Core Profile: Pediatric Biomarkers Core

The Biomarkers Core provides state-of-the-art equipment and up-to-date technology to provide high quality analysis of biological samples in the fastest turnaround time possible to support pediatric research. This core provides equipment and technical expertise to assay samples using methods that combine the features of gas-liquid chromatography and mass spectrometry. Headed by core director Lou Ann Brown, PhD (pictured right) and technical director Frank Harris (pictured far right), the Biomarkers Core is always ready to work with investigators to develop protocols to suit their needs.

Inside this newsletter, check out recent work from the Dawson Lab and the DeGrauw and Kuan Labs that the Biomarkers Core helped support.

Current analyses
- Biomarkers of oxidative stress including reduced and oxidized glutathione, cysteine, cystine, isoprostanes, hydroxynonenal, and malonyldialdehyde
- Fatty Acid Ethyl Esters (FAEE) from biological samples such as meconium, hair, placenta, blood, and plasma as markers of alcohol use and exposure
- Intracellular levels of ribonucleoside triphosphates and deoxynucleoside triphosphates, useful in the study of cellular metabolism and nucleic acid synthesis
- Creatine levels in cells and urine for the study of creatine disorders

Current Equipment
- Waters High Performance Liquid Chromatography with Fluorescence Detector
- HP Agilent Gas Chromatograph/Mass Spectrometer
- Thermo Finnigan TSQ Quantum Triple Quad LC/MS/MS
- Other analyte analysis available, including ELISA, colorimetric, or fluorometric assays, contact core director to inquire

Check out the Biomarkers Core on www.pedsresearch.org
Bile acids and Intestinal Disease: Is there a role for wayward detergents?

Dawson Lab Studying Bile Acids in Disease

Our lab studies the role of bile acids in pediatric and adult disease. Bile acids are made by the liver, stored in the gallbladder, and function as detergents to help absorb dietary fats and fat-soluble vitamins from the intestine and as hormones to regulate hepatic and gastrointestinal function.

Unfortunately, these same detergent properties also have a dark side and can contribute to disease. The liver and intestine express a series of transporters that recirculate bile acids for re-use with each meal, and also work to keep these potentially cytotoxic detergents from accumulating in cells. In the small intestine, bile acids are absorbed from the small intestinal lumen by the enterocyte apical sodium dependent bile acid transporter (ASBT) and then pumped out of the cell by the organic solute transporter α-β (Ostα-Ostβ, SLC51A/B) (1). Our lab discovered that inherited mutations in the ASBT gene (SLC10A2) causes Primary Bile Acid Malabsorption and are responsible for some forms of intractable diarrhea of infancy (OMIM 613291). However, we also wondered if inherited or functional defects in Ostα and bile acid export contributes to neonatal or pediatric disease. Particularly because there may be a connection between bile acids, oxidative stress, and Necrotizing enterocolitis, a common cause of neonatal gastrointestinal-related morbidity and mortality in low birth weight preterm infants (2-6). We reasoned that impeding export of bile acids from enterocytes may cause these detergents to accumulate in the cell and induce intestinal injury. For these studies, we used a mouse model in which the export transporter gene Ostα (SLC51A) was inactivated and looked for signs of injury during neonatal development. We found morphological, histological, and gene expression evidence of early intestinal injury followed by a healing response. The injury correlated with bile acid trapping, and may involve bile acid-induced oxidative stress. However, we needed a validated and sensitive assay system to measure biomarkers of oxidative stress in very small amounts of intestinal tissue to investigate this mechanism.

Collaborating with the Biomarkers Core

We were familiar with Dr. Lou Ann Brown’s Biomarkers Core and interacted with Frank Harris on several occasions regarding the use of their LC-MS method for our research. It was natural and beneficial then to enlist the Biomarkers Core lab to measure the levels of reduced and oxidized glutathione, cysteine, and cystine in intestinal tissue samples for our study. Frank was terrific. He was able to process and analyze the samples, provide the data, and answer questions in a prompt manner. The results from the Biomarkers Core complemented our original findings. In aggregate, the data suggests that enterocytes engage several repair mechanisms to protect against bile acid-induced injury and oxidative stress. Some of this work was presented as a plenary session talk at the American Gastroenterological Association Meeting in 2016 (7). We are grateful for the assistance of Dr. Lou Ann Brown and Frank. We look forward to continuing our relationship with the Biomarkers Core as we work to translate these findings to patients with pediatric intestinal disease and ultimately new therapies.

“...submitted by Courtney B. Ferrebee, Graduate Student (pictured) & Paul A. Dawson, PhD, Professor of Pediatrics

Developing Tools to Study Creatine Deficiency

Creatine Transporter-Deficient Mice

Drs. Chia-Yi (Alex) Kuan and Ton DeGrauw are collaborating to study the neuropathology and experimental therapies of Creatine Transporter (CrT) deficiency using CrT-deficient mice. The CrT deficiency, first reported by Dr. DeGrauw in 2001, is the second most common cause of X-linked mental retardation (next to the Fragile X syndrome), but far less studied. Patients with this condition lack creatine (Cr) in the central nervous system, as shown by the proton magnetic resonance spectroscopy (1H-MRS). The neuropathological basis of mental retardation in CrT deficiency is not known; children with CrT deficiency do not respond to Cr supplement therapy. Thus, there is a need to better understand CrT deficiency in order to develop effective treatments.

Moreover, since creatine phosphate (PCr) is believed to be the major energy reserve to replenish cellular ATP (of a smaller quantity) when it is exhausted, it is of high interest to investigate how neurons cope with stress in the absence of Cr and PCr. To this end, Drs. Kuan and DeGrauw have generated mice lacking CrT, which is also located in X-chromosome in the mouse genome. They have confirmed great reduction of the brain Cr and PCr levels on MRS (collaboration with Dr. Hui Mao) and uncovered, for the first time, severe dendritic spine dysgenesis in CrT mutant mice (Figure: A: CrT+/y wildtype; B: CrT-/y mutant).

Biomarkers Core Partnership

Next, Drs. Kuan and DeGrauw set out to determine whether CrT is needed merely at the blood-brain-barrier or also at neuronal surface to transport Cr from blood and the extracellular milieu, respectively. Dr. Lou Ann Brown and Mr. Frank Harris helped the research team to develop a Cr uptake assay using gas chromatography-mass spectrometry (GC-MS) to compare the abilities of wildtype and CrT-null neurons to absorb Cr from the culture. This study is still ongoing, but the pilot data strongly suggest that CrT-null neurons fail to uptake Cr from the culture medium. This finding has profound implications on how to treat children with CrT deficiency. It suggests that the Cr supplement therapy is unlikely to help these patients (since CrT-deficient neurons cannot absorb Cr from outside). Rather, gene therapy or supplement of Cr-analogues that can cross the blood-brain-barrier and neuronal cell body are more promising options.

Again, the Biomarker Core made important contributions to Drs. Kuan and DeGrauw’s research of CrT deficiency.

~submitted by Chia-Yi (Alex) Kuan, MD, PhD, Associate Professor of Pediatrics
Getting to Know the Animal Physiology Core

After joining Emory 14 years ago, I recently became the technical director of the Pediatric Animal Physiology Core. My most passionate area of interest is animal survival surgeries, but I also have significant experience in Molecular biology, Cellular biology, Immunology and Hematology. My major role in the Core is to provide technical support in both small animal survival surgery and small animal ultrasound.

Surgical services currently offered by the Core include Pulmonary Aortic Banding, Ascending Aortic Banding, Transverse Aortic Constriction, Myocardial Infarction, 5/6th Nephrectomy for Chronic Kidney Disease, Mouse Liver Ischemia and Reperfusion, Hind Limb Ischemia, Rodent Heterotopic Ear-Heart Transplant, Bone Marrow Transplant, and Rat Jugular and Carotid Cannulation. I also work with investigators to develop new surgical and imaging services to meet each principal investigator’s needs.

I utilize the Visualsonics Vevo 2100 High Frequency Ultrasound System which is a high resolution small animal ultrasound system in the Core. This equipment is critical for researchers who wish to perform non-invasive measure of structure and function in small animals. In particular, comprehensive cardiac exams, characterization of liver and kidney blood flow, measures of arterial stiffness and imaging of tumor growth are some examples of my specialties.

The newly-acquired Vevo LAZR add-on system for the Visualsonic incorporates photoacoustic imaging into high-resolution ultrasound for anatomical, functional, and molecular imaging. Photoacoustic imaging is a new in vivo hybrid imaging modality that combines the sensitivity and contrast of optical imaging with the depth and resolution of ultrasound. The ultrasound imaging provides a high-resolution frame of reference for identifying anatomy, while the photoacoustic imaging enables functional measurements such as oxygen saturation, total hemoglobin and the microdistribution of biomarkers.

Animal Physiology Core Team

We have an ideal team to perform the best service for all the principle investigators. Dr. Josh Maxwell, the director of the Core, assists investigators in accessing the Core, developing strategies suited to their needs, and utilizing all that we have to offer. Kristen Herzegh, the administrator of the core, handles much of the budgeting details which allows us focus on the scientific research and technique development and ensures the investigators have no worries about administrative affairs.

Outside the Lab

Beside the life of the lab, I am a wife, a mom of two teenage boys, a dog lover, and a volleyball enthusiast. I participate in Zumba and Pilates after work. I am also a volunteer at the children missionary in my church.

~submitted by Ming Shen, Animal Physiology Core
**Instrument Profile: Lattice Light Sheet**

**Brand New Technology in the ICI Core**

Light sheet microscopes introduce one or more additional lenses into the standard single lens setup, and with it brings several advantages, such as increased imaging distance for larger samples, more uniform 3D resolution, faster volume acquisitions, and dramatically reduced photodamage. These benefits allow researchers to follow life in 4D (x,y,z & time) in a way not previously possible. The Lattice Light Sheet (LLS) microscope based in the Winship Cancer Institute has been developed for imaging small samples (single cell level) at high resolution and speed for long durations. The dramatic reduction in photodamage (of cells) and photobleaching (of fluorescent labels) allows intrinsically rapid cellular processes to be followed for longer than any previously available microscope.

To learn more about this technology, visit [https://www.intelligent-imaging.com/lattice](https://www.intelligent-imaging.com/lattice)

~submitted by Neil Anthony, PhD, Integrated Cellular Imaging Core

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**Core Pilot Awardees**

In the fall of 2016, the ICI Core awarded pilots to support education on fluorescence microscopy in pediatric research. The Flow Cytometry Core awarded mini-pilots for subsidized used of the Amnis ImageStream instrument.

**ICI Pilots Winter 2016-2017**

- Katherine Minson, MD—The Role of Nuclear-localized MERTK in Pediatric Acute Leukemia
- Periasamy Selvaraj, PhD—Targeted Delivery of Tumor Vaccines to Antigen Presenting Cells of the Immune System
- Joanna Goldberg, PhD—Interactions of Cystic Fibrosis Pathogens Visualized by Fluorescence Microscopy

**Flow Cytometry Core Mini-Pilots Fall 2016**

- Edward Quach (Li Lab)—Elucidating the mechanism of IVIG resistance in Immune Thrombocytopenia
- Mandy Ford, MD—ImageStream Analysis of CD45 Isoform Localization on Memory CD8+ T cells
- Lokesh Guglani, MD—Detection of premature aging markers in cystic fibrosis children using FISH-IFC
- Glaivy Batsuli, MD—Determining Factor VIII B-cell Epitopes Recognized by Anti-Factor VIII Monoclonal Antibodies That Mediate FVIII Uptake by Dendritic Cells
- Charles Searles, PHD—The Use of ImageStream to Study Microparticles
Publication Highlight: GPIb-IX-V complex and platelet signaling


Editor’s Note: Dr. Deng’s recent publication in Nature Communications included work produced in the ICI and Flow Cytometry Cores. The work continues with another project from Rehano Li’s lab, which is supported by a Flow Cytometry Core mini-pilot

GPIb-IX-V Glycoprotein

GPIb-IX-V is a glycoprotein complex expressed abundantly on the platelet surface. GPIb-IX-V is the primary receptor for Von Willebrand Factor (VWF) that is immobilized to the site of injury in blood vessel endothelia. Once it detects a ligand, GPIb-IX-V quickly transfers signals into the platelet to stimulate its activation, which further triggers the downstream coagulation cascade. Interestingly, the VWF binding site on GPIb-IX-V complex is located at the N-terminus of GPIba, distal from the membrane surface. How, then, does the GPIb-IX-V complex turn distal binding signals into activation signals and transduce them into the platelet? We now know that mechanical shear force is required for GPIb-IX-V mediated signaling. Indeed, changing the applied shear force significantly alters the level of GPIb-IX-V induced platelet activation.

This knowledge supports the hypothesis that there is a “mechanosensory” domain in GPIb-IX-V that is responsible for sensing the shear force in the blood stream and regulating GPIb-IX-V signaling. A mutation in this domain could turn on or off the GPIb-IX-V signaling automatically. However, testing this hypothesis directly in platelet has been greatly hindered by the difficulty of making mutagenic platelets. Instead, making a mutant cell line expressing GPIb-IX-V complex is convenient and practical. It has also been established that filopodia (a long, slender cytoplasmic projections) from the GPIb-IX-V transfected cells is mediated by GPIb-IX-V signaling. Herein, we were seeking to test the aforementioned hypothesis using cell imaging technology. The screening of the positively transfected cells was conducted with the assistance of Aaron Rae, from the Pediatric Flow Cytometry Core, who did fantastic job in high-speed cell sorting.

While I was searching for resources to image the cells transfected with different mutants of the GPIb-IX-V complex, I attended an ICI Data Club seminar and become aware that the ICI core is equipped with several high-resolution confocal optical fluorescence microscopes. Afterward, I discussed the experimental plan with Neil Anthony, PhD, who later assisted me in optimizing the method and acquiring image data. Meanwhile, during the data collection period, the new DeltaVision OMX super resolution fluorescence microscope was also equipped in ICI core.

One limitation of traditional confocal fluorescence microscopy is optical bleaching. Due to the slow data acquiring speed, a sample is often lighted for minutes to get a 10-µm z-stack scan. The signal intensity becomes so weak in the end that the upper portion of the image was often too dim to tell any information. The OMX super-resolution fluorescence microscope has a much faster data acquiring speed and improved resolution so that the details in the middle or upper portion of the cells become clear. I became the first user of OMX in campus and spent several months optimizing and improving the data quality. I was also granted ICI Pilot funding to support my research. Thanks to indispensable support from the ICI core, I have recently published our study in Nature Communications in a paper addressing how the mutations in the mechanosensory domain affect GPIb-IX-V signaling.

I am very grateful to the ICI Core for their collaboration on this cutting edge study. I look forward to continued collaboration with the ICI Core and Flow Cytometry Core.

~submitted by Wei Deng, PhD, Pediatrics Instructor (Renhao Li, PhD Lab)
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How to Acknowledge the Cores:

These cores are generously supported by Children's Healthcare of Atlanta and Emory University. When presenting or publishing work completed using the core, please include "Children's Healthcare of Atlanta and Emory University [core name]" in the acknowledgments.

Mark your calendars for the Emory School of Medicine Core Day: April 28, 2017!

This newsletter serves to highlight the activities of the cores supported by Emory University’s Department of Pediatrics and Children’s Healthcare of Atlanta. If you have a story idea for a future edition, please contact Karen Kennedy (kmurra5@emory.edu).