Charles H. Hood FOUNDATION

Charles H. Hood Foundation Child Health Research Awards Program
July 2018 Award Recipients

- **David Breslow, Ph.D.**
  Assistant Professor of Molecular, Cellular & Developmental Biology
  Yale University

  "Defining a cilia-lysosome axis in developmental signaling and cilium-based disease"

  Key Words: Primary cilia, Ciliopathies, Signaling, Lysosome, Hedgehog pathway, MTor pathway, Intellectual disability, Congenital heart defects, Medulloblastoma

---

The primary cilium is a protrusion from the cell surface that serves as a specialized center for signal transduction. A number of signaling pathways that control embryonic development, tissue homeostasis, and sensory signaling depend on cilia, and many signaling molecules localize to cilia during signaling. Consistent with key roles for cilia in signaling, ciliary defects promote tumorigenesis and also cause a group of inherited pediatric disorders known collectively as ciliopathies. Critically, while the importance of cilia is now recognized, the molecular mechanisms that support the assembly and function of cilia are not fully understood. Consequently, many ciliary disorders remain poorly characterized, and no effective treatments are available for children with ciliopathies.

Hedgehog pathway signaling is strictly dependent on primary cilia, and aberrant Hedgehog signaling contributes to congenital heart defects, brain malformations, and childhood medulloblastoma. To systematically investigate ciliary signaling, we recently conducted a genome-wide CRISPR-based screen using a Hedgehog pathway reporter. Strikingly, this unbiased screen identified many hit genes that act at lysosomes and late endosomes, suggesting that lysosomes are required for cilium function. Thus, our central hypothesis is that primary cilia depend upon lysosomes and that a cilia-lysosome axis plays key roles in ciliary signaling and ciliopathies. We will examine this hypothesis by 1) characterizing the lysosome-associated GTPases and their associated regulators and effectors, 2) dissecting the contribution of lysosomal nutrient sensing and intracellular positioning to specific aspects of cilium assembly and ciliary signaling, and 3) defining the role of the cilia-lysosome axis in stem cell fate determination and proliferation of medulloblastoma cells.

Together, these studies will provide new insights into how inter-organelle communication between cilia and lysosomes contributes to developmental signaling and childhood disease. We expect this work will also provide a foundation for future translational studies aimed understanding the cilia-lysosome axis in clinical and therapeutic contexts.

Charles H. Hood FOUNDATION

Charles H. Hood Foundation Child Health Research Awards Program
July 2018 Award Recipients

- **Eliezer Calo, Ph.D.**
  Assistant Professor of Biology
  Massachusetts Institute of Technology

  "Elucidating Mechanisms Underlying Phenotypic Variation in Craniofacial Disorders"

  Key Words: Craniofacial Anomalies, Congenital Disorders, Treacher Collins Syndrome, Neural Crest Cells, Phenotypic Variation, DNA Damage

---

Due to its anatomical complexity, the head is highly prone to genetic and environmental insults. Although 1% of newborns exhibit developmental deformities, one-third of these include craniofacial abnormalities. Over 700 distinct craniofacial syndromes exist so far, but the underlying causes for most remains unknown. Craniofacial anomalies exhibit significant variation in penetrance and severity of the phenotype to the extent that it is challenging to predict who will present with a particular craniofacial condition even when carrying an identified causative mutation. This greatly precludes our ability to understand the cause and devise treatment strategies for these devastating childhood disorders. Although genetic and environmental factors are known to contribute to phenotypic variation, at the molecular level, we do not fully understand how, where and when phenotypic variation arises and why is so pervasive in these disorders. My goal is to elucidate molecular mechanisms leading to phenotypic variation in craniofacial disorders. I will focus on Treacher Collins syndrome (TCS), one of the most severe and phenotypically variable disorders of craniofacial development. TCS is caused by heterozygous mutations in the RNA polymerase I (Pol I) cofactor TCOF1 or subunits POLR1D and POLR1C. I have successfully developed models of TCS in zebrafish that faithfully recapitulate the human disease. I have also generated a cellular model of TCS by introducing patient mutations into human embryonic stem cells using CRISPR-Cas9. I will leverage these models to elucidate mechanism downstream of TCS-mutations leading to phenotypic variability. This study will provide novel insights into phenotypic variation and the etiology of craniofacial malformations.

Charles H. Hood FOUNDATION

Charles H. Hood Foundation Child Health Research Awards Program
July 2018 Award Recipients

- **Luke Chao, Ph.D.**
  Assistant Professor
  Massachusetts General Hospital

"Characterization of OPA1 membrane phenotypes in childhood blindness"

Key Words: Dominant optic atrophy, Membrane dynamics, Membrane morphology

---

Mutation in OPA1 results in dominant optic atrophy, the most frequent form of hereditary optic neuropathy resulting from devastating degeneration of retinal ganglion cells. A dynamin family GTPase, OPA1 catalyzes mitochondrial inner membrane fusion, maintains the mitochondrial network and mediates cristae structure. OPA1's multiple roles make it an essential regulator of mitochondrial bioenergetics and an initiator of cytochrome-c mediated cell death. The importance of OPA1 in membrane homeostasis is underscored by the prevalence of mutations across the entire protein. Rescuing OPA1 activity is an attractive strategy to prevent the onset of childhood blindness. Yet, targeted design of treatments is limited by lack of molecular understanding of how specific mutations alter OPA1's different activities.

Dynamin family GTPases mediate membrane rearrangement through a GTP-coupled series of conformational rearrangements. A central outstanding question is how OPA1 protein conformation relates to its membrane functions. We hypothesize that OPA1 conformational states sampled during membrane fusion are essential mediators of membrane morphology.

To test idea, we will reconstitute biochemically OPA1 activity and resultant membrane outcomes *in vitro* to decouple the functional effects of specific mutations. I have previously reconstituted and captured snapshots of flavivirus membrane fusion with single particle fluorescence imaging. This format allows controlled dissection of the interplay between membrane composition and protein state inaccessible in cellular environments. I have now reconstituted OPA1 to build an experimental system that allows us to functionally decouple the different stages of membrane fusion and membrane remodeling. We will investigate the effect of patient-derived mutations on membrane fusion using this system to determine specific functional signatures (Aim1). We will determine the effects of patient mutation on protein and membrane conformation to identify states important for therapeutic intervention (Aim 2). This work makes the essential first steps towards targeted treatment to relieve a devastating form of child blindness.

Charles H. Hood Foundation Child Health Research Awards Program
July 2018 Award Recipients

- **Michela Frascoli, Ph.D.**
  Instructor
  University of Massachusetts Medical School

  "Identification of the Embryonic Lymphoid Progenitors of Neonatal IL-17 Producing T Cells"

  Key Words: Embryonic Progenitors, IL-17, γδ T Cells, In-utero Transplantation

---

Every year, immune-related disorders affect millions of children worldwide. The fetal immune system during pregnancy plays a central role in determining the child's future health. In particular, immune cells that secrete IL-17 promote tissue development and homeostasis, and serve as sentinels for the detection of microbes. For example, murine IL-17 dysregulation due to microbiota disruption after antibiotics administration is linked to obesity later in life, and increased amounts of IL-17 in pregnancy cause behavioral abnormalities, reminiscent of autism spectrum disorder. Therefore, a better understanding of the origin and development of immune cells with the ability to mediate immune effector functions in fetal life has important clinical implications, especially in providing novel insights into how child-specific diseases arise. This proposal focuses on the developmental origin of fetal-derived murine and human IL-17 producing T cell subsets.

We plan to identify and characterize the murine embryonic progenitors of IL-17 producing γδ T cell (Tγδ17), exploiting our recent discovery of a network of transcription factors (TFs) that shape the identity of Tγδ17 cells. The progenitor subsets will be identified in hematopoietic organs of the fetus using fluorescent reporters for the TFs, analyzed at a single cell level, and transplanted into animals to determine their generative potential. In parallel, we will study the human Tγδ17 progenitor counterpart from umbilical cord blood. We will test culture conditions that support and expand progenitors that preferentially give rise to human Tγδ17 cells. A detailed phenotypical and transcriptional analyses of the *in vitro* generated and *ex vivo* isolated subsets will be performed.

Dissecting the molecular requirements for Tγδ17 cells development has critical clinical implications, as early immune events during the fetal stage will shape the immune status during childhood and adult life.

Charles H. Hood FOUNDATION

Charles H. Hood Foundation Child Health Research Awards Program
July 2018 Award Recipients

- **Alisa Khan, M.D., M.P.H.**
  Instructor in Pediatrics
  Boston Children's Hospital

"A Mobile Application to Engage Families of Hospitalized Children in Safety Reporting"

Key Words: Family Safety Reporting, Family Engagement, Medical Errors and Adverse Events, Hospital Safety, mHealth

---

Over 250,000 US patients die yearly from medical errors, making errors a leading cause of death. Families of hospitalized pediatric patients are high-yield reporters of medical errors and adverse events (AEs). We previously found that 9-26% of families of hospitalized children report safety concerns, many of which are otherwise undocumented. Families report similar rates of errors/AEs as providers (when providers' reports are actively solicited) and 3-5 times higher rates than voluntary hospital incident reporting systems (which typically exclude patients/families). However, family safety reporting has not been operationalized in hospitals.

Leveraging health information technology (IT) can help fill the gap between researching and operationalizing family safety reporting. A few recent efforts to engage adult patients/families in safety reporting through health IT demonstrate that IT tools work well and identify important safety issues. However, these tools suffer from poor reporting rates that are orders of magnitude lower than those found in our prior research.

We seek to leverage the efficiencies of health IT while incorporating lessons learned in our research to develop a family safety reporting approach that is active, health-literacy-informed, and engages families and interprofessional team members in order to achieve—operationally—the high rates of reporting we observed previously.

We propose to develop a mobile family safety reporting application for routine operational hospital use adapted from prior research and IT tools, bolstered by: a health-literacy-informed curriculum to activate and educate families about hospital safety, a program to train and encourage providers to engage families in hospital safety, and a unit-wide, multimodal, interprofessional safety culture campaign.

We hypothesize that family safety reporting rates will increase significantly following our intervention. By leveraging the unique expertise and partnership of families, our project has the potential to identify and prevent medical errors/AEs, thereby improving the safety and quality of care hospitals provide to children.