Charles H. Hood Foundation

January 2016 Award Recipients

- **Jenna Galloway, Ph.D.**
  Assistant Professor of Orthopaedic Surgery
  MGH Center for Regenerative Medicine

"The Role of a Novel Pathway in Vertebrate Tendon Development"

Key Words: Tendon formation, Musculoskeletal development, Congenital skeletal disorders and contractures

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Tendons provide the connections between your muscles and bones and enable coordinated movement. Defects in their development can result in contractures and clubfoot, congenital abnormalities in which tendons and skeletal structures are malformed causing restricted movement. Treatments for children with these conditions involve multiple surgeries and extensive physical therapy. Although the primary underlying causes of these conditions are unknown, they are thought to be multifactorial and involve the aberrant development of the connective tissues. Therefore, comprehensive knowledge of the molecular pathways that guide tendon development would have broad impact in our understanding of the etiology of these types of congenital defects. Surprisingly, we understand very little about the induction and development of tendon progenitor cells.

To address this gap in our knowledge, we are using the zebrafish model system to discover new pathways that affect early tendon formation. We have shown that zebrafish and mammalian tendons are similar in gene expression, developmental regulation, and ultrastructural properties, making them an excellent system for studying tendon biology. Previous screening efforts identified a new pathway to have an important role in regulating tendon progenitor cells in the developing zebrafish embryo. Inhibition of this pathway caused a significant expansion of tendon markers in zebrafish and mouse systems, demonstrating conservation of activity across vertebrates. This unique phenotype will allow us to dissect the pathways regulating tendon cell populations during musculoskeletal development. We propose to elucidate the mechanisms underlying the expansion of tendon tissues using molecular and genetic assays in the zebrafish. We propose that by understanding how this pathway regulates tendon progenitor cell numbers, we will gain valuable mechanistic insight into tendon developmental mechanisms. Knowledge of these processes will further impact our understanding of congenital musculoskeletal abnormalities.

• **Shangqin Guo, Ph.D.**
Assistant Professor of Cell Biology
Yale School of Medicine

"Molecular Definition of Cancer Cell-of-Origin in B-ALL"

Key Words: Cell-of-origin, B-ALL, cell fate, hematopoietic progenitors, MLL-AF9

Acute lymphoblastic leukemia (ALL) is the most common cancer type in children. Elucidating its etiology is crucial for devising strategies to alleviate this leading cause of childhood morbidity and mortality. The prevailing models for how cancers arise cannot explain why malignancy develops so early in life without multiple genetic abnormalities. A new conceptual framework for how malignancy arises from the cell-of-origin is necessary to better understand how ALL develops. Our recent work identified that transient cell cycle acceleration to a critical threshold speed (~8 hours/cycle) is a key step limiting pluripotency induction from somatic cells. Since the cell fate control mechanism subverted in pluripotency induction is fundamentally analogous to that altered during malignant transformation, our work provided the conceptual foundation where cell cycle kinetics dictate how the epigenome responds to genetic insult. Thus, I propose to test whether the transiently ultrafast-cycling hematopoietic progenitors are permissive for transformation due to their unique epigenetic state, using one of the rearranged MLL-fusion oncogene MLL-AF9 as a model. We have generated novel oncogene models and obtained supportive preliminary data in the initiation of acute myeloid leukemias. We propose to use these models to test whether the initiation of ALL follows the same principle at cellular and molecular level. While it is long appreciated that proliferative cell types are more prone to malignancies, this has been attributed to the replicative errors accumulated during successive divisions. An alternative explanation, which distinguishes my hypothesis, is that the epigenetic state of fast cycling cells is inherently more amenable to cell fate altering genetic activities. By defining the leukemia cell-of-origin in molecular details, this project promises new insights into basic cancer mechanisms and etiology, as well as critical implications for cancer prevention and treatment.

- **Read Pukkila-Worley, M.D.**
  Assistant Professor of Medicine
  University of Massachusetts Medical School

  "Homeostatic Regulation of Innate Immune Activation in Intestinal Epithelial Cells"

  Key Words: Innate Immune Activation, p38 MAP Kinase Pathway, Inflammatory Bowel Disease

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Inflammatory Bowel Disease (IBD) is a debilitating, chronic illness of the gastrointestinal tract. Up to one fifth of patients with IBD present as children, and those that do often have fulminant disease marked by severe intestinal inflammation. For reasons that are not fully understood, intestinal epithelial cells in patients with IBD mount inappropriate immune responses towards normally innocuous intestinal flora. The induction of inflammatory cascades in these cells occurs via the p38 Mitogen-Activated Protein Kinase (MAPK) signaling cassette, a highly conserved defense regulatory pathway found in nematodes and mammals. We have uncovered an ancient mechanism of p38 MAPK pathway regulation using the powerful model Caenorhabditis elegans. In a classical genetic screen, we identified a G protein-coupled receptor, which we have shown acts to control p38 MAPK-driven inflammatory responses. Here we will characterize the mechanism of immune suppression by this GPCR in C. elegans (Aim 1) and define a conserved role for this receptor in the regulation of inflammatory responses in mammalian intestinal epithelial cells (Aim 2). The long-term goal of this work is to facilitate the development of new therapies for inflammatory disorders of the intestine by characterizing a novel and ancient mechanism of homeostatic innate immune pathway regulation.

• **Vijay Rathinam, Ph.D.**
Assistant Professor
UConn Health School of Medicine

"Inflammatory Mechanisms in Enteric Bacterial Disease"

Key Words: Enteric bacteria, LPS, Caspase-11, Inflammasome, Innate

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Diarrheal diseases due to microbial infections are the second most common cause of mortality in children worldwide. Enterohemorrhagic E. coli (EHEC) is the causative agent of hemorrhagic colitis and the life-threatening hemolytic uremic syndrome (HUS) in children. Owing to our poor understanding of immunological and microbial mechanisms involved in gastrointestinal illnesses, effective vaccines and therapies are still lacking. The unmet need therefore is to identify pathogenic mechanisms responsible for diarrheal diseases.

Innate immune system is central to the sensing of invading pathogens and the activation of the host immune response. A diverse set of germ-line encoded innate immune receptors survey nearly all-cellular compartments for the presence of pathogens and their products. Inflammasomes are multi-protein scaffolds in the cytosol containing a NLR receptor, an adapter ASC, and an effector, caspase-1. Inflammasome is an integral part of the immunosurveillance of the cytosol. Inflammasomes directly detect various "signature" microbial products or indirectly sense signs associated with an infection. Although lipopolysaccharide (LPS) of Gram-negative bacteria was believed to be exclusively detected at the cell surface by Toll-like receptor-4 (TLR4), it has very recently been described that the LPS is sensed in the cytosol in a TLR4-independent manner by caspase-11, an inflammatory caspase. Activation of caspase-11 by intracellular LPS leads to the proteolytic activation of caspase-1, which then executes the activation of IL-1beta and IL-18. Importantly, active caspase-11 triggers an inflammatory form of cell death (pyroptosis) and the release of endogenous alarmin or danger molecules that perpetuate the inflammatory reactions. The overall goal of this study is to define the role of cytosolic LPS sensing during enteric infections.

July 2016 Award Recipients

- **April Craft, Ph.D.**
  Assistant Professor of Orthopaedic Surgery
  Boston Children's Hospital

  "Identifying Mechanisms of Cartilage Homeostasis and Degeneration Through the Use of Patient Specific iPSCs"

  Key Words: Joint Degeneration, Musculoskeletal Disease, Cartilage, Pluripotent Stem Cells

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Novel strategies are needed when standard approaches fail to uncover the mechanism by which a mutation causes disease. This is the case for the severe, degenerative joint disease that occurs in children with Progressive Pseudorheumatoid Arthropathy of Childhood (PPAC), caused by loss of function mutations in WISP3. PPAC becomes symptomatic between the ages of 3 and 8, and then rapidly progresses to end-stage articular cartilage failure. PPAC children thus require total hip and knee replacement surgeries in their teenage years.

Researchers have failed to determine why WISP3 deficient cartilage fails precociously because patient samples collected at the time of arthroplasty resemble end-stage osteoarthritis, and mice lacking Wisp3 have no disease phenotype. In vitro cell culture studies have suggested multiple biologic activities for WISP3; yet the cartilage-specific relevance of these findings is uncertain. Therefore, to better understand PPAC, we have generated induced pluripotent stem cells (iPSCs) from 5 patients with PPAC and will now differentiate these iPSCs into two different cartilage lineages, articular and growth plate cartilage. In an unbiased approach, we will compare RNA, histologic, cell biologic, and biochemical profiles of these in vitro cartilages to those generated from wild-type iPSCs, PPAC iPSCs that had their WISP3 mutation corrected using Crispr/Cas9, and wild-type iPSCs that had WISP3 inactivated using Crispr/Cas9. Because cartilage degeneration occurs during childhood and is not present at birth, we hypothesize that mechanical stress can contribute to disease pathogenesis. Thus we will characterize the response of PPAC cartilages to mechanical and environmental stresses to identify mechanisms by which cartilage homeostasis is perturbed.

The knowledge we gain about mechanisms that lead to cartilage failure will benefit patients with PPAC, and could also point to new approaches for protecting cartilage from damage associated with other skeletal dysplasias and more common forms of degenerative joint disease.

- **George Dragoi, M.D., Ph.D.**
  Assistant Professor of Psychiatry and Neuroscience
  Massachusetts Institute of Technology

  "Brain mechanisms underlying cognitive development during childhood"

  Key Words: Hippocampus, Spatial representation, Developing rat, Electrophysiology, Neuropsychiatric disorders

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The long-term objective of this proposal is to reveal the brain mechanisms underlying the formation of internal representations of the external spatial world within hippocampal-neocortical networks which support innate and learned behavior from childhood to adult. Our previous work has shown that, in adulthood, learned information is not encoded in isolation, but is integrated within a network of pre-existing knowledge stored in patterns of neuronal ensemble functional connectivity which can be thought as pre-representations. Particularly, novel spatial experiences can be encoded in the hippocampus, in part, by the selection of blocks of pre-existing neuronal firing sequences from a larger internal repertoire identifiable during the preceding sleep (i.e., pre-representations), rather than by exclusively creating the sequences in response to external cues. The rapid selection of pre-existing cellular firing sequences could be essential to the role of the hippocampus in rapid learning, internally generated spatial-temporal representations, and in ascribing specific valence to particular new experiences based on prior knowledge. However, what remains unknown is how the emergence of these pre-representation patterns in the brain is modified by specific external factors active during childhood, and how they are disrupted in early-onset neuropsychiatric diseases.

In this proposal, we plan to investigate how early-life environmental factors affect the rapid formation of new memories and their consolidation during sleep in normally-raised and in deprived developing juvenile animals. To achieve these goals, we combine large-scale electrophysiological recordings of neuronal ensembles in freely-behaving rats, behavioral manipulations, and computational methods for decoding neuronal population activity. Our goal is to gain a detailed understanding of the emergence, organization, and function of neuronal ensembles and to use this to identify neural mechanisms underlying the formation of spatial-temporal representations in the normal brain, with implications for understanding and treatment of early-onset neuropsychiatric disorders like autism and schizophrenia.

- **Benjamin Lee, M.D.**
  Assistant Professor of Pediatrics
  University of Vermont College of Medicine

  "The Effects of Increased Inoculum on Oral Rotavirus Vaccine Take and Immunogenicity"

  Key Words: Rotavirus, Vaccine take, Fecal shedding, Immunogenicity, Correlates of protection

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The long-term goal of this project is to identify strategies to improve oral rotavirus vaccine performance in low-income countries. The burden of rotavirus disease remains unacceptably high in low-income countries, where current vaccines show markedly diminished efficacy. The reason(s) for vaccine underperformance remain unclear.

One potential mechanism is rapid inhibition of the live-attenuated vaccine-strain virus in the infant gut due to gut inflammation from enteric coinfections and/or maternal antibodies, preventing establishment of replicating infection by the vaccine, or vaccine "take." Vaccine take is an absolute requirement for immune recognition and subsequent protection (i.e. effective vaccination), and is identified by detection of fecal vaccine shedding or by host antibody response. Previous experiments with oral cholera vaccines have demonstrated that an increased inoculum of vaccine improves host immune responses, but this strategy remains unexplored for rotavirus. Furthermore, the currently accepted marker vaccine immunogenicity, rotavirus-specific serum IgA, correlates poorly with vaccine efficacy in low-income countries; improved markers are urgently needed to inform future vaccine trials.

Therefore, we will perform a randomized, controlled trial of standard versus high dose oral rotavirus vaccine among infants in Dhaka, Bangladesh. In Aim 1, we will evaluate the effects of increased vaccine inoculum on vaccine take as measured by fecal shedding and serum IgA seroconversion. In Aim 2, we will evaluate the performance of serum antibodies directed against the rotavirus outer capsid structural proteins VP4 and VP7 as alternate markers of immunogenicity as compared to serum IgA. If vaccine take and markers of immunogenicity can be improved using this approach, these strategies could be rapidly implemented in the field to improve rotavirus vaccine performance and inform next-generation vaccine trials design. Together, these findings have the potential to significantly decrease the burden of rotavirus disease and prevent thousands of deaths in children around the globe.

- **Farrah Mateen, M.D., Ph.D.**
  Assistant Professor of Neurology
  Massachusetts General Hospital

  “Remote diagnosis of pediatric epilepsy through a smartphone-based EEG”

  Key Words: Brain Disorders, Epilepsy, Neurodevelopment, Neurology.

The majority of children with epilepsy (CWE) live in low- and middle-income countries (LMICs). Electroencephalography (EEG) aids the diagnosis and characterization of epilepsy in higher income settings, but can be especially difficult to access among CWE in resource-limited locations and LMICs. Smartphones, owned by more than 2.6 billion people, now offer a potential solution for the distribution of EEG to populations globally. In this study in the lower-income, Himalayan Kingdom of Bhutan, we will test and implement a multi-platform, open-source, software application that combines off-the-shelf EEG caps with a smartphone to acquire EEG in children with seizures and epilepsy. This represents a low-cost, real-time, fully portable EEG imaging system that obviates the need for paper tracings, EEG technicians, and consistent electrical supplies. The smartphone based EEG will be clinically tested in 150 children with poorly controlled epilepsy -- operationally defined as children who have experienced at least two unprovoked seizures in the past year while taking at least one anti-epileptic drug. The stated aim of this prospective interaction study, involving children from newborns to 18 years old is the estimation of the number of children with poorly controlled epilepsy who will receive improved characterization of their epilepsy syndrome on a smartphone based EEG. This includes a team led by applicant at the Jigme Dorji Wangchuck National Referral Hospital in Bhutan in collaboration with the Institute for Traditional Medicines in Bhutan and colleagues at the Massachusetts General Hospital's Department of Neurology. Accomplishment of the above aims will provide the necessary data to randomize smartphone based versus usual epilepsy care for children in several lower income countries through the scale up of this model alongside complementary "disruptive" smartphone-based health technologies for pediatric epilepsy care in the longer term.

- **Annapurna Poduri, M.D.**
  Assistant Professor in Neurology
  Boston Children's Hospital

"Zebrafish Models of PCDH19-related Pediatric Epilepsy"

Key Words: Epilepsy, Genetics, Zebrafish, Animal Models, Drug Screens, PCDH19

Epilepsy affects one in 200 children, many with a genetic etiology. Inherited and de novo heterozygous mutations in the X-linked PCDH19 gene are associated with "female-limited epilepsy," characterized by refractory early onset seizures, intellectual disability, autism, and behavioral problems. To date, there are no PCDH19-specific treatments or animal models to study this condition. Zebrafish have emerged as a simple, robust vertebrate model to study epilepsy and other neurodevelopmental diseases. Our preliminary data indicate that genome-edited zebrafish models of PCDH19-related epilepsy display behavioral and electrophysiological abnormalities. The focused goals of this proposal are to establish clinically relevant pcdh19 zebrafish models and to screen the effects of available AEDs.

Our goal is to establish genetic models of PCDH19-related epilepsy in zebrafish to study the cellular and network abnormalities underlying PCDH19-related epilepsy. Using the CRISPR/Cas9 system, we will introduce patients' PCDH19 into this model system to evaluate for spontaneous seizures and proconvulsant-induced seizures that can be detected visually and with locomotion detection. In addition, we will perform electrophysiological recording to assess for tendency to seizures and to confirm that a given model is 'epileptogenic.' We then expose these models to anti-seizure medications to evaluate for reduction in seizures. These studies will set the stage for high-throughput drug screens of additional compound libraries, thus moving us toward the goal of "precision medicine" for this and other pediatric epilepsies.

- **Dorothy Schafer, Ph.D.**
  Assistant Professor
  University of Massachusetts Medical School

  "The function of Immune cells and Sensory Experience on Brain Circuit Development"

  Key Words: Immune, Neural Circuit, Sensory Experience, Synapse, Microglia, Plasticity, Development, Brain

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Aberrant synaptic connectivity and abnormal sensory perception are hallmark features of autism spectrum disorders (ASDs). Interestingly, these defects are often accompanied by abnormally reactive microglia, resident brain immune cells. However, it is unknown whether microglia and circuit abnormalities are mechanistically linked and whether they manifest in behavioral changes. The goal of my research is to determine how neuron-microglia interactions regulate neural circuit development. Long term, we will apply this basic science to identify new therapeutic targets in ASDs. This line of research builds off my unexpected and exciting finding that microglia sculpt synaptic connectivity (Schafer et al. Neuron 2012). During development, synaptic connections first form in excess. Sensory experience (touch, vision, etc.) then regulates the removal of less active synapses and maintenance of more active synapses. We found that microglia engulf and remove less active synapses in the developing visual system. This finding compels us to consider microglia as regulators of brain wiring and inspires exciting new questions: Is microglia-mediated synapse removal a universal mechanism regulating circuit refinement across sensory modalities and different types of synapses? Do changes in sensory experience directly regulate microglial gene expression and synaptic engulfment? To address these questions, we will manipulate somatosensory and visual experience in the developing mouse (whisker manipulation and dark rearing). We will then fluorescently label microglia and synapses and assess microglia-mediated synapse removal using our newly developed super-resolution imaging and 2-photon in vivo live imaging approaches. We will also identify new microglia-specific genes that are regulated by sensory experience using next generation RNA sequencing followed by rapid validation with a new CRISPR/Cas9 in vitro screen. Answers will revolutionize our understanding of how sensory experience regulates neural circuit refinement, will identify novel molecular mechanisms underlying microglia function, and will provide new insight into ASDs with underlying defects in microglia, sensory perception, and synapses.