytogenes* infection after H&E staining, along with anti-CD90.1 staining for ovalbumin specific CD8+ T cells (red) and DAPI nuclear immunofluorescence staining (blue). High-magnification fields show placental tissue intersecting the decidua basalis (DB) and junctional zone (JZ). Brackets in the low-magnification fields indicate the source of decidual tissue harvested for analysis by flow cytometry. Myo = myometrium; Lab = labyrinth; CP = chorionic plate.

"Restricting harmful immune cells' access to developmentally delicate placental tissue represents a highly innovative therapeutic strategy."

Frequent Interruptions Most Common Cause of Lapsed Nursing Care in Neonatal ICUs



Heather Tubbs-Cooley, PhD, RN

RESEARCH AND TRAINING DETAILS

Faculty	12
Joint Appointment Faculty	15
Direct Annual Grant Support	$8M
Peer Reviewed Publications	52

Tubbs-Cooley HL, Pickler RH, Younger JB, Mark BA. A descriptive study of nurse-reported missed care in neonatal intensive care units. *J Adv Nurs.* 2015;71(4):813-824.

PUBLISHED ONLINE NOV. 27, 2014
Journal of Advanced Nursing

Researchers examining nursing care lapses in neonatal intensive care units (NICUs), including missed rounds, found that the most commonly cited reasons were frequent interruptions and urgent situations involving other patients.

The lapses themselves were wide-ranging, including oral care for ventilated infants, educating and involving parents in care, and oral feedings. The least common lapses were hand hygiene, safety and physical assessment, and medication administration.

The study, first published online Nov. 27, 2014, in the *Journal of Advanced Nursing*, achieved significant national attention for calling attention to nursing care quality in NICUs.

The team was led by Heather Tubbs-Cooley, PhD, RN, a faculty member in Research in Patient Services with a secondary appointment with the James M. Anderson Center for Health Systems Excellence. The study focused on the frequency of nurse-reported missed care, and nurses' reports of factors contributing to missed care on their last shift worked. While previous studies highlighted the frequency of missed nursing care in adult settings, there was little or no such information on incidents in NICUs.

Using a cross-sectional web-based survey, researchers took a random sample of certified neonatal ICU nurses in seven states. Descriptive statistics constituted the primary analytic approach. The team concluded that system factors might have contributed to missed care. The most frequent reasons nurses cited were: frequent interruptions (73 percent), urgent patient situations (66 percent) and an unexpected rise in patient volume and/or acuity on the unit (61 percent).

Approximately half of respondents reported that an inadequate number of nurses and missing equipment/supplies were reasons for missed care.

Tubbs-Cooley and colleagues are analyzing data from a separate longitudinal study in one NICU to examine relationships between nurse workload, specific instances of missed care, and the occurrence of adverse events.

The neonatal intensive care unit (NICU) is home to extremely delicate lives. In caring for these patients, nurses are frequently interrupted, often to tend to other patients. In a study published in the *Journal of Advanced Nursing* that gained national attention, researchers used a cross-sectional web-based survey involving NICUs in seven states. The survey respondents? Nurses themselves.

Approximately half of respondents reported that an inadequate number of nurses and missing equipment/supplies were reasons for missed care.

Mayerson Collaborations Drive Insights Into Childhood Adversity

Robert Shapiro, MD

The Mayerson Center for Safe and Healthy Children created a new key collaboration this year with its colleagues in Biostatistics and Epidemiology to co-explore the effects of — and solutions to — childhood adversity.

"As a division, we are most excited about the research focus we have developed and are expanding that revolves around childhood adversity and toxic stress," says Robert Shapiro, MD, Director of the center's Child Abuse Team.

Living in an adverse, unsafe, or unstable environment can lead to the release of stress hormones throughout the body and developing brain of a child. If a child does not have the resources to effectively cope with this stress, the prolonged release of these hormones can have a toxic impact.

This "toxic stress" can cause harmful physiological changes and damage to the regions of the brain essential for memory, learning, and behavior. These changes can alter a child's capacity to learn and reason, to develop healthy attachments and behaviors, and to navigate social relationships.

A child may also adopt risky behaviors to help them cope with the stress that may compromise their health. Childhood adversity has been strongly associated with an extensive list of health problems in adulthood.

The Mayerson Center is teaming up with Biostatistics and Epidemiology to design and conduct studies examining the biological processes of how childhood adversity disrupts normal brain development, the impact of parental adverse childhood experiences on child development, and the prevention, detection, and early intervention for adversity in families.

The Mayerson Center has partnered with local pediatricians to begin adversity screening and brief interventions in primary care practices to identify and address social risk factors in families. The Center also has created a new community-wide initiative called Joining Forces for Children to build a public health response system to childhood adversity.

RESEARCH AND TRAINING DETAILS

Faculty	3
Joint Appointment Faculty	9
Research Students	1
Support Personnel	7
Direct Annual Grant Support	$415,593
Peer Reviewed Publications	4

Pinwheels symbolize healthy and happy childhoods. The Mayerson Center is a leader in drawing attention to child-abuse treatment and prevention, including events such as this display in Cincinnati. Each pinwheel represents a case of reported child abuse in Hamilton County that year. that year.

Insights into Cell Growth, Cell Death Offer New Pathways to Treat Kidney Disease

Elif Erkan, MD, MS

RESEARCH AND TRAINING DETAILS

Faculty	15
Joint Appointment Faculty	1
Research Students	8
Support Personnel	9
Direct Annual Grant Support	$1.5M
Direct Annual Industry Support	$120,539
Peer Reviewed Publications	53

Koral K, Li H, Ganesh N, Birnbaum MJ, Hallows KR, Erkan E. Akt recruits Dab2 to albumin endocytosis in the proximal tubule. *Am J Physiol Renal Physiol*. 2014;307(12):F1380-1389.

PUBLISHED ONLINE SEPT. 24, 2014
American Journal of Physiology - Renal Physiology

One of the challenges for kidney specialists is protecting the kidney from too much protein, which can contribute to progressive damage that leads to end-stage renal disease. Proximal tubule epithelial cells internalize albumin by receptor-mediated endocytosis and undergo apoptosis – programmed cell death – when exposed to too much albumin, the primary protein in the filtrate.

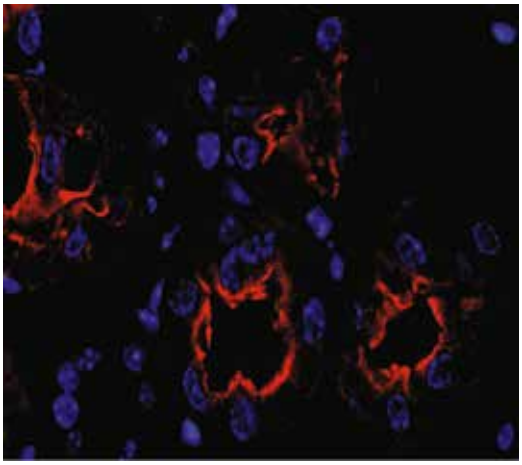
A team led by Elif Erkan, MD, MS, in the Division of Nephrology, has identified an interaction between receptors and proteins in the proximal tubule that support albumin endocytosis and cell survival. The link involves protein kinase B (Akt), a pivotal protein involved in cell survival, megalin-cubilin complex receptor and the endocytic adaptor disabled-2 (Dab2). Detailed findings appeared online Sept. 24, 2014, in the *American Journal of Physiology - Renal Physiology*.

Specifically, Erkan's team found that both Akt1 and Akt2 are involved in mediating albumin endocytosis in proximal tubule epithelial cells, and that Akt phosphorylates Dab2. In Akt1 and Akt2 knock-out mice, the location of Dab2 is shifted from the cell membrane to the perinuclear area of the proximal tubule indicating the role of Akt in trafficking of Dab2.

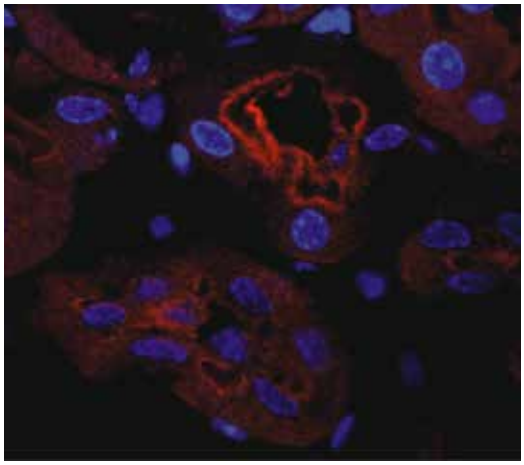
Erkan says the highlight of the work is the discovery of the link between the endocytic pathway and cell survival/cell death. "Because Akt mediates albumin endocytosis, its expression is down-regulated in tubular epithelial cells in kidney disease, leading to apoptosis" she says. "If we can figure out a way to up-regulate Akt in tubular epithelial cells, perhaps we can promote cell survival and prevent progression in glomerular diseases."

Erkan and her team are continuing to study the link between Akt and Dab2 in mice. Additional research may identify other potential targets or additional regulating roles for Akt in a search for novel pharmaceutical agents to reduce kidney damage.

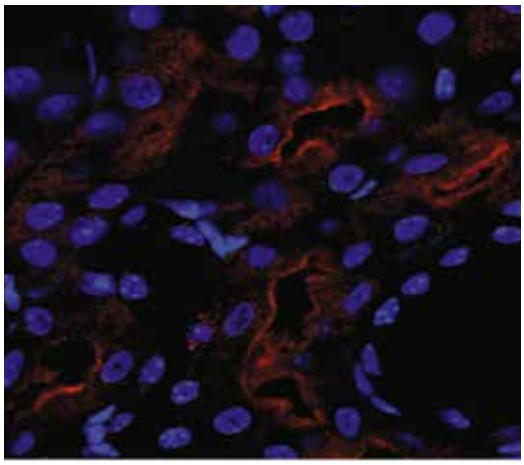
WT



AKT1KO



AKT2KO



Dab2 expression was examined in WT, Akt1 KO and Akt2 KO mice kidneys. Apical location of Dab2 was prominent in WT mouse proximal tubule cells in parallel with its function as an adaptor protein harboring receptor-mediated endocytosis. There was a decrease in apical location of Dab2 in Akt1 and Akt2 KO mice proximal tubule cells.

Up-regulating Akt in tubular epithelial cells may promote cell survival and prevent progression in glomerular diseases.

Variable Adherence Has Significant Impact on Seizure Outcomes

Avani Modi, PhD

Tracy Glauser, MD

RESEARCH AND TRAINING DETAILS

Faculty	43
Joint Appointment Faculty	3
Research Fellows	5
Research Students	45
Support Personnel	35
Direct Annual Grant Support	$3.6M
Direct Annual Industry Support	$1,818,692
Peer Reviewed Publications	105

Modi AC, Wu YP, Rausch JR, Peugh JL, Glauser TA. Antiepileptic drug nonadherence predicts pediatric epilepsy seizure outcomes. *Neurology.* 2014;83(22):2085-2090.

PUBLISHED ONLINE OCT. 29, 2014
Neurology

A two-year collaborative study provides solid scientific backing to the age-old parents' admonition: "It's important to take your medicine."

Avani Modi, PhD, Director of the Center for Adherence and Co-Director of the New Onset Seizure Clinic, led a research team including colleagues from the divisions of Neurology and Behavioral Medicine and Clinical Psychology, who tracked drug adherence and seizure outcomes in children with epilepsy for two years. Findings appeared online Oct. 29, 2014, in *Neurology*.

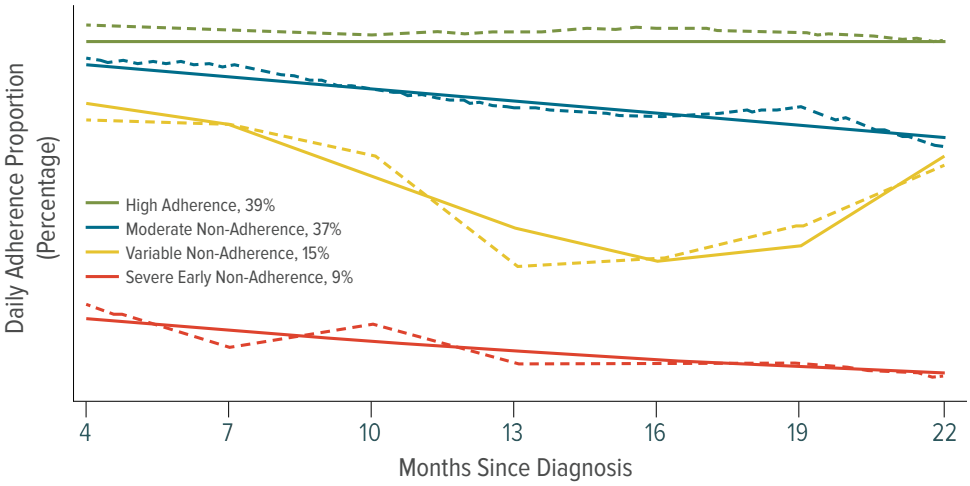
The study used electronic monitoring devices to track adherence patterns and seizure patterns in 109 children. The researchers found that patients fell into four distinct adherence groups: severe early non-adherence (9 percent), variable non-adherence (15 percent), moderate non-adherence (37 percent) and high adherence (39 percent). Children with epilepsy also fell into two distinct seizure groups: high or low seizure probability. Overall, children whose adherence patterns changed significantly had the worse seizure outcomes.

Children in the variable non-adherence group were more likely to be in the high seizure probability group, even after accounting for important medical characteristics such as seizure type and brain abnormalities. Their drug adherence started at 71 percent, dropped to 32 percent and then improved to 58 percent by the end of the study — a variability rate that put them at high risk for ongoing seizures.

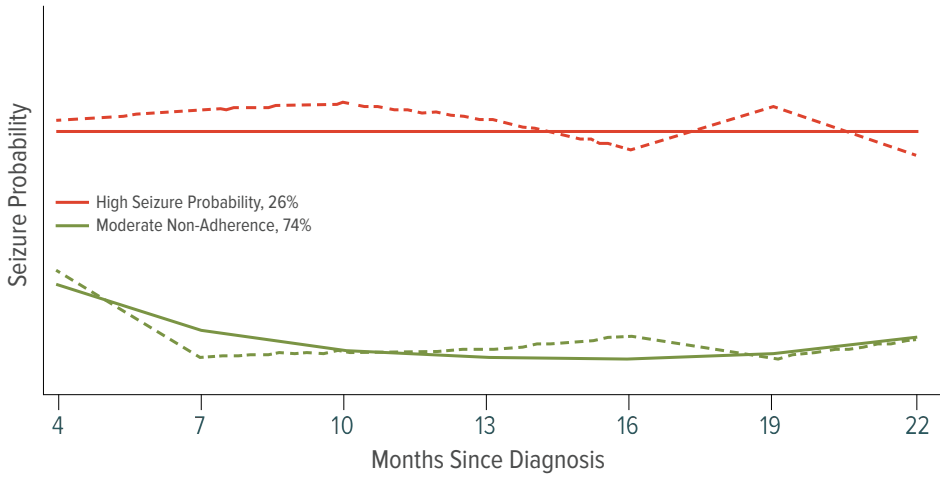
"What was previously unrecognized," Modi says, "is that monitoring and addressing drug adherence is a highly important, modifiable variable that can impact long-term seizure outcome."

This new understanding of the role of drug adherence "empowers the family to really contribute to the child's outcome in a way that no one previously understood," says co-author Tracy Glauser, MD, Director of the Comprehensive Epilepsy Center at Cincinnati Children's.

"Now," Modi says, "we can say with more confidence to families, 'Our job is to give you the best medications and treatments for your child's particular epilepsy diagnosis, and your job is to take the medications.' "

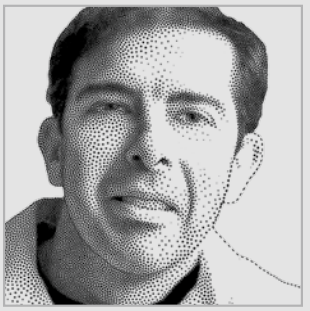


These figures show four distinct adherence patterns among children with pediatric epilepsy (shown here) and two distinct seizure patterns. Researchers at Cincinnati Children's have found that children who have Variable Non-Adherence are more likely to have a High Seizure Probability. These data demonstrate that the relationship between non-adherence and seizures is not linear.



This new understanding of the role of drug adherence empowers the family to really contribute to the child's outcome in a way that no one previously understood.

Novel Surgical Protocol Helps Eliminate Seizures for Some Children With TSC-Related Epilepsy



Francesco Mangano, DO, FACS

RESEARCH AND TRAINING DETAILS

Faculty	7
Joint Appointment Faculty	4
Research Fellows	1
Research Students	2
Support Personnel	11
Direct Annual Industry Support	$292,544
Peer Reviewed Publications	20

Arya R, Tenney JR, Horn PS, Greiner HM, Holland KD, Leach JL, Gelfand MJ, Rozhkov L, Fujiwara H, Rose DF, Franz DN, Mangano FT. Long-term outcomes of resective epilepsy surgery after invasive presurgical evaluation in children with tuberous sclerosis complex and bilateral multiple lesions. *J Neurosurg Pediatr.* 2015;15(1):26-33.

PUBLISHED JANUARY 2015
Journal of Neurosurgery: Pediatrics

Some children with a form of epilepsy that previously made them poor candidates for surgery are now able to live without seizures, experience drastically reduced seizure episodes and symptoms, or take fewer anti-seizure medications as the result of a novel pre-surgical evaluation protocol developed by researchers in the Pediatric Epilepsy Surgery Program at Cincinnati Children's.

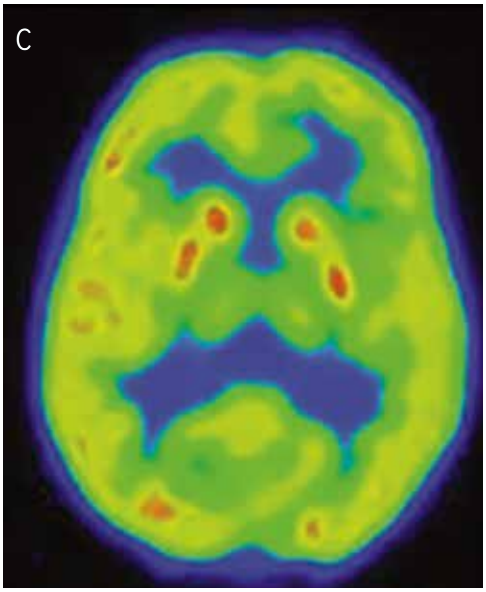
A research team led by Francesco Mangano, DO, FACS, FACOS, Chief of the Division of Pediatric Neurosurgery, studied 37 children who developed epilepsy as a result of tuberous sclerosis complex (TSC), a genetic disorder in which non-malignant tumors form in different organs, including the brain. More than 80 percent of children with TSC develop epilepsy that involves multi-focal brain abnormalities that vary from child to child. Their epilepsy symptoms vary as well.

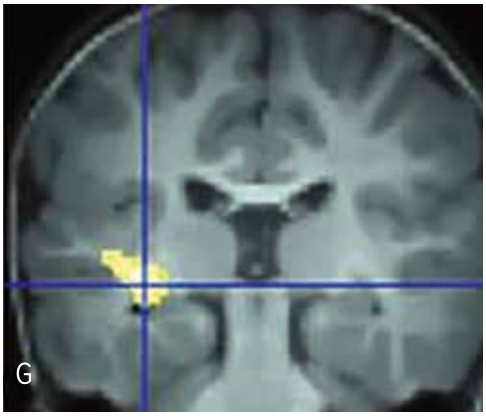
The study, thought to be the largest single-center study in this pediatric epilepsy population, appeared in the January 2015 issue of the *Journal of Neurosurgery: Pediatrics*.

Pre-surgery evaluations relied on non-invasive and invasive brain mapping to identify the origin of seizure patterns in each patient. Neurosurgeons then decided which types of resective surgery to pursue — craniotomy to remove tuberous tissue, lobar resections, and even hemispherectomy in some cases.

After five years, 56 percent of the children were seizure-free, and 87 percent had far fewer seizures and significantly less severe seizures, based on a scale developed by the International League Against Epilepsy (ILAE).

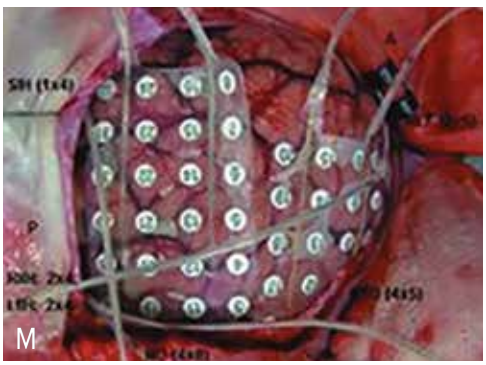
"In those children who were not seizure-free, we were able to decrease the number of anti-epileptic drugs needed to continue to control their disease, and we were able to reduce their medication frequencies and doses to improve their side effect profiles," Mangano says.





Neurosurgeons at Cincinnati Children's employed a novel surgical protocol to help a 5-year-old girl with tuberous sclerosis complex (TSC) who developed multiple types of seizures. At the time of surgical referral, four anti-seizure medications had failed and she had mild global delay. Among several diagnostic scans, FDG-PET showed multiple focal areas of decreased metabolism (image C). SPM imaging revealed a prominent area of hypometabolism involving the left frontal, temporal, and anterior parietal lobes (image G). Subdural grids and interhemispheric strips were placed for invasive monitoring (Image M) in preparation for a right occipital lobectomy. At the two-year follow-up, the child was completely seizure-free.

More than 80 percent of children with TSC develop epilepsy involving multi-focal brain abnormalities that vary from child to child. Their epilepsy symptoms vary as well.



Drosophila Research Leads to Light-Bulb Moment About Body Temperature Regulation

Fumika Hamada, PhD

RESEARCH AND TRAINING DETAILS

Faculty	13
Research Fellows	7
Research Students	6
Support Personnel	14
Direct Annual Grant Support	$832,188
Peer Reviewed Publications	16

Head LM, Tang X, Hayley SE, Goda T, Umezaki Y, Chang EC, Leslie JR, Fujiwara M, Garrity PA, Hamada FN. The influence of light on temperature preference in *Drosophila*. *Curr Biol*. 2015;25(8):1063-1068.

PUBLISHED APRIL 20, 2015
Current Biology

Researchers have long known that human body temperature rises when people are exposed to light during the night. Understanding the molecular mechanisms that cause such temperature changes could advance the study of sleep disorders, seasonal affective disorders and temperature regulation.

A study published in April 2015 in *Current Biology* reports finding important clues about these mechanisms in a most-unusual place: the temperature sensitivities of the tiny, cold-blooded fruit fly, *Drosophila melanogaster*.

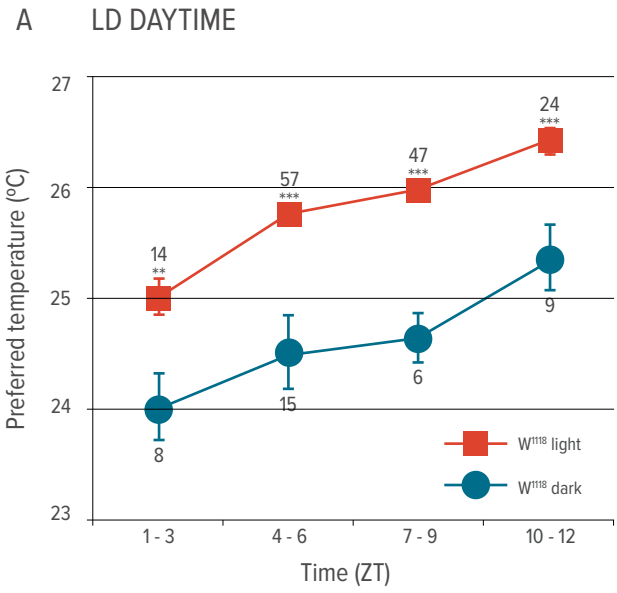
A research team led by Fumika Hamada, PhD, Division of Ophthalmology, found that *Drosophila* exhibit light-dependent temperature preference (LDTP) in which the flies prefer a one-degree higher temperature in light than in dark. Because the flies are cold-blooded, their body temperature is also higher in light.

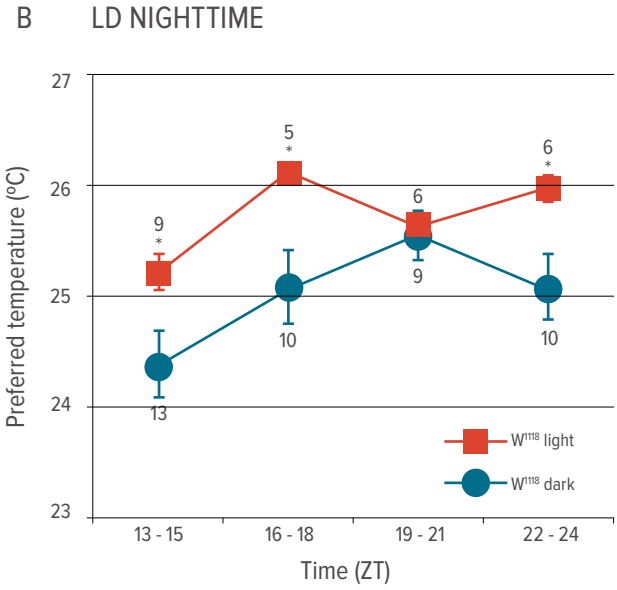
The team uncovered the molecular mechanisms that control LDTP in flies, and theorize that acute light on temperature regulation may be conserved evolutionarily between flies and humans. "Light affects many physiological responses, but the underlying mechanisms of it are unclear," Hamada says.

The team also found a well-known circadian clock molecule — pigment dispersing factor receptor — in subsets of the circadian clock cells that control the flies' LDTP. The result suggests a connection between light and circadian clock neurons. Given that LDTP occurs irrespective of the state of the circadian rhythms, the research provides new insights into how circadian clock mechanisms impact light and temperature regulation.

Heat generation is different between humans and flies, but the light/temperature connection has similarities. Eventually, this line of research could benefit people who work night shifts and are exposed to light in nighttime.

"Long-term sleep deprivation may increase the risk of obesity, diabetes and cardiovascular disease," Hamada says. "This evening light exposure increases body temperature, which causes abnormal circadian rhythmicity."



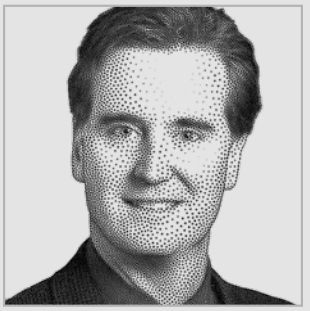


The two figures here show how acute light positively influences temperature preference in cold-blooded *Drosophila*, commonly known as fruit flies. Understanding these elusive molecular mechanisms could one day advance how light affects sleeping humans, leading to improved treatment of sleep disorders.

Chart A (top) compares preferred temperature between light and dark conditions for w1118 flies during the daytime.

Chart B (bottom) does the same for nighttime. The w1118 flies were raised in alternating 12-hour cycles of light and dark. Ambient light was either on or off when the behavioral experiments were performed for 30 minutes.

Orthopaedic Surgeons Help Form ROCK Group to Battle Degenerative Knee Condition in Adolescents



Eric Wall, MD

RESEARCH AND TRAINING DETAILS

Faculty	15
Research Fellows	2
Research Students	5
Support Personnel	7
Direct Annual Grant Support	$87,950
Direct Annual Industry Support	$21,961
Peer Reviewed Publications	40

Wall EJ, Polousky JD, Shea KG, Carey JL, Ganley TJ, Grimm NL, Jacobs JC, Jr., Edmonds EW, Eismann EA, Anderson AF, Heyworth BE, Lyon R, Research on OsteoChondritis Dissecans of the Knee Study G. Novel radiographic feature classification of knee osteochondritis dissecans: a multicenter reliability study. *Am. J. Sports Med*. 2015;43(2):303-309.

PUBLISHED FEBRUARY 2015
The American Journal of Sports Medicine

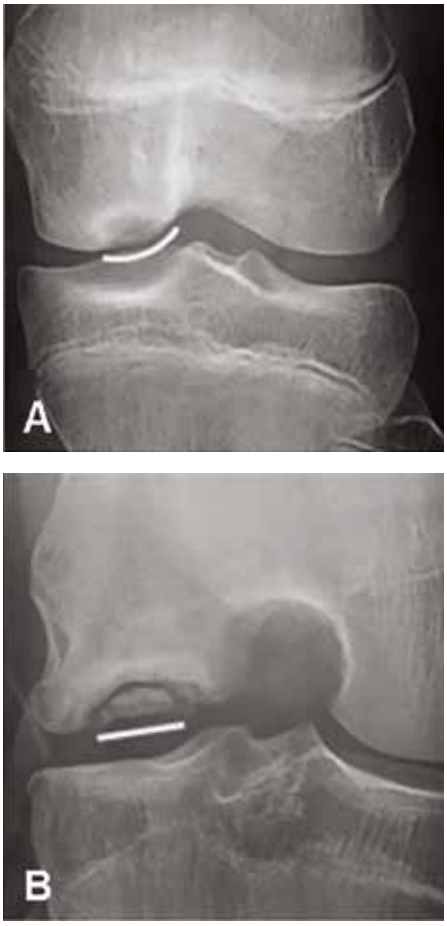
Pediatric orthopaedic surgeons around the country remain baffled by a rare condition called osteochondritis dissecans (OCD), a degenerative cartilage disorder that strikes the knees of active, athletic teenagers. The surgeons teleconference frequently and meet several times a year to share information about their rare cases to better understand a condition with no known cause, and no proven best treatment.

Their collaborative team, Research in OsteoChondritis of the Knee (ROCK) Group, has cleared one hurdle by reaching baseline agreement on the key anatomical features that mark the condition. Results of the team's first Cincinnati-led, multi-center study appeared in the February 2015 issue of *The American Journal of Sports Medicine*.

In the ROCK study, seven orthopaedic specialists rated X-rays of 45 different knees for nine or more specific OCD characteristics. The study showed highly reliable agreement among the doctors on features such as growth plate maturity, condylar width and lesion size, and the ability to differentiate medial and lateral lesions in the knee. Other characteristics were less reliable for identifying OCD.

"We all hate osteochondritis dissecans because it's so nebulous and so debilitating," according to Eric Wall, MD, study coordinator and Director of Orthopaedic Sports Medicine. Aided by new data, doctors will now focus on how specific OCD features correlate to treatment outcomes, Wall says.

With treatment failure rates of 30 percent, some OCD-diagnosed adolescents must drastically reduce physical activities for up to two years as cartilage recovers, and others can develop early onset degenerative arthritis. Affected knee tissue, Wall says, "looks like a Cincinnati road after a hard winter, with a big deep pothole in the knee cartilage. But asking a 10-year-old to rest for up to two years — that's a lot of time out of a young life. Our goal is to cure it in the next decade."



These knee images show the articular side of osteochondritis dissecans (OCD) lesions with predominantly (A) convex, (B) linear, or (C) concave contours. The images were part of a multi-center study reporting that certain radiographic features can be reliably classified by multiple observers. This will allow for the determination of predictors of OCD healing with non-operative or operative treatment.

"We all hate osteochondritis dissecans because it's so nebulous and so debilitating."

Evidence-Based Decision Making Found Lacking in Sleep Apnea Management

Stacey Ishman, MD, MPH

Most care decisions for the management of obstructive sleep apnea (OSA) in children are based on widely varying clinical experience rather than being evidence-based, including when to refer patients for subspecialty clinic follow-up and when to order follow-up overnight-sleep studies, according to research led by members of the Division of Otolaryngology.

The research team presented new findings from an ongoing research effort at the Triological Society Combined Sections Meeting in San Diego in January 2015. One article based on the presented findings has been accepted for publication in *The Laryngoscope*, while another has been submitted to the journal *Otolaryngology — Head & Neck Surgery*.

In the initial, smaller study, Stacey Ishman, MD, MPH, led a team that examined 324 decisions on 58 patients made at clinics and care conferences over a one-week period. Subspecialists explained the basis of their decisions, which were then classified into 10 categories. The findings: only 34 percent of decisions were evidence-based, while 59 percent were non-evidence-based and 7 percent were based on parental preference. Providers were able to cite specific studies for less than 20 percent of their decisions.

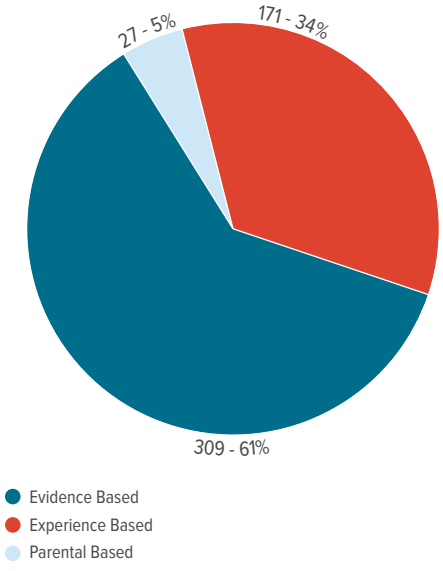
In a companion study for *The Laryngoscope*, the team analyzed these gaps over a two-month period. That study of 507 decisions found that the proportion of non-evidence-based decisions actually increased two percentage points from the shorter study, while parental-based decisions dropped by two percentage points.

The most common non-evidence-based decisions analyzed included the timing and appropriate subspecialty clinic follow-up location (38 percent), as well as indications for overnight-sleep studies (11 percent), especially in children at high-risk for persistent OSA such as those with Down syndrome or obesity. Additional gaps included the likelihood of OSA improvement from weight loss and the effectiveness of sleep surgical procedures.

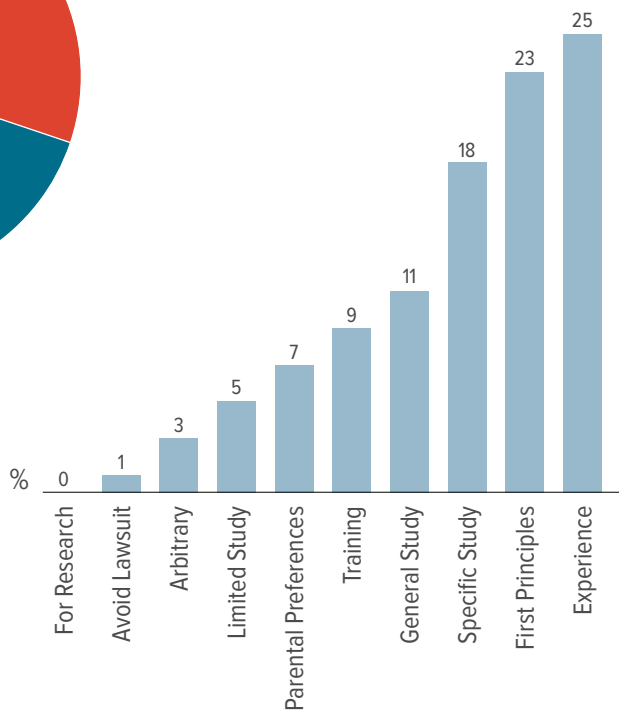
RESEARCH AND TRAINING DETAILS

Faculty	11
Joint Appointment Faculty	3
Research Students	2
Support Personnel	50
Peer Reviewed Publications	27

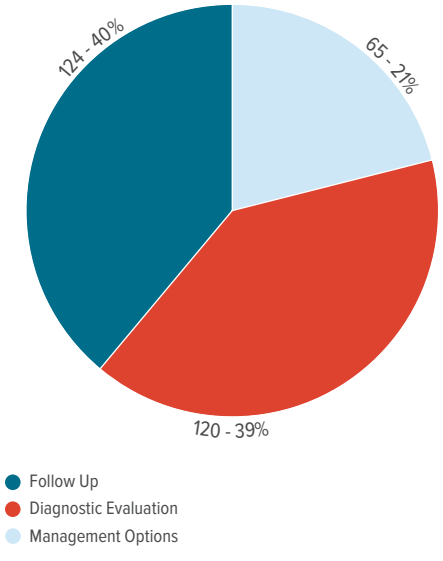
Decision-making by category



Factors that guided decisions



Nature of non-evidence-based decisions



In examining decisions by subspecialists treating children with obstructive sleep apnea (OSA), researchers found that most decisions were not evidence-based (top) and that physician experience was the most common factor in decision-making (above). The team analyzed the kinds of decisions that were not evidence-based (bottom) and learned that the overwhelming majority regarded follow-up options and diagnostic evaluations such as overnight sleep studies.

Glycocholic Acid Proves Effective Against Newly Identified Amidation Defect

Kenneth Setchell, PhD

James Heubi, MD

RESEARCH AND TRAINING DETAILS

Faculty	23
Research Fellows	1
Direct Annual Grant Support	$920,126
Direct Annual Industry Support	$47,541
Peer Reviewed Publications	55

Heubi JE, Setchell KD, Jha P, Buckley D, Zhang W, Rosenthal P, Potter C, Horslen S, Suskind D. Treatment of bile acid amidation defects with glycocholic acid. *Hepatology.* 2015;61(1):268-274.

PUBLISHED ONLINE DEC. 23, 2014
Hepatology

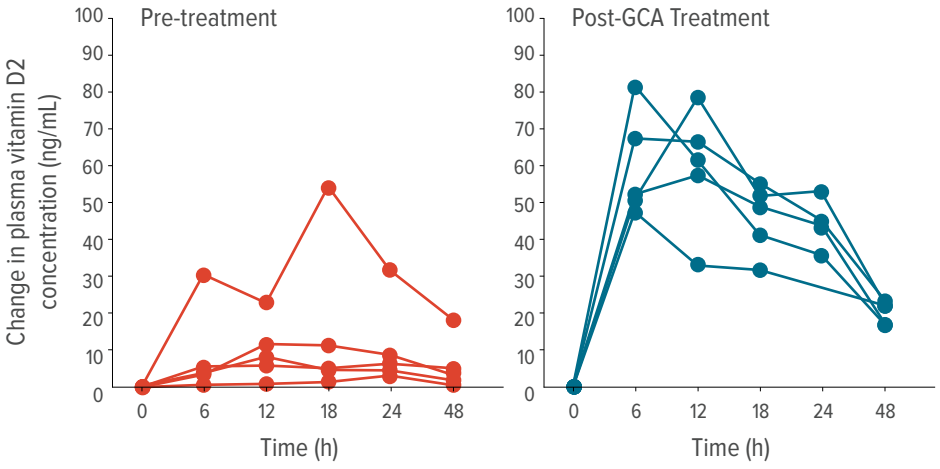
Over the past 30 years, James Heubi, MD, and Kenneth Setchell, PhD, have revolutionized the treatment of liver disease in children, and their novel findings led to the March 2015 FDA approval of the drug Cholbam. This bile acid treatment — when given to children — tricks the liver into thinking it is producing enough of its own healthy bile acid so that it shuts down production of defective bile acids that lead to liver disease.

Now, Heubi and Setchell have identified another liver enzyme irregularity called an amidation defect, in which the liver produces too much unconjugated cholic acid because the amino acids glycine or taurine cannot conjugate it effectively. Normally, cholic acid dissolves fats and helps the body excrete cholesterol.

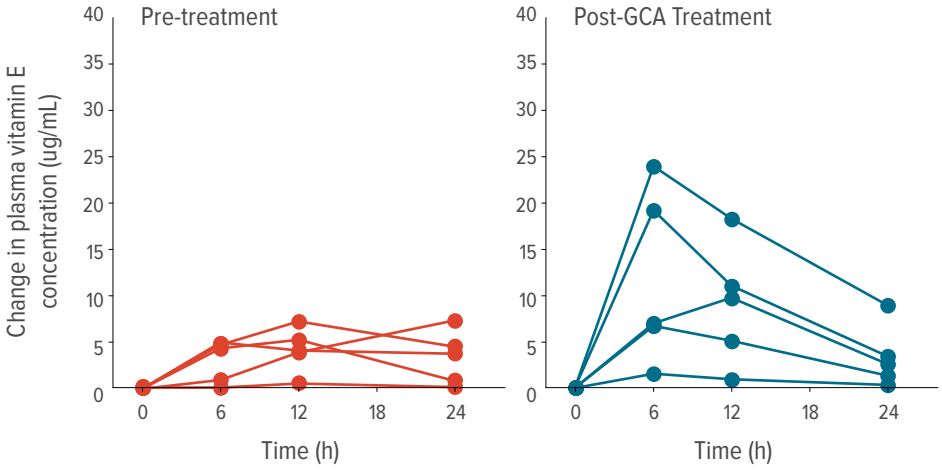
Heubi and Setchell identified five children with the amidation defect, all of whom showed mutations in the BAAT gene and exhibited failure to grow, vitamin absorption deficiencies or choleostasis. After treatment of up to 92 months with glycocholic acid (GCA), the patients were able to absorb the fat-soluble vitamins D-2 and tocopherol, showed improvement in growth, and experienced no side effects.

This is the sixth of 17 known liver enzyme defects that Heubi and Setchell have identified, and they are working on testing and funding for development of a GCA drug. Their work appeared online Dec. 23, 2014, in *Hepatology*.

"All of these defects manifest as fatal forms of liver disease if they're not diagnosed, and there is no other form of liver disease you can reverse like this," Setchell says. "This is lifelong therapy for all these kids. We believe that GCA should be the standard of care and supplemental fat-soluble vitamins should be the standard of care for affected patients."

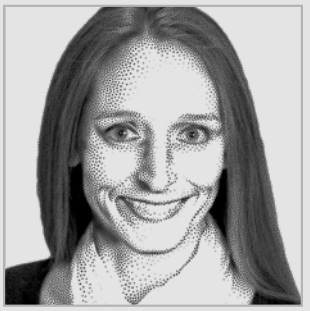


Clinicians and scientists at Cincinnati Children's have identified and treated five patients with defective bile acid amidation due to a genetically confirmed deficiency in bile acid CoA:amino acid N-acyl transferase (BAAT) with the conjugated bile acid, glycocholic acid (GCA). These charts show changes from baseline in plasma vitamin D2 and tocopherol concentrations in response to a single oral bolus dose of vitamin D2 and tocopherol in patients with BAAT deficiency before and after treatment with glycocholic acid.



"This is lifelong therapy for all these kids. We believe that GCA should be the standard of care and supplemental fat-soluble vitamins should be the standard of care for affected patients."

Rope Swings Along Waterways Pose Serious Injury Risks for Girls



Holly Hoefgen, MD

PUBLISHED FEBRUARY 2015
Journal of Pediatric & Adolescent Gynecology

Rope swings hanging from trees along lakes and rivers are a popular recreation for children and teens, but they also pose a risk of severe genital injuries to girls. Moreover, the rural locations of most rope swings complicate providing high quality treatment in a timely manner.

Holly Hoefgen, MD, Co-Director of the Comprehensive Fertility Care and Preservation Program in the Division of Pediatric and Adolescent Gynecology, led a case study review of rope swing injuries with a colleague at the Washington University School of Medicine in St. Louis. Findings were published in February 2015 in the *Journal of Pediatric & Adolescent Gynecology*.

The most commonly reported injuries associated with rope swings are finger fractures, lower extremity trauma, and head and neck trauma. Female genital injuries account for 2.7 percent of overall rope swing injuries, and can result in severe lacerations, hematomas and avulsions. The review specifically analyzed injuries sustained by two girls in swimsuits, ages 13 and 15, who had wrapped their legs around the ropes, began to swing toward the water, but suddenly slid too quickly down the ropes and over knots intended as handgrips or foot grips.

In both cases, the girls required transportation exceeding 100 miles to receive medical attention. One of the two girls required surgery.

"Visitors to inland waterways need to be cognizant of the hazards of rope swings," the authors wrote, "and health care professionals and ED staff should become aware of this mechanism of injury when evaluating patients with vulvar trauma."

RESEARCH AND TRAINING DETAILS

Faculty	4
Research Students	1
Support Personnel	2
Peer Reviewed Publications	3

Hoefgen, H. R. and D. F. Merritt (2015). "Rope swing injuries resulting in vulvar trauma. "*J Pediatr Adolesc Gynecol*. 2015 Feb;28.

The most common injuries related to river tree rope swings are finger fractures, lower extremity trauma, and head and neck trauma. Genital injuries account for 2.7% of all rope swing injuries.

CASE 1 – AGE 13
Incident: Injured while sliding down rope swing and striking knot
Injury: laceration lateral to the clitoral hood extending through the labia majora, avulsing the labia minora, and extending through the rectal mucosa and sphincter into the perirectal space
Medical strategy: Surgery, deep tissue injuries closed in layers, reconstruction of labia majora and minora
Treatment status: post-operative day two
Outcome: Patient doing well

CASE 2 – AGE 15
Incident: Injured while sliding down rope swing and striking knot
Injury: vulvar trauma with bleeding, left vulvar hematoma
Medical strategy: Treated at Emergency Department and released with instructions to apply ice, take sitz baths
Treatment status: Five days later, re-evaluated due to continued vaginal bleeding and pain. Hematoma of left labia was spontaneously draining. Light compression of hematoma resulted in release of a large amount of clotted blood and relief of pain.
Outcome: Two weeks later, pain and hematoma had resolved, previous site of drainage was healing.

Intestinal Organoids Grown from Stem Cells Open Doors for Bioengineered Tissue, Personalized Medicine

Michael Helmrath, MD, MS

RESEARCH AND TRAINING DETAILS

Faculty	26
Research Fellows	9
Research Students	23
Support Personnel	29
Direct Annual Grant Support	$2.8M
Direct Annual Industry Support	$64,173
Peer Reviewed Publications	84

Watson CL, Mahe MM, Munera J, Howell JC, Sundaram N, Poling HM, Schweitzer JI, Vallance JE, Mayhew CN, Sun Y, Grabowski G, Finkbeiner SR, Spence JR, Shroyer NF, Wells JM, Helmrath MA. An *in vivo* model of human small intestine using pluripotent stem cells. *Nat Med.* 2014;20(11):1310-1314.

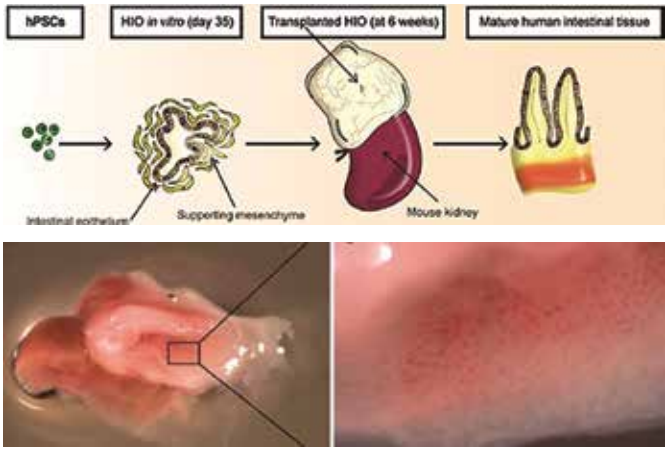
PUBLISHED ONLINE OCT. 19, 2014
Nature Medicine

Doctors in the Division of Pediatric General and Thoracic Surgery are exploring unprecedented areas of tissue bioengineering and personalized medicine through their documented ability to grow human intestinal tissue from stem cells, transplant the tissues into mice and watch them perform as fully functioning human intestines.

In an Oct. 19, 2014, study in *Nature Medicine*, Michael Helmrath, MD, MS, Division of Pediatric General and Thoracic Surgery, reported his team had generated human intestinal organoids (HIOs) by manipulating either human embryonic stem cells (ESCs) or induced pluripotent stem cells (IPSCs). Implanted into mice and connected to the kidney for blood flow, the HIOs developed specialized intestinal epithelial and stem cells, enzymes, and vascular structures of the intestines. They also demonstrated basic digestive functions.

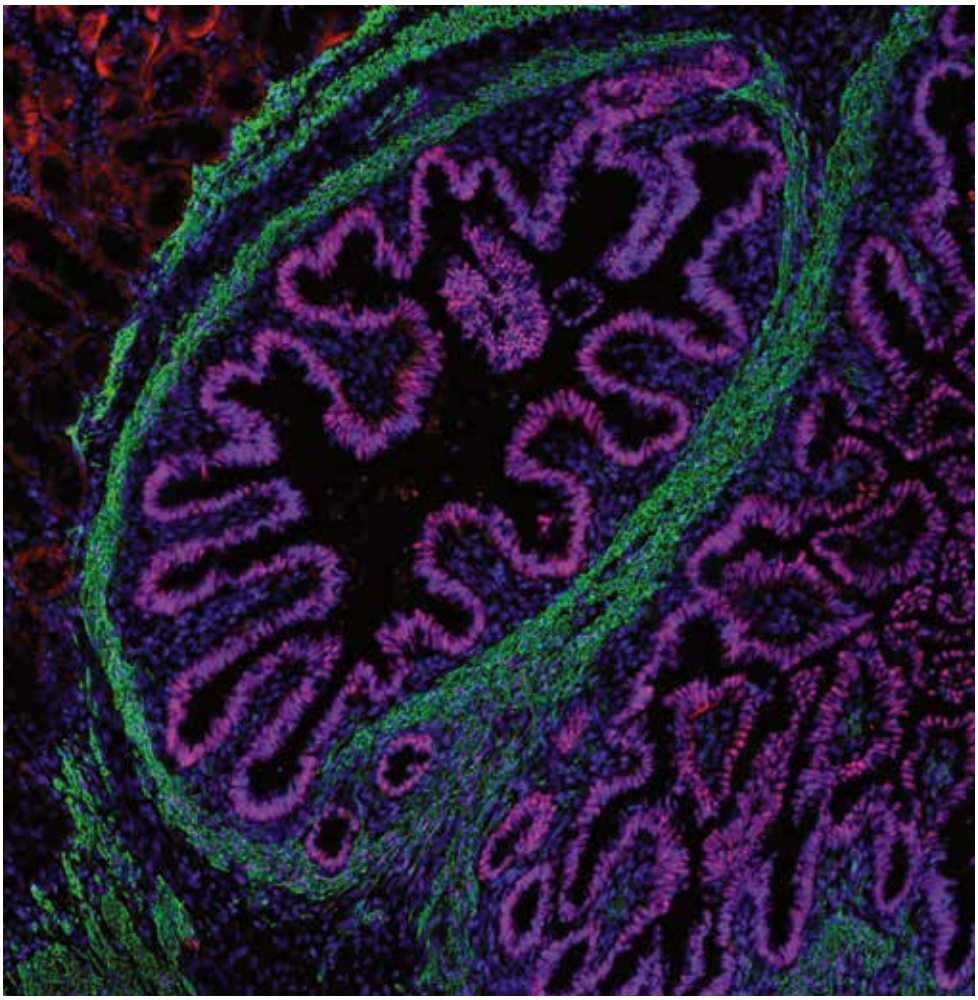
"The ability to regrow an organ is now possible, and it's so impressive how the cells themselves know how to do this," says Helmrath. "These studies support the concept that patient-specific cells can be used to grow intestine, and they provide a new way to study the many diseases and conditions that can cause intestinal failure, from genetic disorders appearing at birth to conditions that strike later in life, such as cancer and Crohn's disease. These studies also advance the longer-term goal of growing tissues that can replace damaged human intestine."

The ability to grow intestines from a patient's own cells also has broad implications for organ transplantation, post-surgical responses, accelerated drug development (by bypassing animal tests), and developing personalized medicine protocols for patients, depending on how their tissues respond to certain drugs or treatments.



This confocal microscopic image shows human intestinal cells that were successfully grown in a mouse model. The finger-like villi that support digestion appear in purple while muscle tissue appears in green.

"The ability to regrow an organ is now possible, and it's so impressive how the cells themselves know how to do this."



SPDEF Transcription Factor Shown to Suppress Prostate Cancer

Tanya Kalin, MD, PhD

The Perinatal Institute includes the Divisions of Neonatology, Perinatal and Pulmonary Biology, Developmental Biology, Reproductive Sciences, the Center for Prevention of Preterm Birth, and the Cincinnati Fetal Center.

THE PERINATAL INSTITUTE RESEARCH AND TRAINING DETAILS

Faculty	60
Joint Appointment Faculty	1
Research Fellows	14
Research Students	14
Support Personnel	95
Direct Annual Grant Support	$10.5M
Direct Annual Industry Support	$167,672
Peer Reviewed Publications	134

Cheng XH, Black M, Ustiyan V, Le T, Fulford L, Sridharan A, Medvedovic M, Kalinichenko VV, Whitsett JA, Kalin TV. SPDEF inhibits prostate carcinogenesis by disrupting a positive feedback loop in regulation of the Foxm1 oncogene. *PLoS Genet*. 2014;10(9):e1004656.

PUBLISHED SEPT. 25, 2014
PLOS Genetics

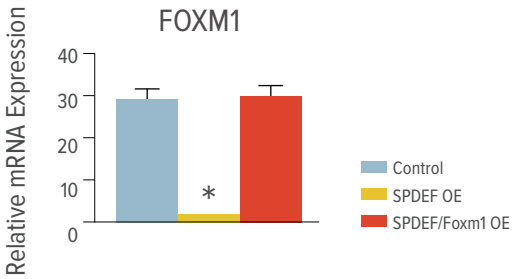
Prostate cancer continues to be the most common malignancy diagnosed in American men and the second leading cause of male cancer mortality.

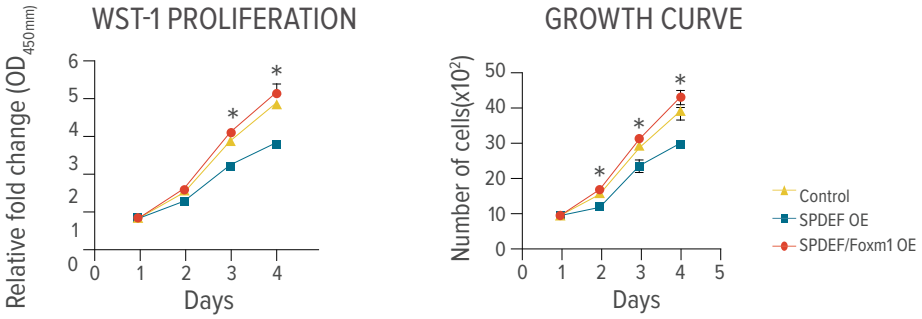
Tanya Kalin, MD, PhD, leads a research team at Cincinnati Children's that seeks to identify the direct role of several transcription factors (Foxm1, Foxf1, Foxf2, SPDEF) in prostate cancer. The team's latest findings, published Sept. 25, 2014, in *PLOS Genetics*, explain how SPDEF transcription factor expression changes during prostate carcinogenesis, which suggests that new treatments could be developed that target Foxm1 via SPDEF dependent pathways.

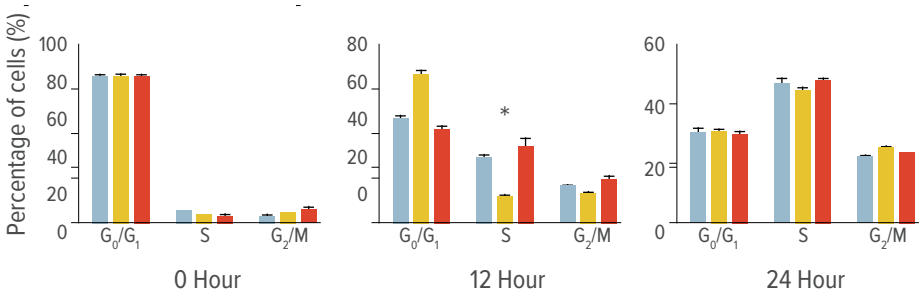
"Our data demonstrate that SPDEF functions as a tumor suppressor in prostate cancers by inhibiting tumor cell proliferation via disruption of an auto-regulatory element in the Foxm1 promoter," Kalin says. "It is possible that the loss of SPDEF causes increased expression of oncogenic Foxm1, accelerating tumor cell proliferation and leading to poor outcome in prostate cancer patients."

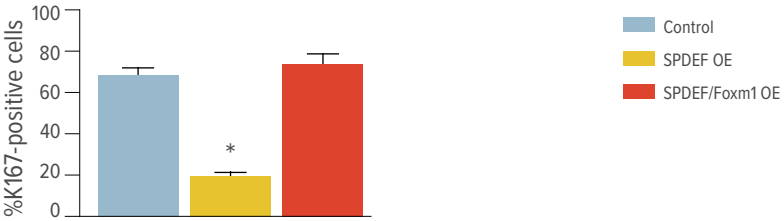
Until now, researchers lacked useful transgenic mouse models to study the role of SPDEF in prostate cancer. Kalin and colleagues generated mice that either lacked or over-expressed SPDEF function. The mice revealed that loss of SPDEF increased cancer progression and tumor cell proliferation, whereas over-expression inhibited carcinogenesis and reduced tumor cell proliferation *in vivo* and *in vitro*.

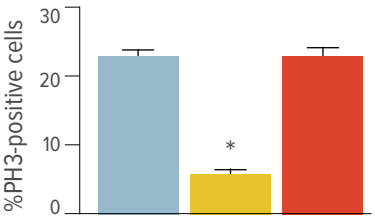
Specifically, over-expression of SPDEF inhibited RNA and protein levels of Foxm1, a transcription factor critical for tumor cell proliferation, and reduced expression of Foxm1 target genes, including Cdc25b, cyclin B1, cyclin A2, Plk-1, AuroraB, CKS1 and Topo2alpha. Furthermore, an inverse correlation between SPDEF and Foxm1 levels was found in human prostate cancers, with the two-gene signature of low SPDEF and high Foxm1 predicting poor survival.











Re-expression of Foxm1 restored cellular proliferation in SPDEF-overexpressing prostate tumor cells as demonstrated by increased numbers of Ki-67-positive (upper panels) and PH3-positive (bottom panels) cells. Percentages of Ki-67-positive and PH3-positive cells were counted in five random microscopic fields.

Macrophage Transplantation Could Become Therapy for hPAP

Bruce Trapnell, MD

THE PERINATAL INSTITUTE RESEARCH AND TRAINING DETAILS

Faculty	60
Joint Appointment Faculty	1
Research Fellows	14
Research Students	14
Support Personnel	95
Direct Annual Grant Support	$10.5M
Direct Annual Industry Support	$167,672
Peer Reviewed Publications	134

Suzuki T, Arumugam P, Sakagami T, Lachmann N, Chalk C, Sallese A, Abe S, Trapnell C, Carey B, Moritz T, Malik P, Lutzko C, Wood RE, Trapnell BC. Pulmonary macrophage transplantation therapy. *Nature*. 2014;514(7523):450-454.

PUBLISHED ONLINE OCT. 1, 2014
Nature

A new type of cell transplantation may one day become a treatment for hereditary pulmonary alveolar proteinosis (hPAP) and certain other rare lung diseases.

Bruce Trapnell, MD, and Takuji Suzuki, MD, PhD, discovered hPAP at Cincinnati Children's and first reported it in 2008. Children with hPAP have mutations in the genes of GM-CSF receptor alpha or beta (CSFR2RA or CSFR2RB). These mutations reduce the ability of alveolar macrophages to remove used surfactant from the lungs, which can lead to respiratory failure. The only current treatment is repeated, invasive whole-lung lavage.

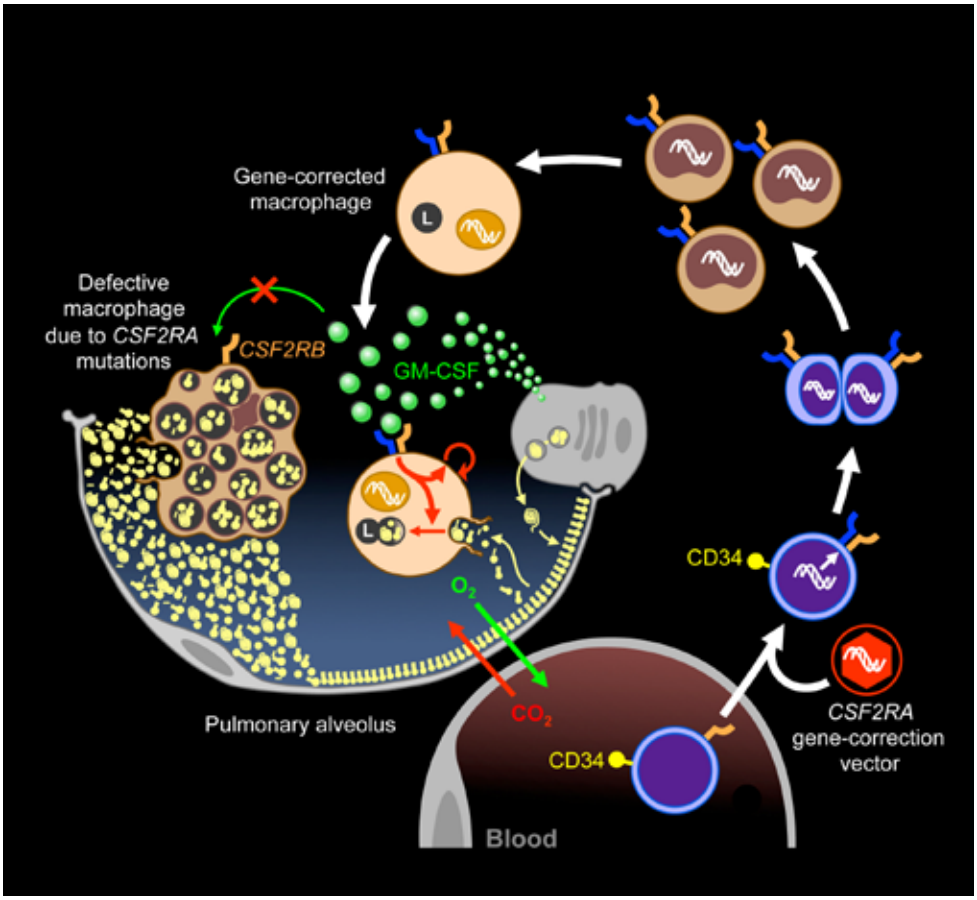
In a recent study published in *Nature*, Suzuki and Trapnell report that macrophage transplantation (involving normal or gene-corrected cells) fully reversed the disease in mice bred to mimic hPAP. The treatment also prevented disease-specific mortality for at least one year.

"These are significant findings with potential implications beyond the treatment of a rare lung disease," says Trapnell, senior author, and a researcher in the Translational Pulmonary Science Center at Cincinnati Children's. "Our findings support the feasibility of pulmonary macrophage transplantation as the first specific therapy for children with hPAP."

The research team utilized mice with the homologous CSFR2RB gene that mimics hPAP knocked out. The team then used a viral vector to deliver a correct version of CSFR2RB to abnormal alveolar macrophages taken from the animals. The gene-corrected cells were returned to the mice by direct instillation into the lungs.

Since publication, the researchers have begun the pre-clinical studies needed to prepare for human clinical trials.

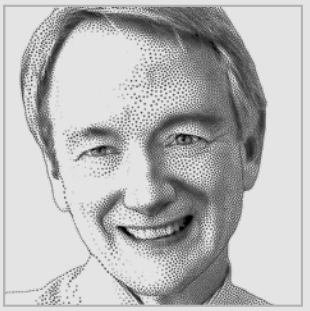
PULMONARY MACROPHAGE TRANSPLANTATION THERAPY



Scientists at Cincinnati Children's have demonstrated in mice bred to mimic hereditary pulmonary alveolar proteinosis (hPAP) that pulmonary macrophage transplantation of either wild-type or Csf2rb-gene-corrected macrophages without myeloablation was safe, well-tolerated, and that one administration corrected the lung disease. This illustration outlines the transplantation process planned for therapy of hPAP in children.

"Our findings support the feasibility of pulmonary macrophage transplantation as the first specific therapy for children with hPAP."

Two Genes Expressed in Airway Epithelial Cells Play Important Roles in the Development of Asthma



Jeffrey Whitsett, MD

THE PERINATAL INSTITUTE
RESEARCH AND TRAINING DETAILS

Faculty	60
Joint Appointment Faculty	1
Research Fellows	14
Research Students	14
Support Personnel	95
Direct Annual Grant Support	$10.5M
Direct Annual Industry Support	$167,672
Peer Reviewed Publications	134

Rajavelu P, Chen G, Xu Y, Kitzmiller JA, Korfhagen TR, Whitsett JA. Airway epithelial SPDEF integrates goblet cell differentiation and pulmonary Th2 inflammation. *J Clin Invest*. 2015;125(5):2021-2031.

PUBLISHED MAY 4, 2015
The Journal of Clinical Investigation

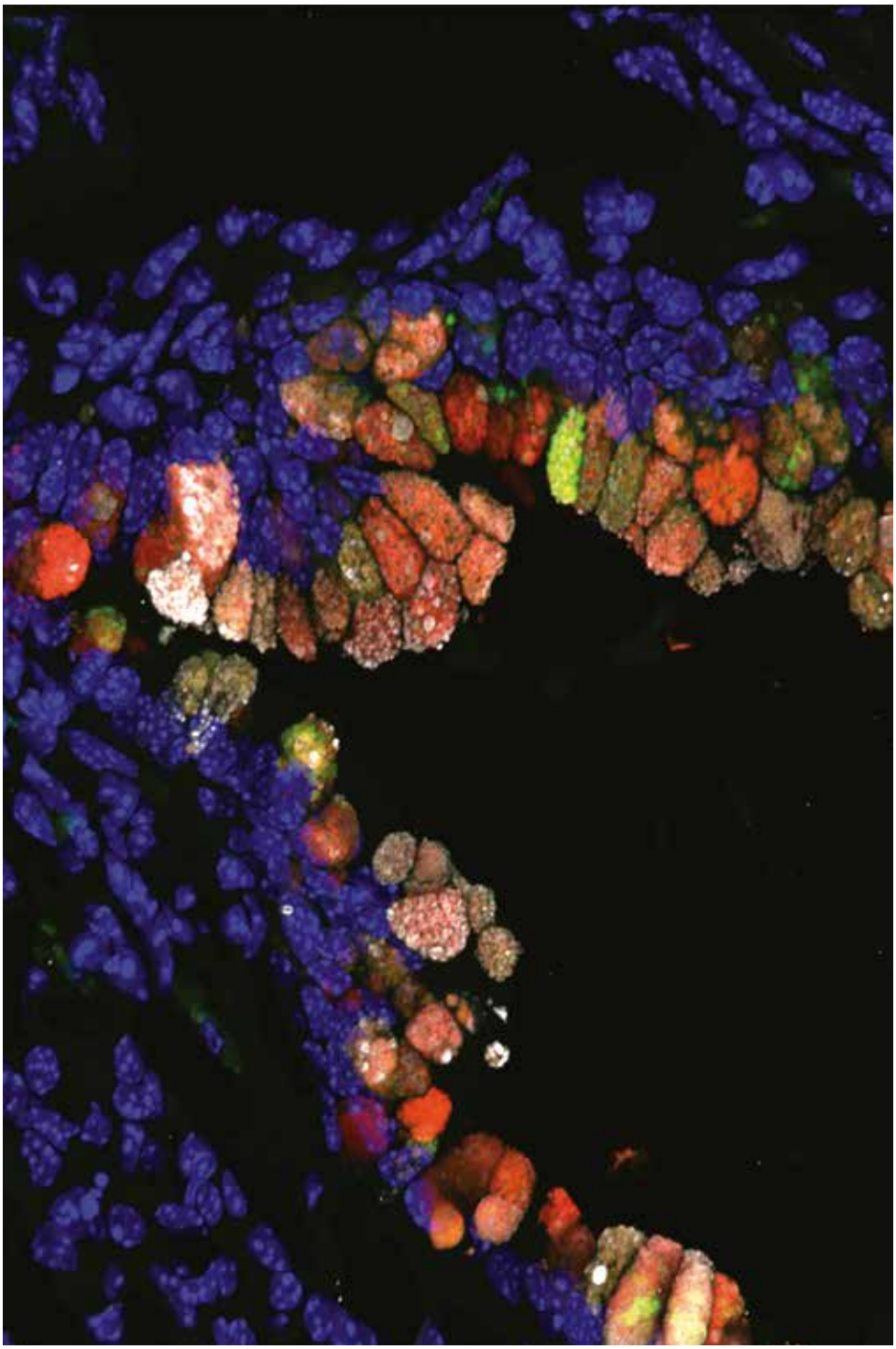
Epithelial cells lining the airways are the first line of defense against infections and allergens, and doctors are increasingly understanding the role played by pulmonary immune responses — initiated early in development, *in utero*, and during infancy — in the development of asthma and other lung disorders.

Jeffrey Whitsett, MD, Co-Director of the Perinatal Institute, and a team of pulmonary biology researchers have shown that airway epithelial cells orchestrate immune responses after birth that influence subsequent allergic inflammation, leading to asthma.

Specifically, the researchers found that the genes SPDEF and FOXA3, which control mucus production and goblet cell differentiation, program pulmonary immune responses early in life and are sufficient and required to induce asthma. Goblet cells secrete the major components of mucus. The SDPEF and FOXA3 genes, expressed only in airway epithelial cells, control inflammatory responses to allergens and infections, programming subsequent asthma-like responses.

Whitsett's study, which measured immune system responses in the lungs of neonatal mice, appeared May 4, 2015, in *The Journal of Clinical Investigation*. It concludes that exposure to commensal and pathogenic microbes and antigens influences goblet cells in the airways that determine the acquisition of immune responses after birth, responses that are likely to have long-term effects on the patterning of subsequent immune and inflammatory responses of the lung, leading to asthma.

"Inhibition of mucus cell hyperactivity induced by SPDEF following lung infections or exposure to allergies," says Whitsett, "provides a novel, therapeutic approach for treatment and prevention of chronic airway diseases associated with excess mucus, including asthma and cystic fibrosis, common causes of severe lung disease in children."



This confocal microscope image shows airway goblet cells and mucus accumulation in the airways of mice caused by expression of FOXA3 and SPDEF. The mice develop “asthma” induced by expression of the genes controlling mucus production.

Online Family Problem-Solving Therapy Can Offset Impact of Traumatic Brain Injury

Shari Wade, PhD

RESEARCH AND TRAINING DETAILS

Faculty	7
Research Fellows	2
Research Students	8
Support Personnel	10
Direct Annual Grant Support	$957,022
Direct Annual Industry Support	$1,230
Peer Reviewed Publications	13

Wade SL, Kurowski BG, Kirkwood MW, Zhang N, Cassedy A, Brown TM, Nielsen B, Stancin T, Taylor HG. Online problem-solving therapy after traumatic brain injury: a randomized controlled trial. *Pediatrics.* 2015;135(2):e487-495.

PUBLISHED MAY 18, 2015
Pediatrics

Children and teens who experience traumatic brain injuries (TBI) often struggle with everyday tasks, school performance, jobs and community interactions — and their families also struggle to help them recover and rehabilitate.

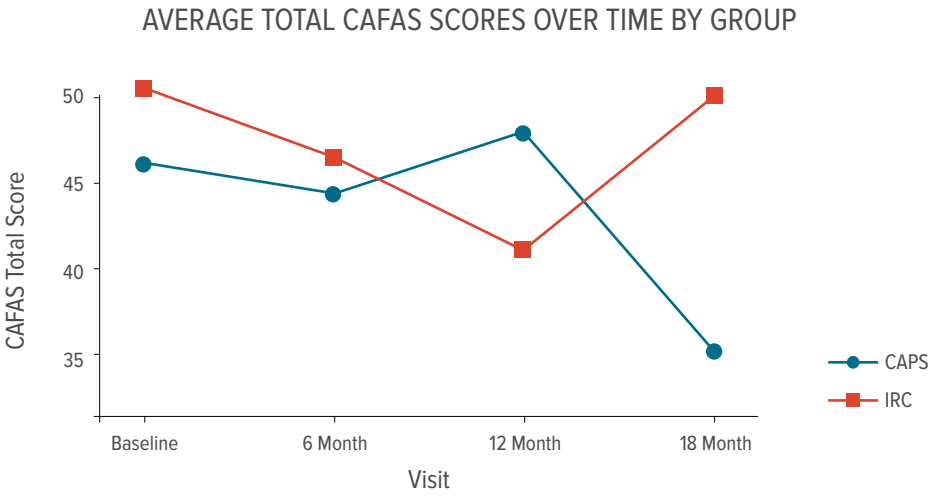
The only study to examine the long-term impact of family intervention programs finds that online sessions can result in long-term improvements in child functioning, particularly among families of lower socioeconomic status (SES). The study, led by Shari Wade, PhD, Director of Research in the Division of Physical Medicine and Rehabilitation, appeared May 18, 2015, in *Pediatrics*.

Five centers enlisted 132 TBI teens ages 12-17. Sixty-five were randomly assigned to Counselor-Assisted Problem Solving (CAPS), a six-month, web-based intervention program in which four licensed therapists used web training and follow-up video conference sessions using Skype to train teens and their families in problem-solving, communication and self-regulation techniques. Another 66 teens were assigned to an Internet Resource Comparison (IRC) control group, which involved self-guided, web-based information and resources that families were encouraged to explore at least one hour a week. Both groups were tracked for 18 months.

Although children of lower SES typically have poorer outcomes following brain injury, findings from this study suggests that online problem solving may be especially beneficial for this population. Differences in functioning between the CAPS and IFC families were not evident until 12 months after sessions ended, "suggesting that the effects of problem-solving therapy delivered soon after injury may successfully generalize to the youth's functioning in everyday settings over time."

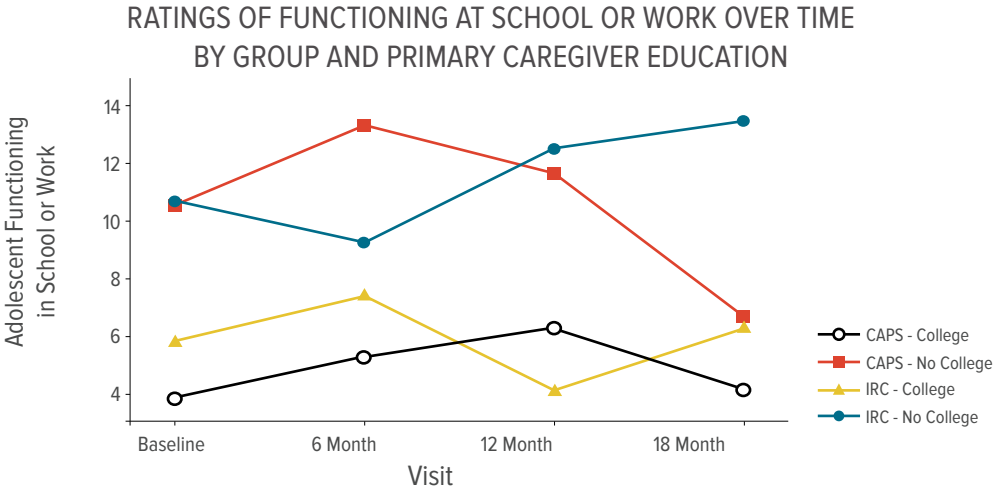
CAPS teens also performed better on tasks outside the home, including at work.

"Given that adolescents with TBI are at risk of deficits in school performance and may have difficulty sustaining employment as they transition into adulthood, improving school and community functioning may be particularly important for long-term success," said Wade.



Significant group x visit interaction, F(3, 301) = 4.18, p = .006. CAPS group is significantly less than IRC group at visit 4, p < .05.

Providing Counselor-Assisted Problem Solving (CAPS) therapy shortly after a traumatic brain injury may result in long-term improvements in child functioning, particularly among families of lower socioeconomic status. Average Child and Functional Assessment Scale (CAFAS) scores (above) indicate a significant group x visit interaction, suggesting that improvements in everyday functioning emerged over time after the intervention. In ratings of school or work functioning (below), there was a significant group x time x caregiver education interaction. Overall, the CAPS group demonstrated significantly better functioning than an internet resource comparison (IRC) group particularly among families with lower levels of education.



Significant group x time x caregiver education interaction, F(3, 315) = 3.26, p = .02. Post hoc contrasts indicate significant group difference between the low education CAPS and IRC groups at visit 4 t(351) = -2.20, p = .03.

Whole-Genome Sequencing Confirms Avian Model of Human Disease

Ching-Fang Chang, PhD

Samantha Brugmann, PhD

RESEARCH AND TRAINING DETAILS

Faculty	10
Joint Appointment Faculty	1
Research Fellows	1
Research Students	2
Support Personnel	1
Direct Annual Grant Support	$325,000
Direct Annual Industry Support	$147,572
Peer Reviewed Publications	20

Chang CF, Schock EN, O'Hare EA, Dodgson J, Cheng HH, Muir WM, Edelmann RE, Delany ME, Brugmann SA. The cellular and molecular etiology of the craniofacial defects in the avian ciliopathic mutant talpid^2. *Development*. 2014;141(15):3003-3012.

PUBLISHED AUGUST 2014
Development

For more than 60 years, developmental biologists have studied a naturally occurring avian mutant called talpid^2 because of its interesting phenotypes, including cleft lip/palate, oral dysmorphologies and limb defects. However, talpid^2 has been of limited utility because researchers did not know what caused the mutation — until now.

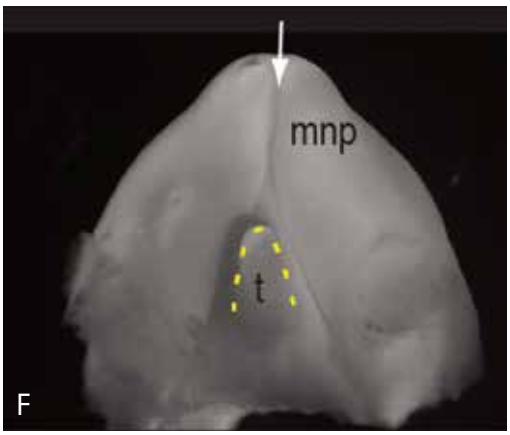
The solution came from the lab of Samantha Brugmann, PhD, which cracked the genetic, molecular and cellular code on how and why the talpid^2 mutation occurs. Their findings appeared in the August 2014 issue of *Development*.

Using whole-genome sequencing, a group of researchers from Cincinnati Children's and four other universities traced the talpid^2 mutation to a ciliary gene called C2CD3, which causes a significant reduction in the number of cells that extend a primary cilium. The team also identified molecular disruptions that occur in the Hedgehog (Hh) signaling pathway, leading to the facial and limb abnormalities associated with talpid^2.

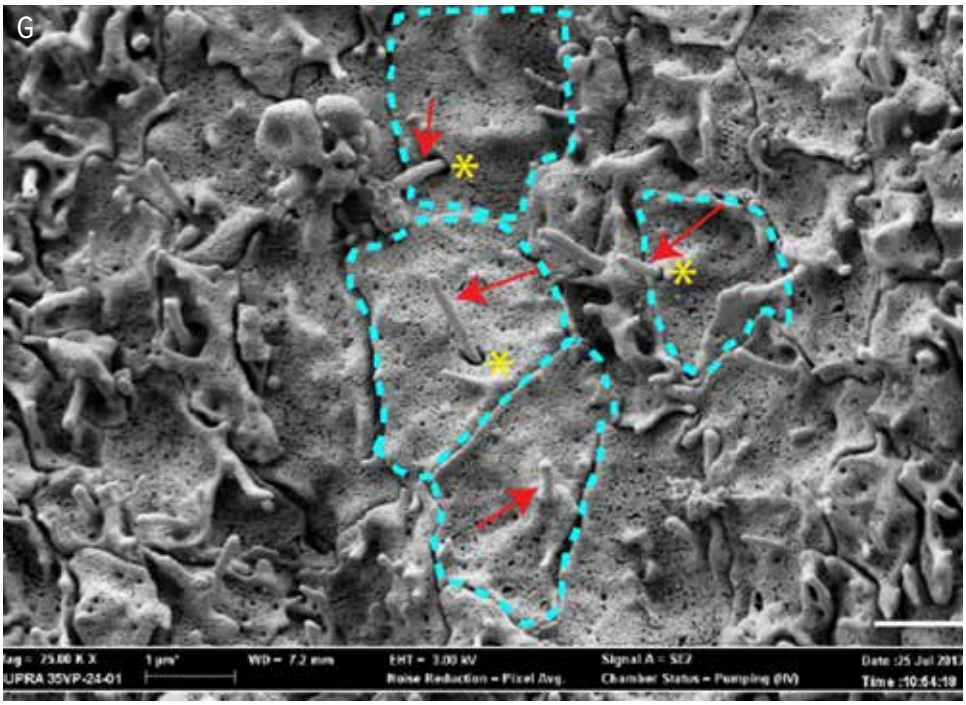
"Recently, the study of primary cilia has exploded because of the identification of a large class of human diseases called ciliopathies," Brugmann says. "Now that we know what gene causes this defect in chickens — as well as the molecular and cellular pathway that is involved — we have an avian model for human disease."

An accompanying *Development* editorial, titled "talpid^2: A Mystery Finally Solved," states: "Identification of the talpid^2 locus has been long awaited, and although there is still much to understand about how C2CD3 regulates cilia formation and function, and SHH signaling, these data provide an important step in this direction."

This prediction has come to fruition as the group, in a subsequent publication (Schock et al., 2015, in *Disease Models & Mechanisms*), has shown that talpid^2 is a bona fide model for the human ciliopathy called Oral-facial-digital Syndrome.



"Now that we know what gene causes this defect in chickens — as well as the molecular and cellular pathway that is involved — we have an avian model for human disease."



Primary cilia are disrupted in talpid[2] mutants as shown in scanning electron microscope images of the ventricular surface of the neuroectoderm. Blue lines outline cells; red arrow indicates axoneme; yellow asterisks mark ciliary pockets.

Alcoholism Drug May Help Treat Fragile X Syndrome-Related Autism Spectrum Disorder

Craig Erickson, MD

RESEARCH AND TRAINING DETAILS

Faculty	20
Joint Appointment Faculty	2
Support Personnel	19
Direct Annual Grant Support	$870,361
Direct Annual Industry Support	$262,242
Peer Reviewed Publications	40

Erickson CA, Ray B, Maloney B, Wink LK, Bowers K, Schaefer TL, McDougle CJ, Sokol DK, Lahiri DK. Impact of acamprosate on plasma amyloid-beta precursor protein in youth: a pilot analysis in fragile X syndrome-associated and idiopathic autism spectrum disorder suggests a pharmacodynamic protein marker. *J Psychiatr Res*. 2014;59:220-228..

PUBLISHED ONLINE AUG. 19, 2014
Journal of Psychiatric Research

A drug originally approved for treating alcoholism is showing promise in modulating the underlying neurochemistry of a type of autism linked to Fragile X Syndrome, an inherited genetic disorder.

Craig Erickson, MD, and his team have discovered that acamprosate, a drug that may modulate glutamate and gaba-aminobutyric acid (GABA) neurotransmission in the brain, normalizes the expression of proteins in the blood known to be dysregulated in people with Fragile X Syndrome-related autism spectrum disorder (ASD), and it produces improved behavior and social interaction skills in some of them.

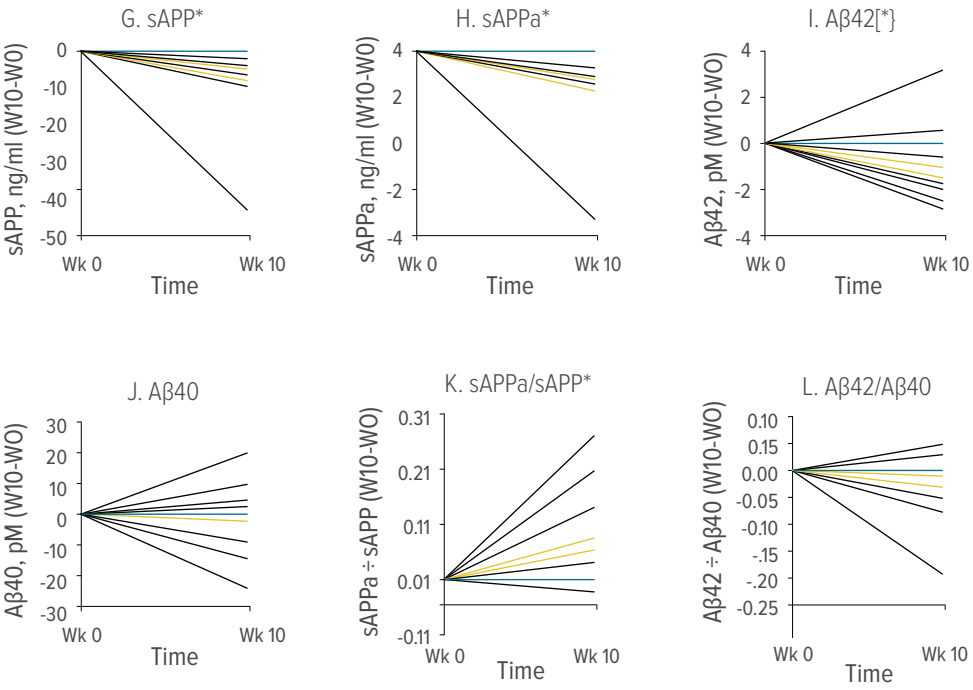
Nearly half of children with Fragile X syndrome exhibit a type of autism marked by development disabilities, distinctive facial characteristics, social anxiety, communication deficiencies or repetitive movement behaviors. They also tend to have higher plasma levels of a derivative of amyloid-β precursor protein, called secreted APPa (sAPPa), as well as excessive levels of glutamate and insufficient levels of GABA, two abundant and critical neurotransmitters.

In collaboration with researchers at the Indiana University School of Medicine, Erickson reported on initial results of the positive impact of acamprosate on APP levels in blood. Now Erickson is collaborating with Rush Medical Center in Chicago on a 48-participant clinical trial of the drug, in which researchers will analyze blood levels for sAPPa, eye-tracking data, social interactions, and other ASD behavioral markers.

"Acamprosate is really a targeted treatment based on the physiology and neurochemistry of the disease," Erickson says. "We're trying to regulate and normalize some of the signaling pathways by use of this drug." Tests on mice, he notes, produce similar molecular neurochemistry results.

Plasma protein derivative analysis, Erickson says, holds promise as a biomarker for ASD-targeted treatment, and acamprosate may have "novel pharmacodynamics properties" to reduce amyloid-β precursor proteins in children with this type of ASD.

PRE- AND POST-ACAMPROSATE IN FXS SUBJECTS



Results from two pilot studies show that treatment with the drug acamprosate produce improved behavior and social interaction skills in some children with Fragile X Syndrome-related autism spectrum disorder. These fan plots show blood test results from FXS patients assayed for selected processing products of the plasma amyloid-b precursor protein (APP), including sAPP, sAPPa, Ab42, Ab40, and Ab42/Ab40. Gray lines show individual subjects. Orange solid lines show mean sample change. Orange dashed lines show mean sample change excluding the most extreme result. Blue lines show "null" zero. Acamprosate use was associated with a significant reduction in plasma sAPP(total) and sAPPa levels, but no change occurred in Ab40 or Ab42 levels.

"We're trying to regulate and normalize some of the signaling pathways by use of this drug."

Understanding of Cystic Fibrosis Leads to Novel Findings About Drug-Induced Diarrhea

Anjaparavanda Naren, PhD

RESEARCH AND TRAINING DETAILS

Faculty	26
Joint Appointment Faculty	5
Research Fellows	7
Research Students	19
Support Personnel	30
Direct Annual Grant Support	$3.6M
Direct Annual Industry Support	$493,675
Peer Reviewed Publications	64

Moon C, Zhang W, Ren A, Arora K, Sinha C, Yarlagadda S, Woodrooffe K, Schuetz JD, Valasani KR, de Jonge HR, Shanmukhappa SK, Shata MT, Buddington RK, Parthasarathi K, Naren AP. Compartmentalized accumulation of cAMP near complexes of multidrug resistance protein 4 (MRP4) and cystic fibrosis transmembrane conductance regulator (CFTR) contributes to drug-induced diarrhea. *J Biol Chem.* 2015;290(18):11246-11257.

PUBLISHED MAY 1, 2015
The Journal of Biological Chemistry

Research by Anjaparavanda Naren, PhD, into the underlying mechanisms of cystic fibrosis — a disease marked by impaired fluid secretions in the lung — is providing insights into why 7 percent of patients develop diarrhea as an adverse effect of certain prescription drugs.

Naren is the Thomas Boat Chair in Cystic Fibrosis Research and Co-Director, Cystic Fibrosis Research Center. He and a Pulmonary Medicine research team that included Chang Suk Moon, PhD, Kavisha Arora, PhD, and Sunitha Yarlagadda have identified an interplay between multidrug resistance protein 4 (MRP4) and the cystic fibrosis transmembrane conduct regulator (CFTR), a chloride channel on epithelial cells that retains and releases fluids. In cystic fibrosis, the channel underperforms, keeping fluids in the lungs; in diarrhea, the channel over-performs and releases fluids into the intestines and bowel.

"Until now, we did not have a good model system to study intestinal biopsies, because the tissue samples are so small," Naren explains. His team deployed stem-cell technologies to create intestinal organoids, called "enteroids," from tissue biopsies from mice. The enteroids were exposed in the laboratory to two drugs and monitored for excessive secretions — the symptoms of diarrhea. The enteroids also were monitored to determine how they responded to anti-diarrhea treatments.

The study in *The Journal of Biological Chemistry* involved irinotecan (a colon cancer drug) and AZT, an antiviral drug for HIV/AIDS. The drugs inhibited MRP4, producing compartmentalized accumulation of cAMP (3'-5'-cyclic adenosine monophosphate) in close proximity to the CFTR, activating the channel function and causing excessive fluid secretion and diarrhea.

"Our findings have broad implications and may help to identify therapeutic targets for ameliorating medication-induced diarrheas," Naren says. "Enteroids allow us to use stem-cell techniques to develop a better index of chloride channel function, giving us the kinds of tools to move into personalized medicine."

A study led by Cincinnati Children's researchers was featured on the May 2015 cover of The *Journal of Biological Chemistry*. The study reports that CFTR-MRP4-containing macromolecular complexes play an important role in the pathogenesis of drug-induced diarrhea, a finding that could have important clinical implications in the hunt for novel agents to mitigate it.

"Enteroids allow us to use stem-cell techniques to develop a better index of chloride channel function, giving us the kinds of tools to move into personalized medicine."

Surgical Anesthesia in Young Children Linked to Effects on IQ, Brain Structure

Andreas Loepke, MD, PhD

Scott Holland, PhD

RESEARCH AND TRAINING DETAILS

Faculty	46
Joint Appointment Faculty	8
Research Students	8
Support Personnel	52
Direct Annual Grant Support	$482,302
Direct Annual Industry Support	$79,184
Peer Reviewed Publications	140

Backeljauw B, Holland SK, Altaye M, Loepke AW. Cognition and Brain Structure Following Early Childhood Surgery With Anesthesia. *Pediatrics*. 2015;136(1):e1-e12.

PUBLISHED ONLINE JUNE 8, 2015
Pediatrics

Scientific understanding of anesthesia's impact on young children took a significant leap forward in June, when a multi-divisional study revealed correlations to slightly lower brain function and IQ.

Researchers were quick to caution that direct causation remains unresolved, and additional studies were needed to determine anesthesia's precise molecular effects on several functions, including language comprehension, in children who underwent surgery before age 4.

The study, published online June 8, 2015, in *Pediatrics*, garnered wide-ranging media coverage, including pieces in *Scientific American*, *U.S. News and World Report* and *Anesthesiology News* and coverage on NPR, CTV and Slate.com. Andreas Loepke, MD, PhD, of the Department of Anesthesia, was the lead author. Scott Holland, PhD, Director of the Pediatric Neuroimaging Research Consortium, led the Division of Radiology's contributions.

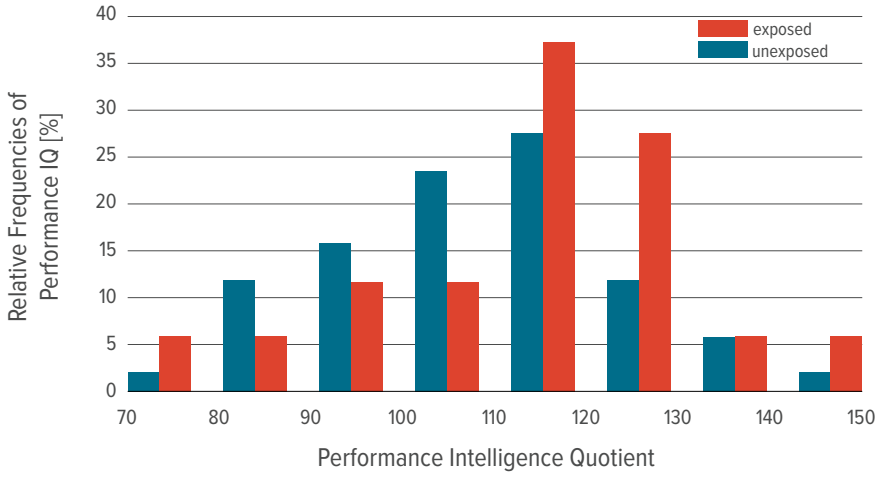
This new knowledge could make it possible to develop mitigating strategies for what scientists describe as a potential dilemma for child health.

"We have to better understand to what extent anesthetics and other factors contribute to learning abnormalities in children before making drastic changes to our current practice, which by all measures has become very safe," Loepke says.

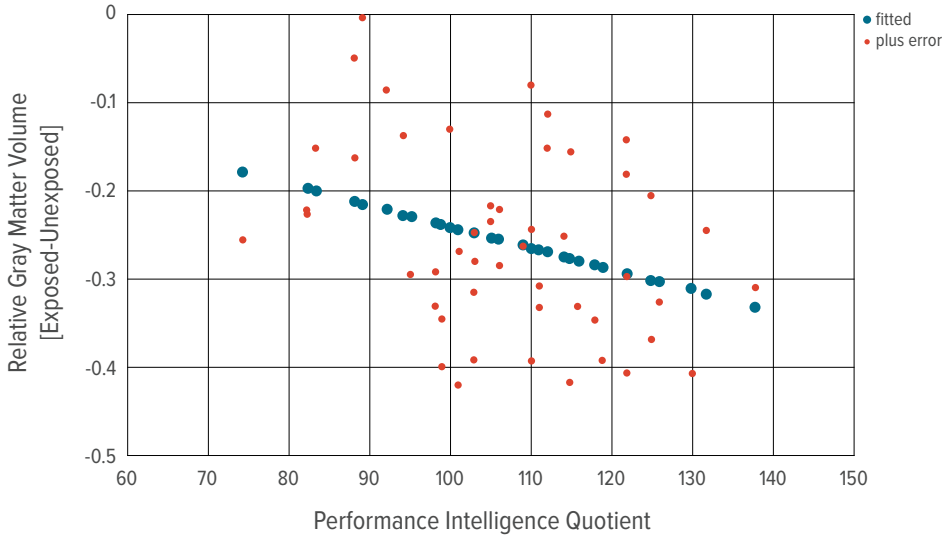
In the study, researchers compared test scores of 53 healthy participants in a language development study (ages 5 to 18 years with no history of surgery) with those of 53 children in the same age range who had undergone surgery before age 4.

The authors emphasized that average test scores for all 106 children were within population norms. Still, compared with children who had not undergone surgery, children exposed to anesthesia scored significantly lower in key areas that warrant additional examination.

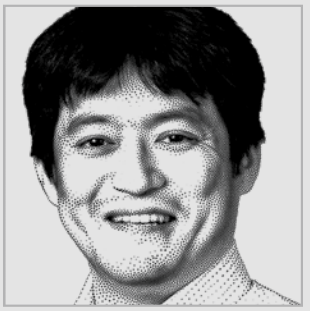
Loepke, Holland and Mekbib Altaye, PhD, Division of Biostatistics and Epidemiology, have submitted an application to the National Institutes of Health seeking funding for a follow up study to investigate more deeply the influence of early anesthesia exposure on brain development.



Substantial concerns have recently been raised regarding the long-term effects of anesthesia and surgery on the developing brain. Brain functional and structural comparisons, conducted by using T1-weighted MRI scans, played a crucial role in a widely-discussed study reporting that exposure to surgical anesthesia can result in diminished language comprehension and IQ. Exposure did not lead to gross elimination of gray matter in regions previously identified as vulnerable in animals. However, decreased performance IQ was associated with diminished gray matter densities in the occipital cortex and cerebellum.



Genomic Research Sheds Light on Possible Causes of Male Infertility



Satoshi Namekawa, PhD

RESEARCH AND TRAINING DETAILS

Faculty	7
Joint Appointment Faculty	3
Research Fellows	14
Research Students	3
Support Personnel	10
Direct Annual Grant Support	$1.4M
Peer Reviewed Publications	17

Hasegawa K, Sin HS, Maezawa S, Broering TJ, Kartashov AV, Alavattam KG, Ichijima Y, Zhang F, Bacon WC, Greis KD, Andreassen PR, Barski A, Namekawa SH. SCML2 establishes the male germline epigenome through regulation of histone H2A ubiquitination. *Dev Cell.* 2015;32(5):574-588.

PUBLISHED ONLINE FEB. 19, 2015
Developmental Cell

Research at Cincinnati Children's has identified a critical determinant of the germline epigenome that has important implications for the genomics and evolution of the male germline.

The study in the journal *Developmental Cell* advances research into the possible causes of male infertility, says senior author Satoshi Namekawa, PhD.

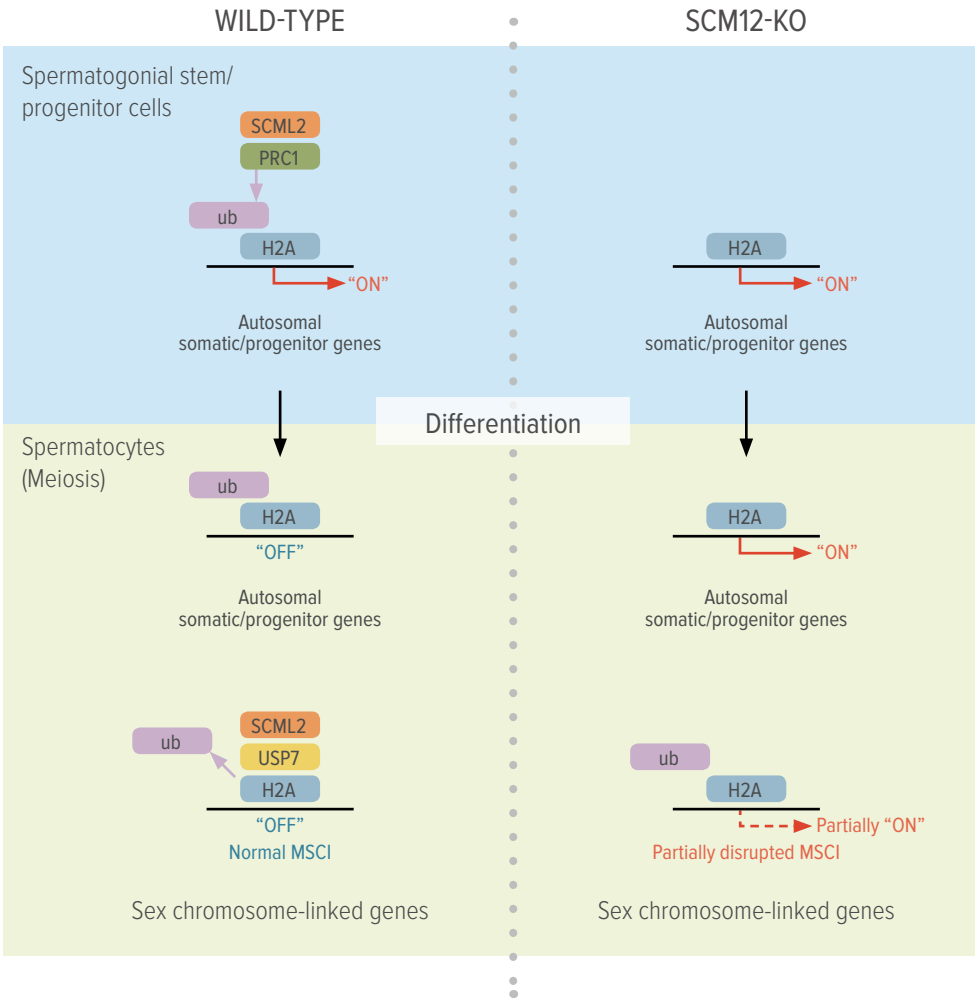
Namekawa and colleagues found that genes commonly expressed in somatic lineages and spermatogenesis-progenitor cells undergo a repression in a genome-wide manner. This repression occurs in the late developmental stages of the male germline, the only lineage that ensures the perpetuation of genetic and epigenetic information across generations.

The research team theorizes that this repression may, in turn, indirectly ensure activation of spermatogenesis-specific genes, an essential step for producing mature spermatozoa. Disruptions to this normal process may explain some forms of male infertility because this mechanism underlies activation of essential genes in spermatogenesis.

The study identifies SCML2, a germline-specific subunit of a polycomb repressive complex 1 (PRC1), as the critical determinant of the germline epigenome. SCML2 establishes the unique epigenome of the germline through two distinct and antithetical mechanisms:

- SCML2 works with PRC1 to promote ubiquitination of histone H2A, a histone modification associated with gene repression, in the stem cell phase of spermatogenesis-progenitor cells. This process appears to mark somatic/progenitor genes on autosomes for repression.
- At the same time, SCML2 also prevents ubiquitination of histone H2A on sex chromosomes during meiosis, thereby enabling unique epigenetic programming of sex chromosomes for male reproduction.

The key finding was revealing essential mechanisms underlying spermatogenesis, which will lead to more focused exploration of how the mechanisms of reproduction operate.



This illustration describes the distinct mechanisms followed by SMCL2 as it regulates histone H2A ubiquitination. By revealing essential mechanisms underlying spermatogenesis, these findings could lead to new understanding of reproduction and reproductive disorders.

Future ACL Injury Risk Runs High After Reconstructive Surgery

Mark Paterno, PhD

RESEARCH AND TRAINING DETAILS

Faculty	12
Joint Appointment Faculty	6
Research Fellows	7
Research Students	37
Support Personnel	34
Direct Annual Grant Support	$645,039
Peer Reviewed Publications	83

Paterno MV, Rauh MJ, Schmitt LC, Ford KR, Hewett TE. Incidence of Second ACL Injuries 2 Years After Primary ACL Reconstruction and Return to Sport. *Am J Sports Med*. 2014;42(7):1567-1573.

PUBLISHED JULY 2014
The American Journal of Sports Medicine

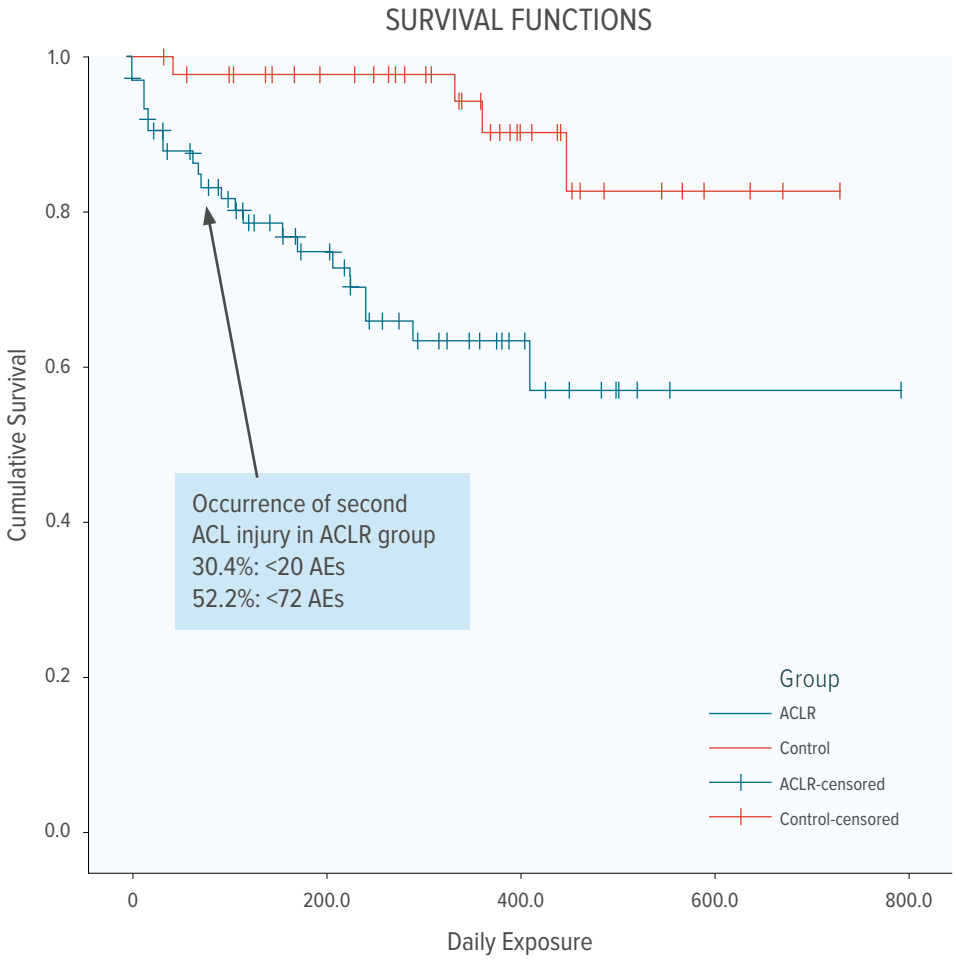
Young athletes who undergo ACL reconstructive knee surgery have a significantly higher rate of suffering a second ACL injury within two years. In addition, girls appear to face greater risk than boys for injuring their other ACL during that time.

Findings of the study, published in July 2014 in *The American Journal of Sports Medicine*, could lead to improved post-operative care and intervention strategies.

"The incidence of repeat injury is much higher than once thought in young, active patients," says Mark Paterno, PhD, Scientific Director in the Division of Occupational Therapy and Physical Therapy and Associate Professor in the Division of Sports Medicine. "Essentially, this highlights the concern that our current outcome after ACL reconstruction is sub-optimal. With this knowledge, it is imperative that we challenge the current management after ACL injuries."

Thirty areas of patient care at Cincinnati Children's work within Patient Services, ranging from OT/PT and ambulatory services to audiology and outpatient clinics. In the field of sports medicine, numerous studies have examined the prevalence of second ACL injuries within the first year of post-reconstruction, but none have reported the incidence normalized to athletic exposure further out. The team found that, overall, 29.5 percent of these young athletes suffered a second ACL injury within two years of returning to sports.

The proportion of athletes reinjuring the same ACL during this period of time was similar between girls and boys. However, 23.7 percent of girls with such an injury later injured the other ACL, compared to just 10.5 percent of boys.



Young athletes who received one anterior cruciate ligament reconstruction (ACLR) were much more likely than non-injured athletes to suffer a subsequent ACL injury upon returning to pivoting or cutting sports activities. This Kaplan-Meier survival curve of ACL integrity shows that more than 30 percent of once-injured athletes experienced a second ACL injury within 20 activity exposures and 52 percent were injured within 72 exposures. Conversely, none of the athletes in the control group were injured in their next 20 exposures and only one was injured in less than 72 exposures.

Tocilizumab Shown to be Safe and Effective as Novel Treatment for Children With Juvenile Idiopathic Arthritis

Hermine Brunner, MD, MSc, MB

RESEARCH AND TRAINING DETAILS

Faculty	10
Joint Appointment Faculty	2
Research Students	5
Support Personnel	16
Direct Annual Grant Support	$1.4M
Direct Annual Industry Support	$839,732
Peer Reviewed Publications	27

Brunner HI, Ruperto N, Zuber Z, Keane C, Harari O, Kenwright A, Lu P, Cuttica R, Keltsev V, Xavier RM, Calvo I, Nikishina I, Rubio-Perez N, Alexeeva E, Chasnyk V, Horneff G, Opoka-Winiarska V, Quartier P, Silva CA, Silverman E, Spindler A, Baildam E, Gamir ML, Martin A, Rietschel C, Siri D, Smolewska E, Lovell D, Martini A, De Benedetti F, Paediatric Rheumatology International Trials Organisation P, Pediatric Rheumatology Collaborative Study G. Efficacy and safety of tocilizumab in patients with polyarticular-course juvenile idiopathic arthritis: results from a phase 3, randomised, double-blind withdrawal trial. *Ann Rheum Dis*. 2015;74(6):1110-1117.

PUBLISHED ONLINE MAY 16, 2014
Annals of Rheumatic Diseases

Children with polyarticular juvenile idiopathic arthritis (JIA) can now be treated safely and effectively with tocilizumab, an interleukin-6 receptor inhibitor drug, according to a 58-center global study coordinated and co-designed at Cincinnati Children's.

An extended report on the results of the Phase III CHERISH study was first published online May 16, 2014, in the *Annals of the Rheumatic Diseases*. The U.S. Food and Drug Administration (FDA) has used positive results of this clinical trial to expand the indication for tocilizumab, which had previously been approved to treat moderate to severe active rheumatoid arthritis and systemic JIA.

Tocilizumab is the first new treatment for polyarticular JIA in five years, and significantly enhances options for treating joint pain and swelling in children with JIA.

Hermine Brunner, MD, MSc, MB, Director of the Division of Rheumatology at Cincinnati Children's and Scientific Director of the multinational Pediatric Rheumatology Collaborative Study Group (PRCSG), led the study. Daniel Lovell, MD, MPH, Chairman of the PRCSG and Clinical Director of Rheumatology, also participated in study design and coordination.

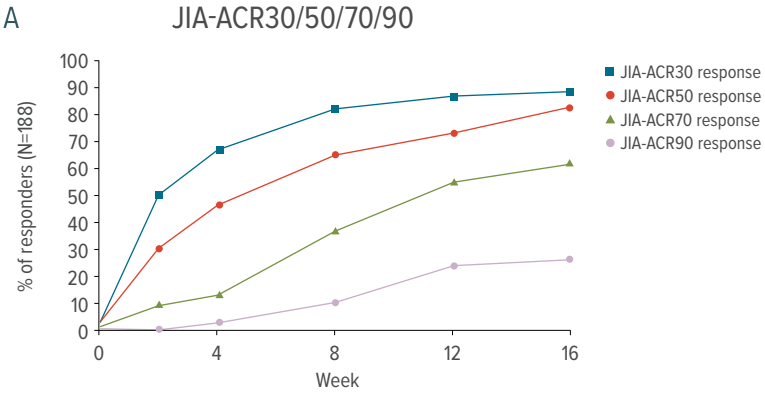
The CHERISH study showed that 89 percent of children treated with tocilizumab markedly improved within 16 weeks, including significantly fewer JIA exacerbations or flares.

The study enrolled 188 patients with active polyarticular JIA, ages 2-17 years. All received monthly infusions of tocilizumab for 16 weeks. Those whose symptoms improved then joined a 24-week, double-blind phase in which half the patients received a placebo and half continued on the drug.

Arthritis symptoms flared in 48 percent of placebo patients versus 26 percent in the tocilizumab group. Side effects, primarily upper respiratory infections, occurred in 8.5 percent of participants.

"The overall pediatric safety profile of tocilizumab was consistent with that seen in adults with rheumatoid arthritis," Brunner says. "Most importantly, tocilizumab provides sustained and clinically meaningful improvement of polyarticular JIA."

A



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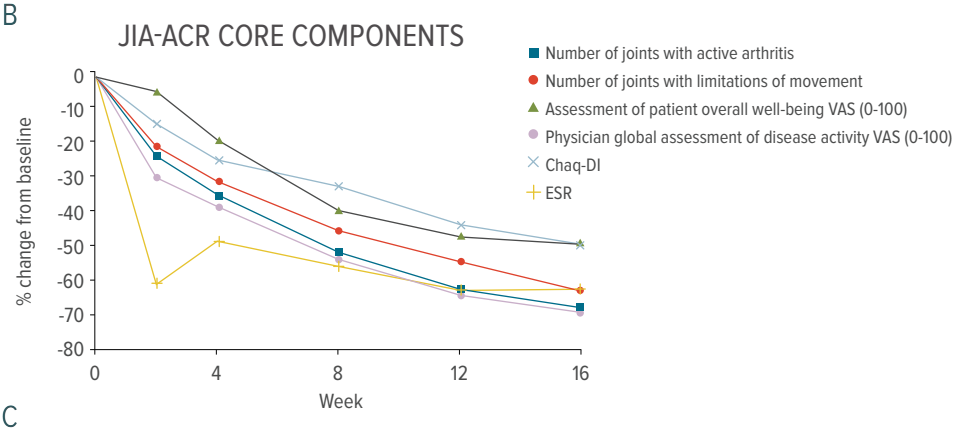

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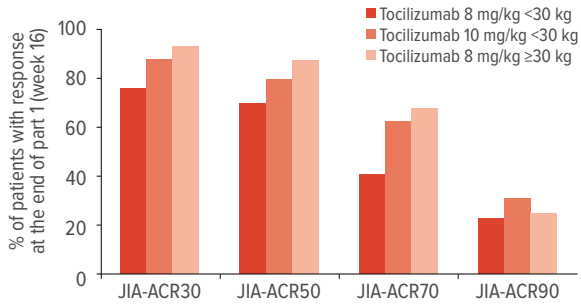

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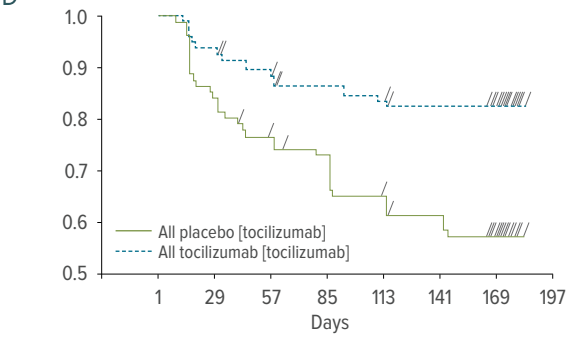


All placebo [tocilizumab] (n)	81	66	58	51	45	43	11	0
All tocilizumab [tocilizumab] (n)	82	75	67	66	61	61	28	0

These four charts, published in the *Annals of Rheumatic Diseases,* show: (A) Improvement of JIA as measured by the American College of Rheumatology Criteria for improvement at JIA-ACR30/50/70/90 levels after open-label treatment with tocilizumab for 16 weeks; (B) Improvements in the individual components contributing to the measurement of JIA-ACR level response of children with polyarticular JIA treated with tocilizumab; (C) Treatment responses considering dosing regimens suggested by pre-trial pharmacokinetic modeling; and (D) Time to JIA-ACR30 flare during the double-blinded phase of the trial when patients with at least a JIA ACR30 response by week 16 were randomized to continue tocilizumab or newly receive placebo.

Football Helmets Do Not Necessarily Protect Against 'Brain Slosh'

Gregory Myer, PhD, FACSM

RESEARCH AND TRAINING DETAILS

Faculty	7
Joint Appointment Faculty	1
Support Personnel	7
Direct Annual Grant Support	$291,407
Direct Annual Industry Support	$164,967
Peer Reviewed Publications	36

Myer GD, Smith D, Barber Foss KD, Dicesare CA, Kiefer AW, Kushner AM, Thomas SM, Sucharew H, Khoury JC. Rates of concussion are lower in National Football League games played at higher altitudes. *J Orthop Sports Phys Ther*. 2014;44(3):164-72.

PUBLISHED JANUARY 2014
Journal of Orthopaedic & Sports Physical Therapy

Can woodpeckers and big horn sheep help solve the troubling and often debilitating problem of football concussions? Does a city's altitude affect the likelihood that its NFL team members will experience concussions? Are better-padded helmets or stricter NFL rules on helmet-to-helmet contact actually solving football's concussion problems?

Yes, yes, and no, according to a study by Gregory Myer, PhD, FACSM, Director of Sports Medicine Research at Cincinnati Children's. Myer led a team of scientists who analyzed 300 concussions sustained by 284 professional football players during the 2012 and 2013 NFL seasons. Their findings, published in the *Journal of Orthopaedic & Sports Physical Therapy*, include:

- "Brain slosh," the rapid acceleration and deceleration of the brain inside the skull, is the suspected common cause of concussions - not direct impact to the head.
- Big horn sheep and woodpeckers, which routinely experience tremendous blows to the head at 10 and 20 times that, respectively, of a head-to-head tackle, appear to be protected from brain injury via modulations of their intracranial blood volume that help make a tighter fit inside their skulls prior to impact. This finding could influence concussion prevention strategies that will better protect human athletes' brains.
- Players in cities at the highest altitudes — Phoenix, Atlanta, Buffalo, Charlotte, Denver, Indianapolis, Kansas City, Minneapolis and Pittsburgh — have a combined 30 percent lower odds of sustaining a concussion relative to players in the NFL's other 23 cities at 644 feet at sea level or lower. At higher altitudes, cerebral blood flow increases, which the research team hypothesized, might influence the brain to fit tighter inside the skull — like a bubble wrapping the brain.

"If we're going to solve this problem, we have to figure out how to protect the brain from the inside out," Myer says. "That's why we think we might be on the leading edge of something that could influence a paradigm shift in concussion-prevention strategies."

HELMET TO HELMET

HELMET TO FIELD

BLOW TO HELMET

body to helmet

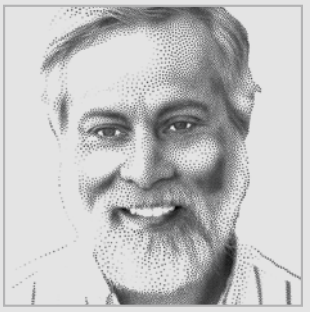
arm to helmet

helmet to facemask

A team of scientists led by Cincinnati Children's analyzed 300 concussions sustained by 284 professional football players during the 2012 and 2013 NFL seasons. Among their findings: Games played in cities with higher altitudes had lower concussion rates, suggesting that topographical mechanisms that increase cerebral blood flow and volume may be useful for protecting the brain from injury during impact sports.

"If we're going to solve this problem, we have to figure out how to protect the brain from the inside out."

Single-Cell Analysis Changes Understanding of Organ Development



Steven Potter, PhD

Joo-Seop Park, PhD

RESEARCH AND TRAINING DETAILS

Faculty	6
Joint Appointment Faculty	2
Research Fellows	1
Support Personnel	15
Direct Annual Grant Support	$220,768
Direct Annual Industry Support	$41,738
Peer Reviewed Publications	18

Brunskill EW, Park JS, Chung E, Chen F, Magella B, Potter SS: Single cell dissection of early kidney development: multilineage priming. *Development* 2014, 141(15):3093-3101

PUBLISHED ONLINE JULY 22, 2014
Development

Researchers examining early kidney development used a recently developed single cell RNA-sequence strategy to create a new atlas of gene expression patterns that has already led to a significant finding on the nature of organ development.

The study, published online in July 2014 in the journal *Development*, included senior author Steven Potter, PhD, Division of Developmental Biology; Joo-Seop Park, PhD, Division of Urology, and research associate Eunah Chung. The study reports that during organogenesis in mice, single cells often express genes related to several developmental pathways — not just a single, predetermined fate. This finding provides powerful evidence of a concept called multilineage priming.

During early development, cells partially activate multiple programs, priming them for their next step in development. This is followed by a combination of gene repressions, which turn off the genes associated with various possible lineages until each cell settles upon its ultimate state. "This was a surprise," Potter says. "We had expected that cells would just gradually fire up the correct program as they assumed their differentiated state."

The team also was surprised to see an abundance of genes with partially degraded noncoding RNA. All these findings are changing our understanding of early organ development, which in turn could lead to new ways to treat or prevent disease.

"As is common in science, this new data raises new questions," Potter says. "How do cells decide which programs to test and how do they make their final decisions? We do not yet know, but new knowledge brings new power. We are moving steadily forward in our quest for groundbreaking therapeutic options."

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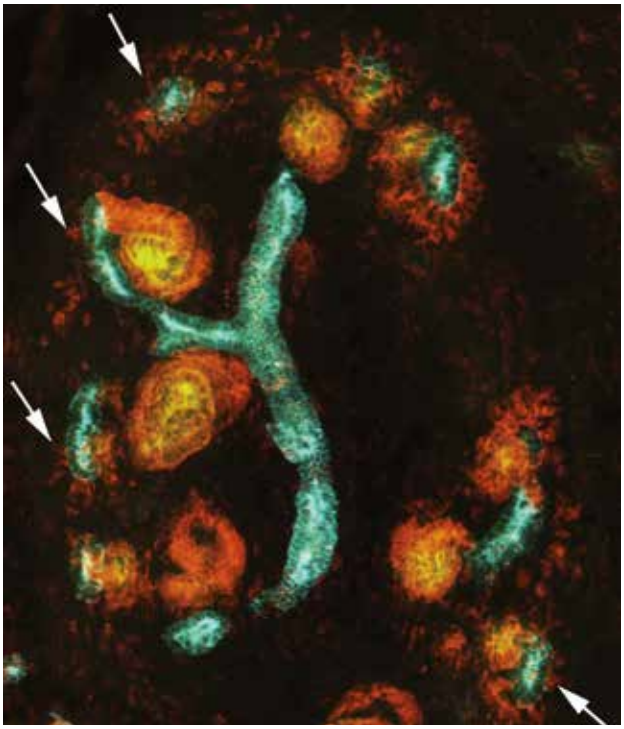
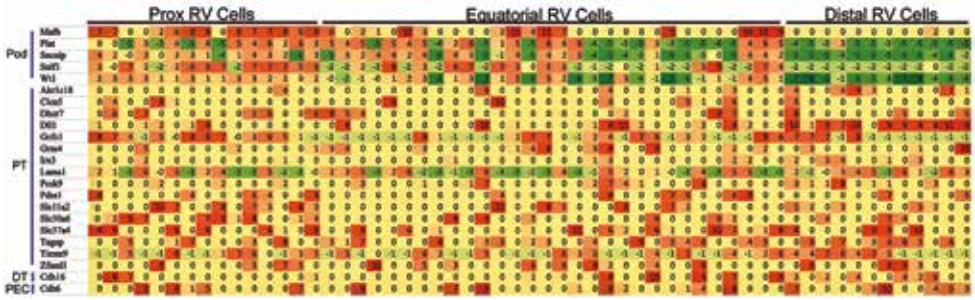


This image shows unexpected Wnt4 expression in progenitor cells that appear to not yet be induced. Wnt4 is normally thought to be expressed only in cells that are induced. Researchers found that during organogenesis in mice, single cells often express genes related to numerous developmental pathways, which bolsters evidence of a concept called multilineage priming.



This heat map shows a diagram of single cells (each column) showing expression of multiple lineages, marked along the left. Individual RV cells express markers of multiple differentiated cell types. Genes normally expressed in differentiated Podocytes (Pod), proximal tubules (PT), distal tubules (DT) and parietal epithelial cells (PEC) are shown on the left. Red represents high expression and green low expression. The numbers represent approximate log, median baseline and normalized expression values.

Shared Facilities

Animal Behavioral Core
Biostatistical Consulting Unit
Cardiovascular Imaging Core
Cell Manipulations Laboratory
Cincinnati Biobank
Comprehensive Mouse and Cancer Core
Confocal Imaging Core
Data Management Center
DNA Sequencing and Genotyping Facility
Gene Expression Core
Imaging Research Center
Mass Spectrometry Facility (Clinical and Biomedical Facility)
MEG Core
Mouse Cytogenetic Core
NMR-Based Metabolomics Core
Pathology Research Core
Pluripotent Stem Cell Facility
Pyrosequencing Core
Research Flow Cytometry Core
Transgenic Animal and Genome Editing Core Facility
Translational Core Labs
Translational Trials Development and Support Laboratory (TTDSL)
Vector Production Facility
Veterinary Services
Viral Vector Core

Accelerating Single-Cell Analysis

Fifteen years ago, in the first important moments of a dawning field, scientists using microarrays carried out cell analysis in what now seem like rudimentary ways.

Say you wanted to know how certain genes operated in kidney development. You would, as S. Steven Potter, PhD, Director of the Gene Expression Core at Cincinnati Children's, explains, take a developing murine kidney sample, "mush it up," amplify its RNA , then see which genes were being expressed.

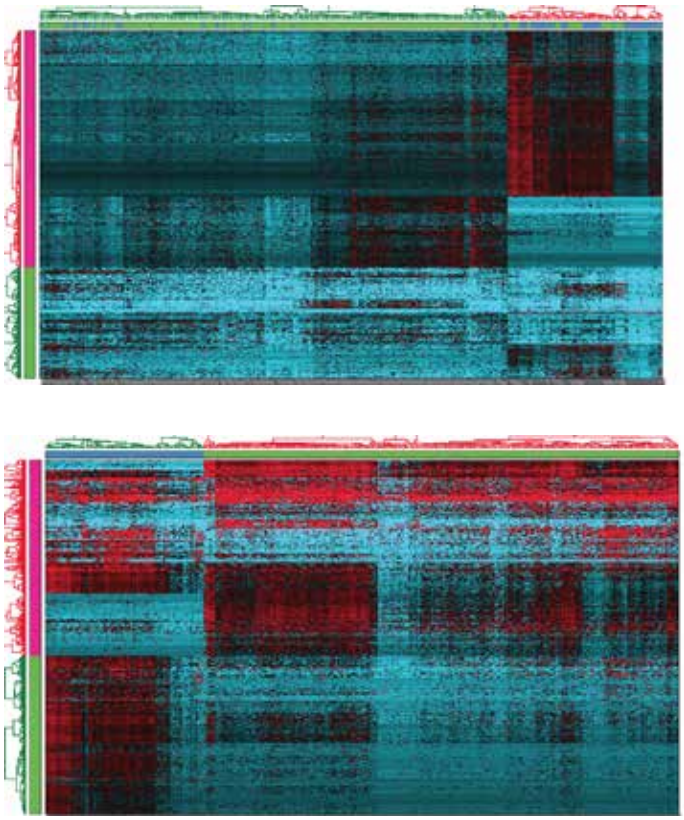
The results were not unlike the early glimpses astronomers had of far off objects in space. The blurry evidence shows something important out there, but with unclear images it was impossible to understand their origins or how they developed. Similarly, in analyzing kidney development using early methods of gene analysis, scientists like Potter could see particular genes expressed in the mush, but could not tell precisely which cells expressed the genes.

> The Gene Expression Core is beta testing a chip that can analzye 800 cells at a time.

Such imperfect, noisy data makes it difficult to understand the normal development of complex organs with multiple cells types, and even harder to detect the points when development goes wrong and leads to congenital malformations and disease.

About five years ago, much better data began to emerge when it became possible for Gene Expression Core scientists to sequence one cell at a time. However, the work was incredibly labor intensive—imagine a project that begins with pipetting one cell at a time into tubes in order to analyze 23,000 genes.

Then two years ago, Cincinnati Children's purchased a $150,000 Fluidigm C1 System, which uses microfluidic chips to sequence up to 96 individual cells, simultaneously. This leap in



capability has supported a wave of important basic science projects at Cincinnati Children's including:

- A study led by Potter, recently published in *Development*, shows that developing kidney stem cells do not differentiate according to a single fated path, but prepare for a variety of developmental paths before committing to one.
- A project led by Potter and James Wells, PhD, Director of Basic Research in the Division of Endocrinology, is developing novel markers for cells in the human gut, defining their complete gene expression profiles for the first time. Their work is beginning to uncover intermediate states between stem cells and differentiated cells.
- Jeffrey Whitsett, PhD, Co-Director of Perinatal Institute, through the NIH-funded LungMap project, is using single cell analysis to better understand gene expressions that drive lung cell formation.

Two years ago, Cincinnati Children's purchased a $150,000 Fluidigm C1 System that can sequence up to 96 individual cells, simultaneously. This leap in capability has supported a wave of important basic science projects.

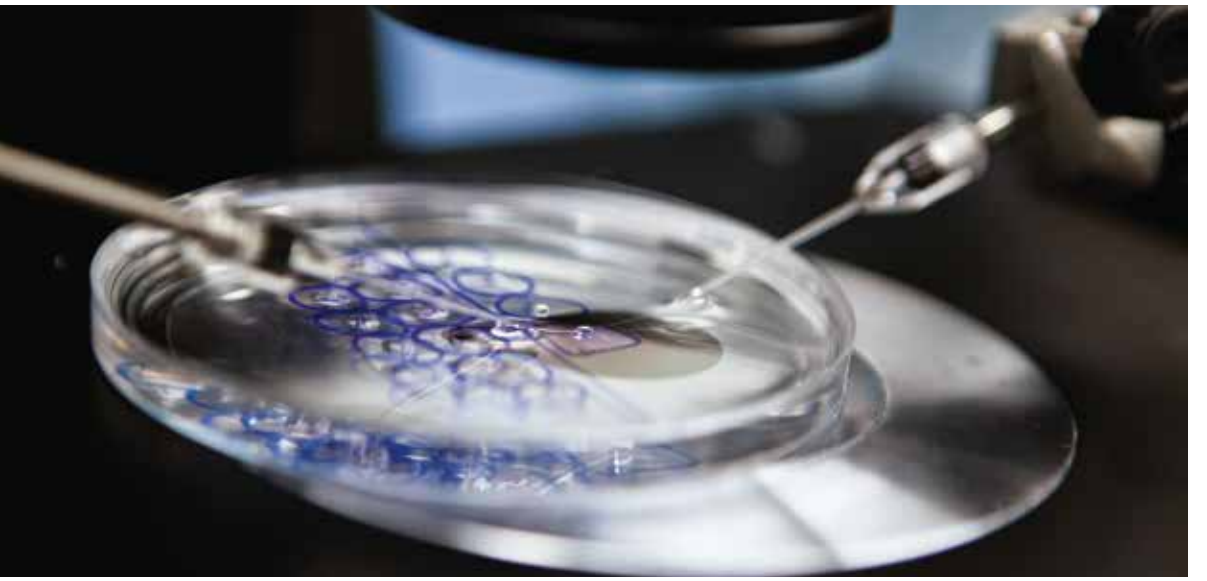
And more automation is coming. The Gene Expression Core is beta testing a new microfluidic chip for Fluidigm, which puts Cincinnati Children's among the first to be able to analyze 800 individual cells at a time.

Now, as researchers here analyze cell development across the kidneys, lungs, gut and immune system, they can obtain a staggeringly detailed series of snapshots along specific developmental pathways.

It is like moving from taking months to find the fuzzy dot of a single far-off galaxy to taking just a few days to see a collection of stars and planets within. With such dramatically improved maps, there is no telling what discoveries the next wave of explorers will make.

Made-to-Order Mice

Transgenic Animal And Genome Editing (TAGE) Core Lab Ushers in a Research Revolution



By knocking in a gene here, knocking out a gene there, Yueh-Chiang Hu, PhD, and colleagues are opening the door to a medical revolution that may soon produce made-to-order treatments for genetic diseases.

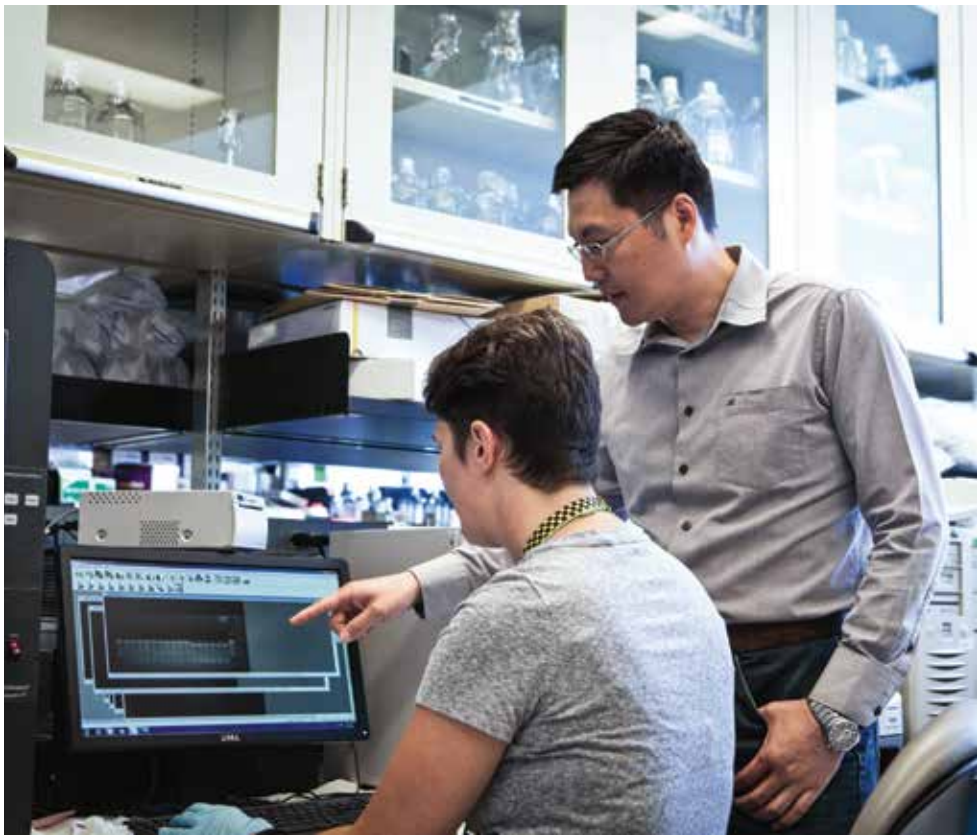
Hu directs the Transgenic Animal and Genome Editing (TAGE) Core at Cincinnati Children's, where a team of scientists use a new technology called CRISPR to rapidly produce gene-edited mice for research, and increasingly, for customized treatment.

> "Usually it takes years to confirm novel genetic variants to be the cause of a disease, but now it is just a matter of few months."

The TAGE core is among the first of its kind in the nation. Hu learned how to use CRISPR technology from one of its pioneers, while completing a post-doctoral fellowship at the MIT-affiliated Whitehead Institute. The technology allows editing, adding or removing any gene in a one-cell (fertilized egg) embryo, dramatically reducing the time it takes to produce biological models expressing specific gene combinations.

"CRISPR is changing the way we do genetic study. You can manipulate the genome in any cell type or organism you want at a previously unimaginable speed," Hu says.

With CRISPR, mouse lines can be developed in a matter of weeks instead of years, and costs have dropped from upwards of $25,000 to as low as $6,000. Since its inception, the core has created 60 new mouse lines, averaging one line a week.



Yueh-Chiang Hu, PhD, and colleagues use CRISPR technology to "edit" mouse genomes by adding or removing genes, allowing mouse lines to be developed within weeks.

The TAGE Core is critical to Cincinnati Children's success in bringing genomics to the bedside, says John Harley, MD, PhD, Director of the Center for Autoimmune Genomics and Etiology. "The facile ability to manipulate chromatin constitution in cells and whole animals opens powerful technologies for genetic insight into normal physiology and the pathophysiology of virtually any disease state," Harley says.

For knock-out mouse lines, in the past six months, 92 percent of mice developed by TAGE were born with the desired mutation. For more challenging knock-in mutations (inserting tags or changing nucleotides), about 50 percent of mice were born with the desired mutation.

Already, TAGE is making a difference for children with rare genetic diseases. Earlier this year, clinical geneticist Taosheng Huang, MD, PhD, was treating three children who suffered a set of identical symptoms including ataxia and seizures. Huang screened the children and found that they shared an otherwise unknown gene mutation.

Within two months, TAGE had developed a line of mice with the children's disease, which has allowed the team to understand disease pathogenesis and test potential treatments. If an off-label treatment can be found, the core lab will have dramatically reduced the cost and time involved in diagnosing and treating a rare disease.

"Usually it takes years to confirm novel genetic variants to be the cause of a disease, but now it is just a matter of few months," Hu says.

Rare Asset: Complete In-House Production to Support Gene Therapy

Gene therapy is one of the most exciting developments in modern medicine. Gene therapy also is one of the most complex capabilities for a medical center to build.

Cincinnati Children's is one of the very few pediatric centers worldwide to successfully assemble every component needed to move this exciting new technology all the way from an intriguing idea to an actual tool for treating disease.

The Translational Core Laboratory at Cincinnati Children's is something special. Here, a Viral Vector Core, Vector Production Facility, Hematopoietic Cell Processing Core, Cell Manipulation Lab and Translational Trial Development and Support Lab (TTDSL) all work in concert to function much like a biopharmaceutical company.

"Other children's hospitals may have some of these facilities, but there are not any that have all of the resources in a single group of laboratories," says Lilith Reeves, Translational Core Administrative Director. "Our benefit is that we can help with most any part of moving cellular and molecular therapies from the researcher's lab through the manufacturing and regulatory steps, all the way into clinic. Then we can continue to do the follow-up on patients after treatment."

Specializing in gene and cell therapies for rare diseases and cancer puts the Translational Core Labs right at the cutting edge of medical research, says Diana Nordling, Translational Core Director. The work happening within the Translational Core supports several Cincinnati Children's investigators as they move potentially breakthrough discoveries far beyond animal studies and into Phase I/II human clinical trials.

The facility produced the carefully manufactured, strictly regulated products to be used for a Phase I/II gene therapy clinical trial for sickle cell disease, which is open to enrollment. Collaborative work is underway with investigators for development of products to be used in new gene therapy treatments for thalassemia and hereditary pulmonary alveolar proteinosis (hPAP).

The facility also has served as a partner in multi-institutional projects, including the recent X-linked severe combined immunodeficiency (X-SCID) gene therapy trial. That trial, which included Boston Children's, UCLA Children's

The 30 team members of our Translational Core Lab have supported projects ranging from a gene therapy clinical trial for sickle cell disease to a multi-center clinical trial to treat X-SCID. The team produces viral vectors and other cell products that meet strict U.S. and European standards.

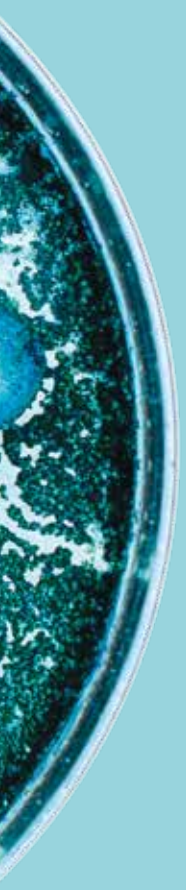
and hospitals in Paris, London and Milan, earned a Distinguished Clinical Research Achievement Award for 2015 and was named one of the Top 10 Clinical Research Awards by the Clinical Research Forum.

This shared facility includes 30 cross-trained researchers and specialists who produce research-quality, pre-clinical and clinical-quality viral vectors, vector modified autologous cell products, along with normal donor hematological cell products. They conduct translational development and process scale-up, translational trials assay support, clinical assay support, assay development, iPSC translation and characterization and more. The lab's key features include:

- Nine ISO-class clean rooms that meet U.S. and EU standards for pharmaceutical grade biologics and cellular-based therapies
- Compliance with Good Manufacturing Practices (GMP) and Good Tissue Practices (GTP)
- A testing lab certified with the American College of Pathology and compliant with the Clinical Laboratory Improvement Amendments (CAP/CLIA)
- Three active Type V Drug Master Files with the FDA.
- FDA Registered GTP Facility

The Translational Core takes on some of the early-stage drug development duties that pharmaceutical makers can be reluctant to perform for products with small potential markets. The core's structure allows a primary investigator to focus on the science while the laboratory team handles the scale-up, validations, and quality oversight.

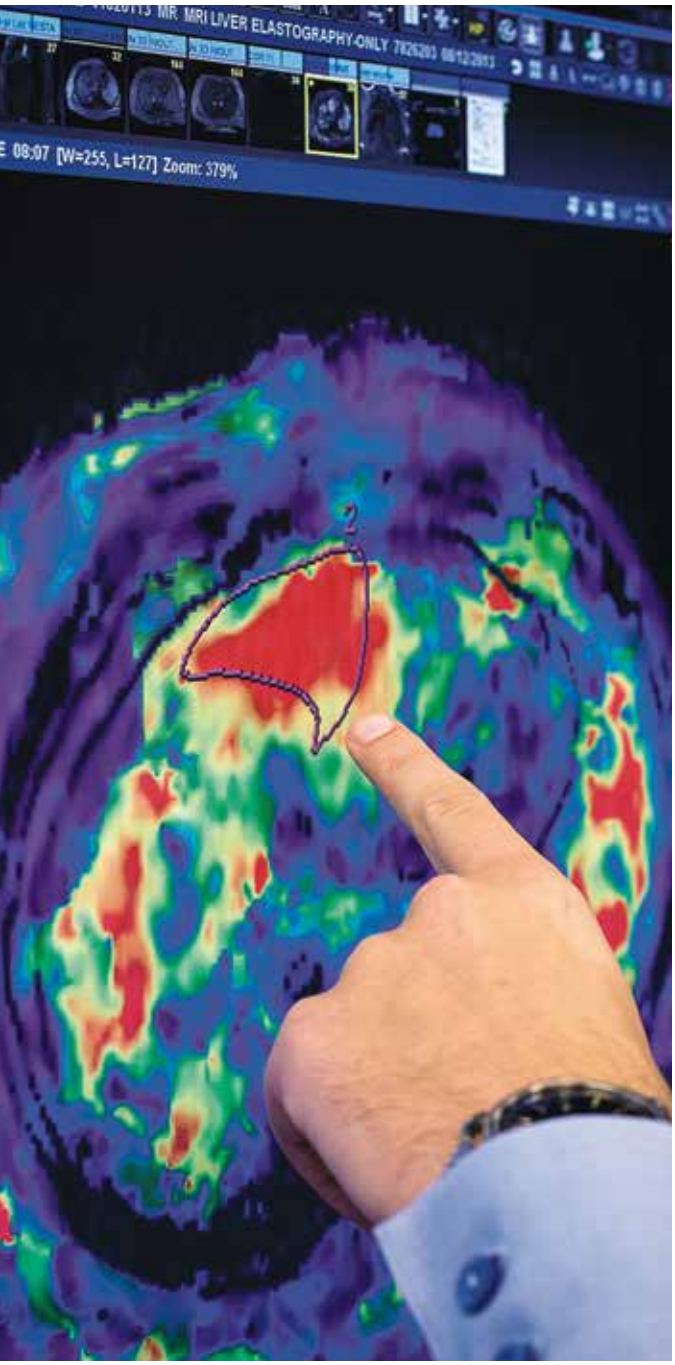
"In addition to performing the manufacturing and testing, we also provide documentation to support the required regulatory submissions," Reeves says.

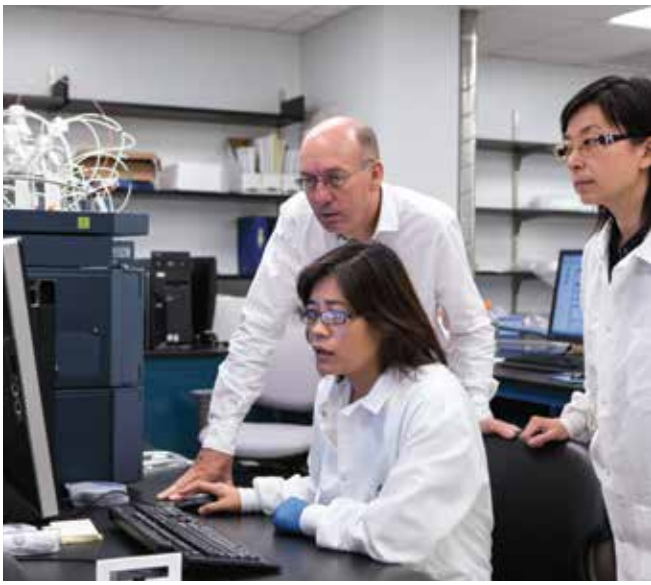


Support Services

The productivity of our research faculty is enhanced by a wide variety of research support programs funded by Cincinnati Children's and the University of Cincinnati. We employ teams of experts who consult with investigators on grant writing, project planning, study design, regulatory compliance, intellectual property protection, and much more. This year, we feature three of these special programs.

Teamwork Behind the Scenes Fuels Major Medical Breakthroughs



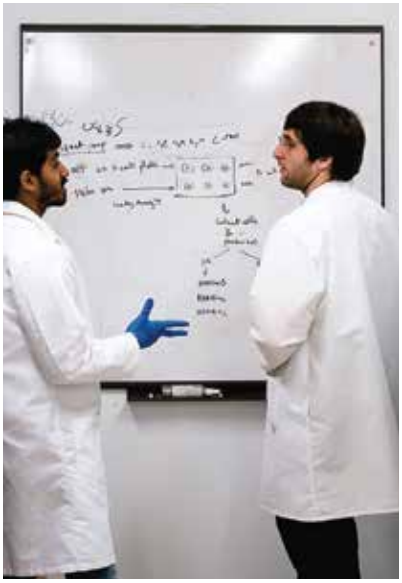


The Center for Clinical and Translational Science and Training (CCTST) provided the investigational backbone that led to the FDA approval of two drugs for rare diseases that have eluded researchers for many years.

The CCTST, based at Cincinnati Children's and led by Director James Heubi, MD, serves the University of Cincinnati and its Academic Health Center partners. Its two most significant accomplishments culminated within a three-month span early in 2015.

An arduous 25-year process led to the March 17 FDA approval of Cholbam (cholic acid) for treatment of bile acid synthesis disorders due to single enzyme defects, conditions estimated to occur once in every 80,000 births.

Spearheaded by Heubi and Kenneth Setchell, PhD, Director of the Clinical Mass Spectrometry Lab at Cincinnati Children's, clinical trials to establish the safety and efficacy of cholic acid therapy were based exclu-



sively in the CCTST's Clinical Translational Research Center (CTRC).

As a result of this investigator-initiated "bench to bedside" drug development — from conceptualization to approval — a daily pill has replaced liver transplant as treatment for these conditions.

Three months later, more major news arrived for the CCTST. Lymphangioleiomyomatosis (LAM) is a rare and frequently fatal lung disease of women, affecting only five people per million. It is characterized by smooth muscle infiltration and cystic destruction of the pulmonary parenchyma. The safety and efficacy of sirolimus (Rapamune) in the treatment of LAM was established by the multi-site MILES study, led by UC's Frank McCormack, MD.

Locally, the trial was based in the CTRC, in collaboration with the Rare Lung Disease Consortium led by Bruce Trapnell, MD, MS, of the Division of Pulmonary Medicine. FDA approval was announced on May 28.

Modern medical research is increasingly about "team science." At Cincinnati Children's, the CCTST is a crucial element of making the goal of team science a functional reality. Consultants with the CCTST help investigators produce the most effective study designs and most competitive grant proposals.

The CCTST was established in 2005 to consult with investigators on research design, help them prepare large multi-institutional grants, and more.

Advancing Promising Discoveries to Market

"Cincinnati Children's researchers discover dozens of health innovations every year. Whether they are prospective molecular targets for treating or diagnosing disease or concepts for new medical devices, many discoveries require additional support get them to the market."

The road to the medical world market crosses the intersection of innovation and collaboration.

The Center for Technology Commercialization (CTC) identifies and protects innovative research and delivers them to the market through licensing and the creation of start-up companies. It also provides crucial funding for emerging projects and facilitates collaborations with external partners.

In the past year, the CTC established three new research collaborations with leading industry partners to identify and advance discoveries towards the market where they can have the greatest impact on patient lives. These partnerships combine Cincinnati Children's research strengths with the drug development and commercial capabilities of world-class pharmaceutical and biotech companies. The collaborations were:

Shire Research Alliance — a multi-year research collaboration designed to discover and develop novel therapies to treat rare diseases with

Vernix is a natural barrier that protects newborn skin, but a synthetic version may someday become part of an anti-aging cream. Meanwhile, a smart sensing catheter conceived at Cincinnati Children's (above) and under early-stage development in Israel may make pediatric surgery safer. Our Center for Technology Commericalization provides the marketing know-how to improve the success of these and other collaborations with companies, including Shire, Alexion and GlaxoSmithKline.

high unmet medical need. This collaboration will provide funding and laboratory research support to selected projects. Shire's therapeutic focus areas include rare diseases, gastroenterology, nephrology and neurology.

Alexion Rare Disease Innovation Award — a unique funding opportunity within Cincinnati Children's Innovation Fund to advance rare disease therapeutics towards commercialization. Alexion is focused on rare and ultra-rare disorders.

GSK Discovery Partnerships with Academia (DPAc) - GlaxoSmithKline R&D is collaborating with Cincinnati Children's to identify and advance innovative therapies. Projects selected are eligible to receive support from GSK such as large scale protein production, medicinal chemistry and PK-PD modeling.

"Cincinnati Children's researchers discover dozens of health innovations every year. Whether they are prospective molecular targets for treating or diagnosing disease or concepts for new medical devices, many discoveries require additional support get them to the market," says Margaret Hostetter, MD, Director, Cincinnati Children's Research Foundation. "This is where our Center for Technology Commercialization comes in—they help innovators leverage internal funding programs and external relationships with industry to advance discoveries towards commercialization."

Blazing the Regulatory Trail From Mouse to Man

The OCTR provides sponsors and investigators with comprehensive support services, research tools, personnel and facilities to support pediatric and adult clinical research studies.

In October 2014, pulmonologist Bruce Trapnell, MD, MS, and his collaborators published a groundbreaking report in the journal *Nature*, demonstrating that transplanting normal or gene-corrected macrophages into the lungs of mice with hereditary pulmonary alveolar proteinosis (PAP) was safe and well-tolerated.

The team showed that a single administration corrected the lung disease and secondary systemic manifestations, normalized disease-related biomarkers, and prevented disease-specific mortality.

This data advanced the future of medicine in which a patient's own cells will be corrected for their disease-causing mutation, reprogrammed and transplanted back into the patient — thus curing their disease.

Translational team members within the Office for Clinical and Translational Research (OCTR) work alongside the basic scientists and clinicians to move these promising results into human studies and treatments. The office pro-

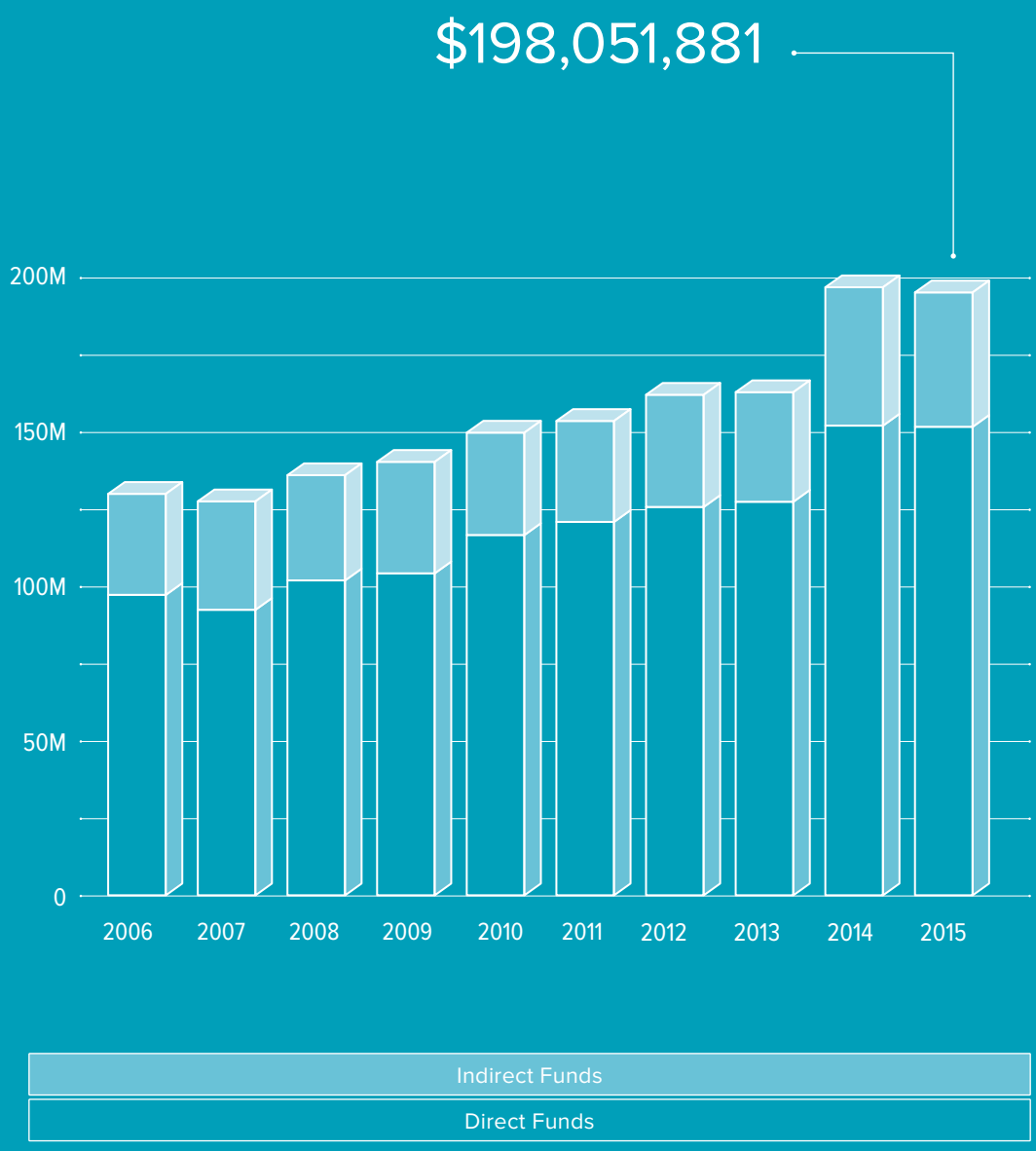
vides sponsors and investigators with comprehensive support services, research tools, personnel and facilities to conduct or facilitate research studies, both pediatric and adult.

During the Trapnell team's pre-clinical work, for instance, an OCTR team of translational specialists concurrently began the exploration of the regulatory pathway forward to begin a treatment in humans based upon the mouse results. This team includes regulatory specialists with strong scientific backgrounds, FDA and IRB expertise and knowledge of clinical trials.

As the work progresses toward first-in-human studies, OCTR experts will navigate the trial through the regulatory approval process, with the safety of all study participants as their foremost concern. Working closely with the Trapnell Lab, the center looks forward to the day on which Cincinnati Children's announces the cure has been found for PAP.

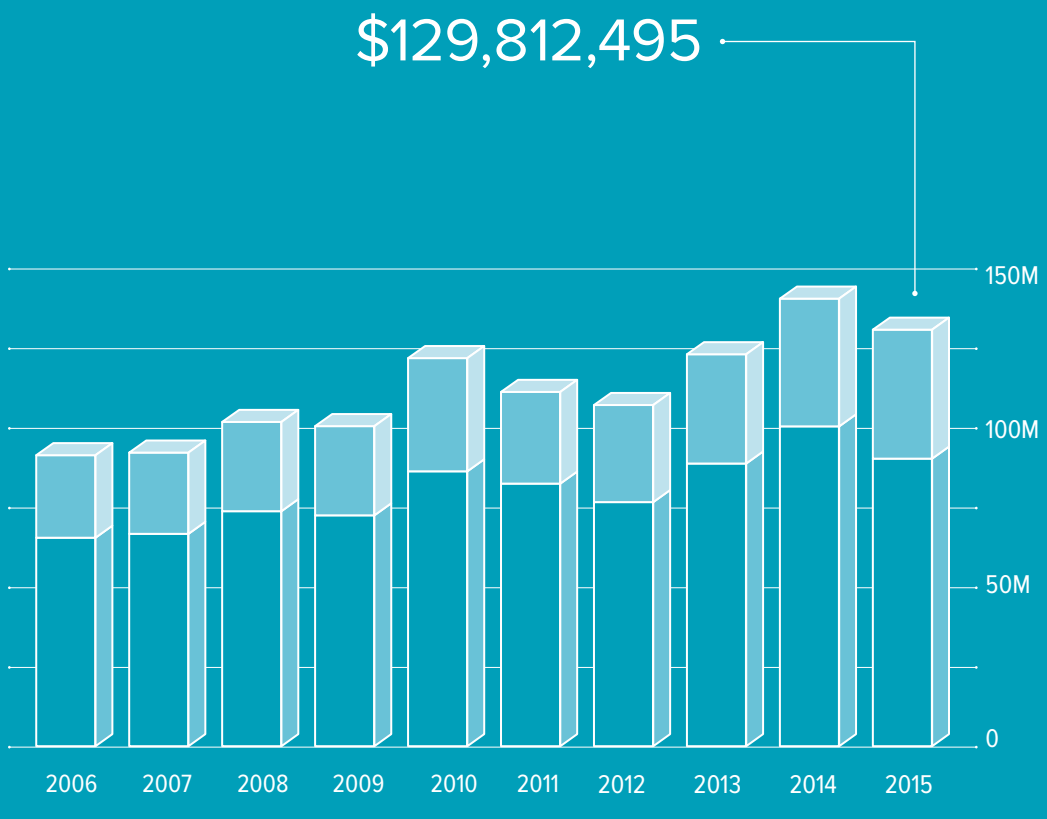
With the 2015 grand opening of our Clinical Sciences Pavilion, families have a state-of-the-art facility to serve their needs as they participate in clinical trials. The OCTR helps clinical trials get off the ground at Cincinnati Children's by assisting investigators as they navigate the complex regulatory requirements involved in human-subjects research.

SPONSORED PROGRAM AWARDS



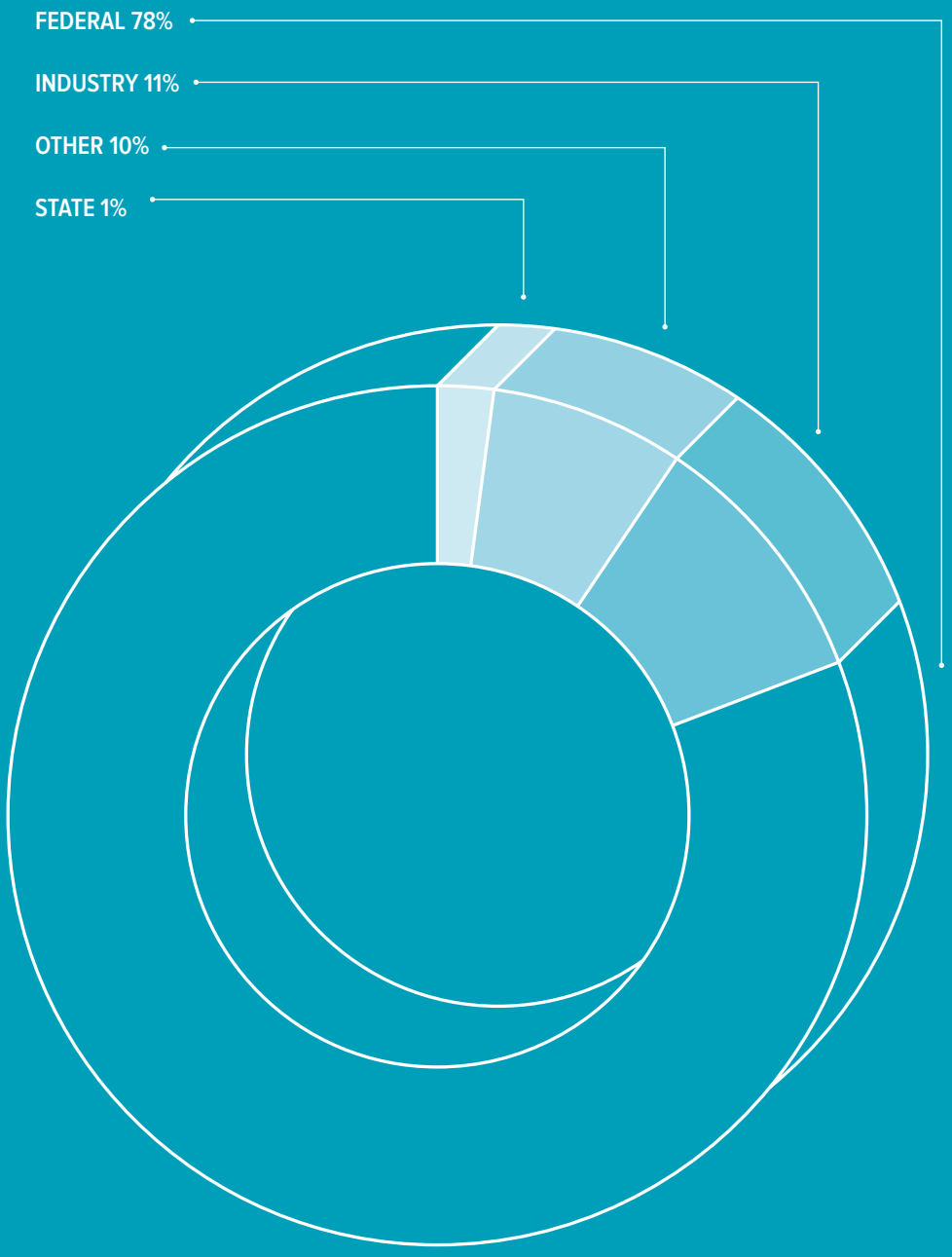
SPONSORED PROGRAM AWARD FIGURES INCLUDE FUNDING AWARDED FOR DIRECT AND INDIRECT COSTS, BUT EXCLUDE FEE-FOR-SERVICE CONTRACTS ($14M IN FY15)

NATIONAL INSTITUTE OF HEALTH AWARDS



APPROXIMATELY $6.8 MILLION OF ARRA AWARDS RECEIVED IN FY10 WERE AWARDED FOR A TWO-YEAR PERIOD. ALL ARE SHOWN IN FY10. APPROXIMATELY $13.7 MILLION OF ARRA AWARDS RECEIVED IN FY11 WERE AWARDED FOR A THREE-YEAR PERIOD. ALL ARE SHOWN IN FY11.

SOURCES OF EXTERNAL FUNDING FY2015



SOURCES OF FEDERAL FUNDING FY2015

National Institutes of Health (NIH)	$129,812,495
Health Resources & Services Administration (HRSA)	$4,607,178
Department of Health and Human Services (DHHS)	$2,910,449
Centers for Disease Control (CDC)	$2,461,323
Agency for Healthcare Research and Quality (AHRQ)	$1,921,412
Center for Medicare/Medicaid Services	$1,625,772
Department of Defense Army (DOD)	$1,620,384
Food & Drug Administration (FDA)	$1,294,474
National Center for Advancing Translational Science	$487,764
U.S. Department of Agriculture	$458,444
National Aeronautics and Space Administration (NASA)	$349,458
U.S. Department of Education	$220,372
Department of Veteran Affairs	$190,266
Department of Homeland Security	$55,544
Department of Justice	$47,087
U.S. Department of Housing & Urban Development (HUD)	$30,651
National Environment Education Foundation	$2,500
TOTAL FUNDING FROM FEDERAL SOURCES	$ 148,086,573

STATE & OTHER FUNDING SOURCES 2015

Patient-Centered Outcome Research Institute
$4,594,603

Bill & Melinda Gates
$4,563,663

March of Dimes
$2,461,897

Ohio Department of Health
$1,810,905

American Heart Association
$1,530,353

Cystic Fibrosis Foundation Therapeutics, Inc
$1,176,959

Crohn's & Colitis Foundation of America
$1,160,558

Miscellaneous Other
$15,004,587

TOTAL STATE AND OTHER FUNDING SOURCES
$ 32,303,525

PHILANTHROPIC GIFTS FOR RESEARCH

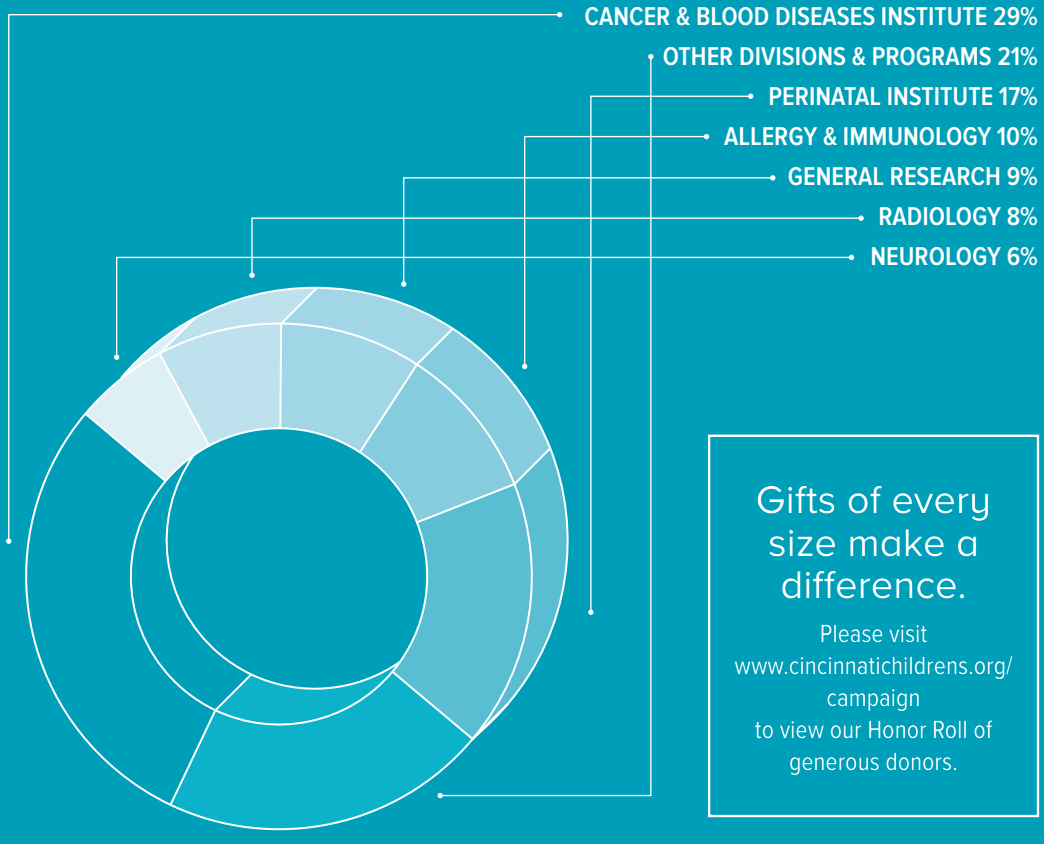
Our commitment to improving care for children through the application of research discovery is the backbone of Cincinnati Children's. And as a nonprofit hospital and research center, private donors play an important role in this work.

OF THE $41 MILLION RAISED THROUGH PHILANTHROPY IN 2015, NEARLY HALF SUPPORTED THE WORK OF OUR RESEARCHERS.

We are profoundly grateful to those who have chosen to partner with Cincinnati Children's to advance scientific innovation and build better futures for kids. Together, we will never stop moving forward to make a difference for children — here in our community and beyond.

$19 MILLION

DONATED TO RESEARCH IN 2015



Gifts of every size make a difference.

Please visit www.cincinnatichildrens.org/campaign to view our Honor Roll of generous donors.

T-32 FELLOWSHIPS

The NIH provides these institutional grants to support research training opportunities at the undergraduate, graduate, and postdoctoral levels. Cincinnati Children's received funding for these T32 programs in FY2015

ADHERENCE PSYCHOLOGY

KEVIN HOMMEL, PHD

BEHAVIORAL MEDICINE & CLINICAL PSYCHOLOGY

SCOTT POWERS, PHD

CLINICAL PHARMACOLOGY

ALEXANDER VINKS, PHARMD, PHD

CRITICAL CARE

HECTOR WONG, MD (CO-P.I.)

GASTROENTEROLOGY

LEE DENSON, MD

GENERAL PEDIATRICS

KRISTEN COPELAND, MD

IMMUNOLOGY

DAVID BERNSTEIN, MD, MA, & MARC ROTHENBERG, MD, PHD

MOLECULAR CARDIOVASCULAR BIOLOGY

JEFFERY MOLKENTIN, PHD

NEONATOLOGY & PULMONARY BIOLOGY

JEFFREY WHITSETT, MD

NEPHROLOGY

PRASAD DEVARAJAN, MD

NEUROLOGY

CHARLES VORHEES, PHD

TRUSTEE AWARDS

This program provides research funds ranging from $30,000 to $75,000 for junior faculty to support rapid achievement of independent, sustained extramural funding for their research. This year's awardees are:

James Bridges, PhD
Neonatology/Pulmonary Biology

Andrew Dimitrijevic, PhD
Communication Sciences Research Center

Joseph Qualls, PhD
Infectious Diseases

Jianqiang Wu, MD
Experimental Hematology and Cancer Biology

Brad Kurowski, MD
Physical Medicine & Rehabilitation

Takahisa Nakamura, PhD
Endocrinology and Developmental Biology

Damien Reynaud, PhD
Experimental Hematology and Cancer Biology

Lilliam Ambroggio, PhD, MPH
Hospital Medicine

Tony DeFalco, PhD
Reproductive Sciences

Yu Lan, PhD
Plastic Surgery and Developmental Biology

Takuji Suzuki, MD, PhD
Pulmonary Biology

Chunyue Yin, PhD
Gastroenterology, Hepatology and Nutrition

Stephen Becker, PhD
Behavioral Medicine & Clinical Psychology

Iouri Chepelev, PhD
Center for Autoimmune Genomics and Etiology

Steven Crone, PhD
Neurosurgery

Michael Rosen, MD, MSCI
Gastroenterology, Hepatology and Nutrition

Matthew Weirauch, PhD
Center for Autoimmune Genomics and Etiology

Mei Xin, PhD,
Experimental Hematology and Cancer Biology

Theresa Alenghat, VMD, PhD
Immunobiology

Zackary Cleveland, PhD
Radiology

Patricia Fulkerson, MD, PhD
Allergery and Immunology

Tzipi Horowitz-Kraus, PhD
General and Community Pediatrics

Douglas Millay, PhD
Heart Institute,
Molecular Cardiovascular Biology

Catherine Quatman-Yates, DPT, PhD
Sports Medicine and Physical Therapy

TRANSLATIONAL RESEARCH AWARDS

These grants, which range from $25,000 to $100,000, support translational research initiatives with potential to transform patient care. The principal investigators receiving funding this year are:

Fred Beyette, PhD, (UC) & Jennifer Kaplan, MD, MS
Critical Care Medicine

H. Leighton Grimes, PhD
Immunobiology

Michael Jordan, MD
Immunobiology

Biplab DasGupta, PhD, MS
CBDI

Parinda Mehta, MD
CBDI

David Haslam, MD
Infection Diseases

Ronald Sacher, MD, (UC) & Yi Zheng, PhD
CBDI

Tsuyoshi Fukuda, PhD
Clinical Pharmacology

Marie-Dominique Filippi, PhD
CBDI

Pranav Shivakumar, PhD
Gastroenterology

Matthew Skelton, PhD
Neurology

Theresa Guilbert, MD, MS
Asthma Center

Kenneth Kaufman, PhD
CAGE

PLACE OUTCOMES RESEARCH AWARDS

This program, administered by the James M. Anderson Center for Health Systems Excellence, provides $60,000 grants to stimulate the development of health services and quality improvement research at Cincinnati Children's and to ensure optimal implementation of clinical and operational innovations in the care delivery system. Awardees receiving funding in FY2015 are:

Katherine Bowers, MPH, PhD
Biostatistics and Epidemiology

Patrick Brady, MD, MSc, & Heather Kaplan, MD, MSCE
Hospital Medicine/Perinatal Center

Rita Pickler, PhD
Nursing

Jayant Pratap, MA, MB, & Lisa Vaughn, PhD
Anesthesia/Emergency Medicine

Joshua Schaffzin, MD, PhD
Hospital Medicine

Julia Anixt, MD, & William Brinkman, MD
Developmental and Behavioral Pediatrics/ General and Community Pediatrics

Samir Shah, MD, MSCE, & Lilliam Ambroggio, PhD, MPH
both with Hospital Medicine

Ni Yizhao, PhD
Biomedical Informatics

Nancy Daraiseh, PhD, & Drew Barzman, MD
CPE Research /Psychiatry

DIVERSITY & HEALTH DISPARITIES RESEARCH AWARD

This award provides $75,000 research grants to junior faculty members who are from underrepresented minorities and/or have a strong commitment to health disparities research. The inaugural recipient of this award is:

Tesfaye Mersha, PhD
Asthma Research,
for "Genetic Ancestry and Racial Disparities In Childhood Asthma"

STRAUSS FELLOWS

The Arnold W. Strauss Fellow Award is a one-year $10,000 funding opportunity awarded to three clinical fellows (MDs) and three postdoctoral fellows (PhDs). It was instituted in 2014 in honor of Dr. Strauss' tireless championship of higher education at Cincinnati Children's. The 2014 Strauss Fellows are:

Nihal Bakeer, MD
Cancer and Blood Diseases Institute

Yoshinobu Odaka, PhD
Ophthalmology

Amanda Schondelmeyer, MD
Hospital Medicine

Changwen Zhang, PhD
Gastroenterology,Hepatology & Nutrition

Fuli Xiang, PhD
Molecular Cardiovascular Biology

Nicole Sheanon, MD
Endocrinology

FIFTH THIRD BANK / CHARLOTTE R. SCHMIDLAPP WOMEN SCHOLARS

This program provides $50,000 grants to support the academic career development of women faculty who have demonstrated academic potential and leadership skills as they progress toward the ranks of associate and full professor. This year's awardees are:

Jareen Meinzen-Derr, PhD
Biostatistics & Epidemiology

Fumika Hamada, PhD
Ophthalmology/Visual Systems Group

PROCTER SCHOLARS

The Procter Scholar Program supports faculty members from the Departments of Pediatrics, Surgery, Radiology, Patient Services, and Anesthesia who are committed to pursuing academic research careers.

3RD YEAR SCHOLARS

Benjamin Mizukawa, MD
Oncology

Andrew Beck, MD, MPH
General & Community Pediatrics

Brian Varisco, MD
Critical Care Medicine

2ND YEAR SCHOLARS

Jennifer Davis, DO
Oncology

Andrew Lindsley, MD, PhD
Allergy & Immunology

Jeffrey Tenney, MD, PhD
Neurology

Patrick McGann, MD
Hematology

1ST YEAR SCHOLARS

Hitesh Deshmukh, MD, PhD
Neonatology

Ernest Pedapati, MD
Psychiatry

CHILD HEALTH RESEARCH CAREER DEVELOPMENT AWARDS (CHRCDA)

This program provides $93,000 grants to support training physician-scientists to stimulate pediatric research across a variety of disciplines. This year's awardees are:

Andrew Lindsley, MD, PhD
Allergy and Immunology

Phil Minar, MD
Gastroenterology, Hepatology and Nutrition

Kasiani Myers, MD
Bone Marrow Transplant and Immune Deficiency (CBDI)

Elizabeth Schlaudecker, MD, MPH
Infectious Diseases/Global Child Health Center

ACADEMIC AND RESEARCH COMMITTEE AWARDS

These grants support multi-disciplinary programs that can become self-sustaining within one to three years. Awardees this year are:

Rashmi Hegde, PhD
Developmental Biology

Carole Lannon, MD, MPH
Anderson Center

Brian Coley, MD
Radiology

Stella Davies, MBBS, PhD, MRCP
CBDI

John van Aalst, MD
Plastic Surgery

Lee Denson, MD
Gastroenterology

James Greenberg, MD
Perinatal Institute

Michael Helmrath, MD, MS
General and Thoracic Surgery

Thomas Inge, MD, PhD
General and Thoracic Surgery

Jaimie Nathan, MD
General and Thoracic Surgery

Nancy Ratner, PhD
CBDI

Harinder Singh, PhD
Immunobiology

Scott Holland, PhD
Research in Patient Services

Richard Lang, PhD
Ophthalmology

Jeffery Molkentin, PhD
Heart Institute

GAP FUNDING AWARDS

THIS PROGRAM PROVIDES ONE YEAR OF SUPPORT AT $75,000 TO MAINTAIN R-LEVEL RESEARCH PROGRAMS AT THE NIH OR THE EQUIVALENT.

Ronald Waclaw, MS, PhD
CBDI

Dimitar Deliyski, PhD
Otolaryngology & Communication

Satoshi Namekawa, PhD
Reproductive Sciences

John Harley, MD, PhD
CAGE

Basilia Zingarelli, PhD
Critical Care

RESEARCH, INNOVATION & PILOT FUNDING AWARDS

This program provides $75,000 grants to support collecting preliminary data for innovative and essential projects considered to be good candidates for future external funding. Awardees receiving funding this year include:

Stephen Waggoner, PhD
CAGE

Richard Strait, MD
Emergency Medicine

Jennifer Vannest, PhD
Neurology

Richard Lang, PhD
Ophthalmology

Yueh-Chiang Hu, PhD
Developmental Biology

Joseph Qualls, PhD
Infectious Disease

Ying Sun, PhD
Human Genetics

Fumika Hamada, PhD
Ophthalmology

Dao Pan, PhD
CBDI

Kenneth Kaufman, PhD
CAGE

Jason Woods, PhD
Pulmonary Medicine

Pooja Khandelwal, MD
CBDI

Lindsey Romick-Rosendale, PhD
Pathology

Senthilkumar Sadhasivam, MD, MPH
Anesthesia

CINCINNATI CHILDREN'S INNOVATION FUND

The Cincinnati Children's Innovation Fund is designed to accelerate the commercialization of discoveries, innovations, projects or products. Recipients this year are:

Daniel von Allmen, MD
General and Thoracic Surgery

Alexander Vinks, PharmD, PhD
Clinical Pharmacology

Michael Helmrath, MD, MS
General and Thoracic Surgery

John Pestian, PhD, MBA
Biomedical Informatics

Sonata Jodele, MD
Bone Marrow Transplantation and Immune Deficiency

CENTER FOR PEDIATRIC GENOMICS PILOT STUDIES

The Center for Pediatric Genomics (CpG) has established a fund to distribute $1 million annually among projects that accelerate innovative research, development, and implementation of genomic science. Awardees this year are:

Hansel Greiner, MD
Neurology

Rolf Stottman, PhD
Human Genetics

Sonata Jodele, MD
Bone Marrow Transplant & Immune Deficiency

Kenneth Kaufman, PhD
CAGE

Taosheng Huang, MD PhD
Human Genetics

Derek Neilson, MD
Human Genetics

S. Steven Potter, PhD
Developmental Biology

Kasiani Myers, MD
Bone Marrow Transplant & Immune Deficiency

James Wells, PhD
Developmental Biology

Senthilkumar Sadhasivam, MD MPH
Pain Management

Chunyue Yin, PhD &
Alexander Miethke, MD
Gastroenterology, Hepatology & Nutrition

ALEXION RARE DISEASE INNOVATION FUND

Alexion Pharmaceuticals and Cincinnati Children's have established a collaboration to fund the advancement of research in rare disease. Following completion of the funded research programs, Alexion will have an exclusive option to enter into a licensing agreement for these programs. This year's recipients are:

Andrew Dauber, MD, MMSc
Endocrinology

Manoj Pandey, PhD
Human Genetics

OUR FACULTY

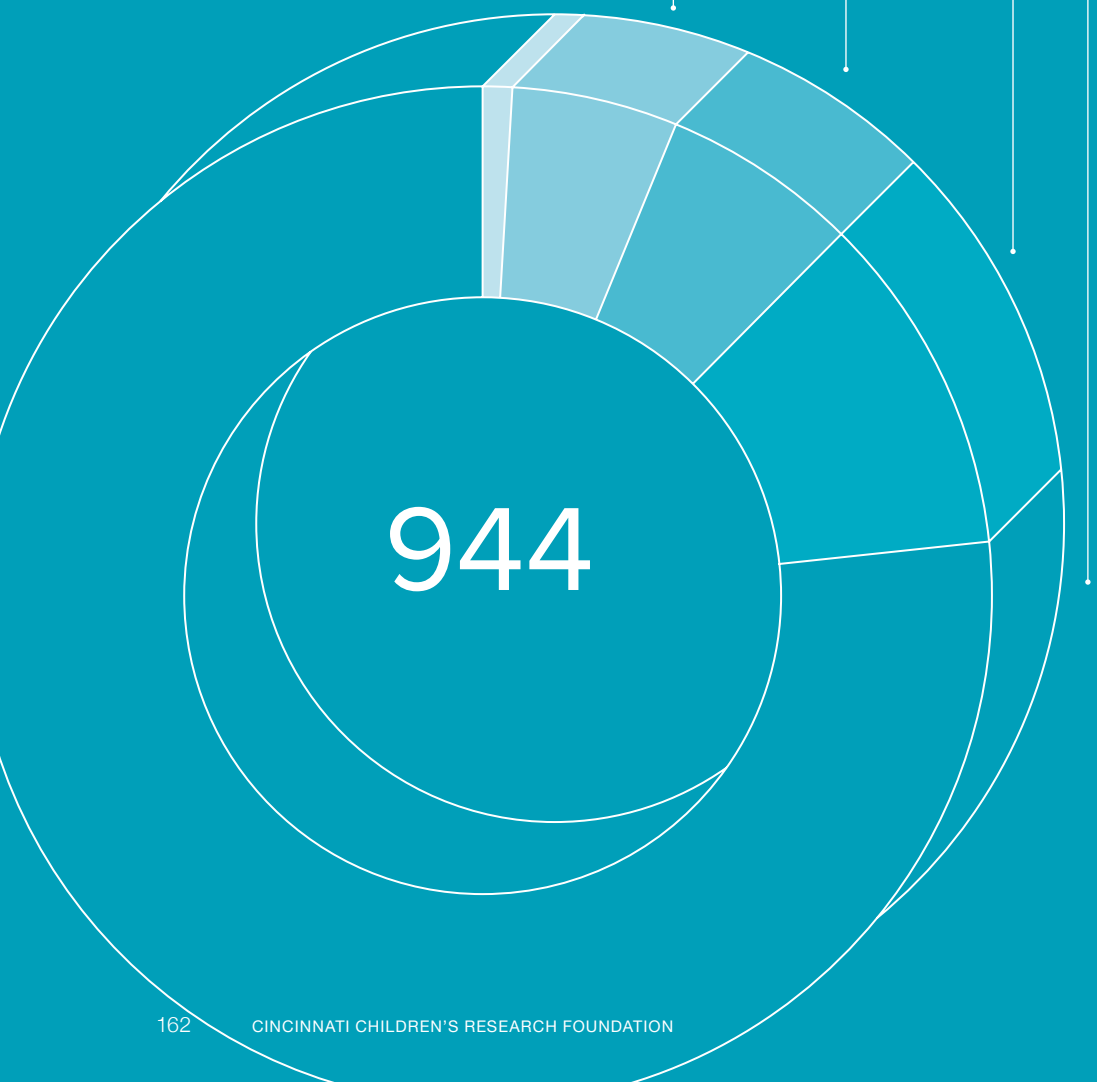
PEDIATRICS
724 total [674 full time / 50 part time]

SURGERY
103 total [93 full time / 10 part time]

ANESTHESIA
59 total [47 full time / 12 part time]

RADIOLOGY
50 total [42 Full time / 8 part time]

PATIENT SERVICES
8 Full time



POSTDOCTORAL FELLOWSHIPS

6	Allergy
5	Anesthesia
2	Biomedical Informatics
1	Biostatistics and Epidemiology
1	Bone Marrow Transplantation
5	CAGE
1	Cardiology
3	Center for Prevention of Preterm Birth
3	Clinical Pharmacology
18	Developmental Biology
3	Endocrinology
26	Experimental Hematology
4	Gastroenterology
2	General Pediatrics
4	Hematology
4	Human Genetics
10	Immunobiology
1	Infectious Diseases
19	Molecular Cardiovascular Biology
5	Neurology
3	Neonatology, Perinatal and Pulmonary Biology
5	Oncology
4	Pathology
2	Patient Services
13	Pediatric Surgery
4	Pulmonary Medicine
8	Reproductive Sciences
162	TOTAL POSTDOCTORAL FELLOWSHIPS

PEDIATRIC HOUSE STAFF RECRUITMENT: 414

Pediatrics	Medicine/ Pediatrics	PM&R	Psychiatry/ Child Psychiatry/ Pediatrics	Human Genetics/ Pediatrics	Neuro/ Pediatrics
275	70	4	26	8	31

CLINICAL FELLOWSHIPS: 263

Adolescent Medicine	6
+ Transition Medicine	0
Allergy/Immunology	5
Anesthesia	12
+ ABA Alternate Pathway	1
+ Adv. Fellowship Quality Improvement & Safety	1
+ Adv. Ped. Anesthesia Fellowship Education	0
+ Intraoperative Neurophysiological Monitoring	1
+ Pediatric & Congenital Cardiac Anesthesia	1
Cardiology	13
+ Adult & Adolescent Congenital Heart Disease	1
+ Cardiac Electrophysiology	1
+ Cardiac Imaging	1
+ Cardiac Intensive Care	0
+ Fetal Cardiology	1
+ Heart Failure, Cadiomyopathy & Transplant	1
+ Interventional Cardiac Cath	1
+ Preventive Cardiology	1
Child Abuse	2
Congenital Cardiac Surgery	1

Critical Care	13
Developmental Behavioral Pediatrics	5
Emergency Medicine	11
Endocrinology	10
Gastroenterology	12
+ Pediatric Transplant Hepatology	1
Division of General Pediatrics	0
+ Pediatric Master Educator	2
+ Pediatric Primary Care Research	4
Genetics, Medical	1
+ Clinical Biochemical Genetics	1
+ Clinical Cytogenics	0
+ Clinical Molecular Genetics	4
Hematology/Oncology	12
+ Academic Research in Pediatric Hem/Onc	2
+ Bone Marrow Transplant	3
+ Clinical Immunodeficiency	0
+ Neuro-Oncology	2
+ Sickle Cell Disease	0
Hospice & Palliative Medicine	2

MEDICAL STUDENTS: 195

Junior Medical Students in the Pediatric Clerkship	Senior Medical Students entered Pediatric Training	Senior Medical Students entered Medicine/ Pediatric Training	Pediatrics/ Neurology
171	14	5	5

MEDICAL RESIDENTS: 438

Hospital Medicine	6
Infectious Disease	4
Neonatology	14
Nephrology	7
+ Acute Care Nephrology & Dialysis	0
Neurology	0
+ Clinical Neurophysiology	1
+ Pediatric Epilepsy	1
+ Neurocritical Care	1
Neurosurgery	1
Ophthalmology	1
Orthopaedics	2
+ Hand & Upper Extremity Surgery	2
+ Surgery of the Spine	1
Otolaryngology	6
Pain Medicine	2
Pathology	2
Plastic Surgery	1
Psychiatry	9
Psychology	17

Pulmonary	9
Radiology	9
+ Body MRI	0
+ Interventional Radiology	0
+ Pediatric Neuroradiology	2
Rehabilitation Medicine	3
Rheumatology	6
Sleep Disorder Medicine	3
Sports Medicine	2
Surgery	2
+ Pediatric and Adolescent Gynecology	2
+ Colorectal Surgery	1
+ ECMO	1
+ Fetal Surgery	1
+ Pediatric International Surgical Fellow	1
+ Trauma Surgery	1
+ Vascular Anomalies	1
Urology	2
+ International Pediatric Urology Fellow	2
TOTAL	263

RESEARCH GRADUATE PROGRAMS

MDB Graduate Program	MSTP Program	Immunology Program	Biomedical Informatics
58 STUDENTS (53 PHD/5 MS)	52 STUDENTS	56 STUDENTS (39 PHD/17 MS)	13 STUDENTS

SUMMER RESEARCH PROGRAMS

Medical Students Program	Undergraduate Students	Summer for Sickle Cell Science Program	High School Interns
18 (INCLUDING 10 IN SMURRF PROGRAM)	106 (SURF)	6	24 (19 High School Senior Summer Interns, 5 BRIMS)

PEDIATRIC SCIENTIST DEVELOPMENT PROGRAM

This program provides intensive training to prepare entry-level faculty for research careers in academic pediatrics.

2014	2015
8 RECIPIENTS	6 RECIPIENTS

ADMINISTRATIVE STAFF

Margaret Hostetter, MD
Director

Derek Wheeler, MD
Associate Chair, Clinical Affairs

Rob Kahn, MD, MPH
Associate Chair, Community Health

Tom DeWitt, MD
Associate Chair, Education

Lori Stark, PhD
Associate Chair, Finance

Evie Alessandrini, MD
Associate Chair, Outcomes

Sandra Degen, PhD
Associate Chair, Promotion & Academic Affairs

Tracy Glauser, MD
Jeff Whitsett, MD
Associate Chairs, Research

Lou Muglia, MD, PhD
Associate Chair, Strategic Partnerships

John Maybury
Vice President, CCRF

Jana Bazzoli
Vice President, Clinical Affairs

Kristine Justus, PhD
Vice President, Research Operations

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PUBLISHED RESEARCH 2015

1,837
PEER-REVIEWED ARTICLES

54
NON-PEER REVIEWED ARTICLES

7
BOOKS
(EDITED OR AUTHORED)

181
CHAPTERS OF BOOKS

17
ONLINE SITE CONTRIBUTIONS

2,096
TOTAL PUBLICATIONS

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EDITORS:
Tim Bonfield
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Vicki Davis
Grant reporting, reviewing and publishing

Clare Douglas
Communication and data coordination

Steven Gentle
IT website developer

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Training and coordination, annual report publications process

Sue MacDonald
Contributing writer, print research annual report

Tom O'Neill
Contributing writer, print research annual report

Sarah Stankorb
Contributing writer, print research annual report

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Reed DeWinter, Julie Kramer, and Michael Wilson

DESIGN:
The Fairview Agency

PRODUCED BY:
Department of Marketing and Communications
Jane Garvey, Vice President
Cincinnati Children's Hospital Medical Center
3333 Burnet Ave., MLC 9012
Cincinnati, OH 45229-3026
513-636-4420

CINCINNATI CHILDREN'S HOSPITAL MEDICAL CENTER
3333 BURNET AVE, MLC 9012
CINCINNATI, OH 45229-3026