CHARLES H. HOOD FOUNDATION
2019 Major Grants Initiative to Advance Child Health

DEADLINES

==Expressions of Interest== (PI Name, Project Title) **by March 20** *(required)*

Internal Competition Deadline: March 27, 2018 at 5:00pm

Sponsor deadline for preliminary proposals: May 21, 2018

FUNDING

$225,000/year for 2 years.   (Total: $450,000, including 10% indirect costs). Award Period: 1/1/19 – 12/31/20

NOMINATION LIMITATION

Yale may submit a letter of intent from one (1) nominee.

PURPOSE

This initiative will support outstanding investigators conducting innovative and transformative translational or clinical research that will improve child health, children's clinical health outcomes, or improve health care access, affordability, and quality.

For grants starting in 2019, requested applications should relate to either of two areas of child health research:
(1) Neonatology or (2) Brain Science & Child Development.

ELIGIBILITY

● **Candidates are eligible at any stage of their faculty careers.** The qualifications of the Principal Investigator and evidence of exceptional creativity are considered important criteria for review. Note that the PI, while responsible for the project, may include other investigators on the research team.

● Major Grants are intended to fund innovative work that may be difficult to fund with traditional grant mechanisms. For example, relatively few child health researchers make use of advanced statistical and computational techniques, ranging from computational biology to health services research to geographic information systems. In addition to traditional project-related costs, PIs may use of funds to bring new techniques or novel collaborations into child health research.

If funds are intended to augment existing funded research programs, *the letter of intent must clearly describe the additional research to be conducted with Charles H. Hood Foundation funding.*

Refer to the attached research summaries for the 2015 -- 2018 recipients to learn more about research funded.

RESEARCH FOCUS

Neonatology

Although the incidence of prematurity has been declining, recent data shows that 10% of babies born in Massachusetts are premature, 7.7% weigh less than 2.5 kg ($\sim$ 5.5 pounds), and 1.3% weigh less than 1.5 kg ($\sim$ 3.3 pounds). These infants require complex and expensive medical care after birth and are at high risk for pulmonary disease, neurological conditions, developmental delay and other issues. Thus, improvements in neonatal care are likely to result in better health outcomes for the infants, and lower initial and lifetime health care costs.

Research projects that focus on pregnant women and their fetuses will not be considered responsive to this RFA.

Brain Science and Child Development

Groundbreaking work, beginning in the late 1990s, has demonstrated that childhood experiences determine important aspects of brain growth and development. **There are at least two critical periods of rapid brain growth: during the first three years of life and, again, during adolescence.** In parallel with the rapid advance of brain science, a new pediatric subspecialty, Developmental and Behavioral Pediatrics, has emerged and is beginning to carve out its own research agenda.

Recent clinical and scientific advances in understanding children's normal and abnormal development have arisen through a variety of approaches: from a basic science understanding of neural development to the epidemiology of the long-term health effects of children's experiences. This focus will support continued innovations that will further the scientific understanding of children's brain and behavioral development that may inform the treatment of children's physical and mental health.

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<mark>**INTERNAL COMPETITION PROCEDURES**</mark>

1. Email **Expressions of Interest** (PI Name, Project Title) **by March 20 to** melanie.smith@yale.edu

2. For this internal competition, please follow the instructions below. These instructions are nearly identical to the initial "letter of intent" instructions outlined by the Hood Foundation, plus the Scholar Awards "facepage."

Format requirements: Arial 11 font. One-inch margins on all sides (other than biosketch and face page).
        Double-space between paragraphs.   Single or double-space within paragraph.

Combine the following documents, in this order, as a single PDF:

2.1.   Scholar Awards face page   (skip referee section)

2.2   Lay Summary (up to 350 words) – This should be included within the 3-page research project section below. The Lay Summary should be a non-technical summary that addresses the project's relevance to one of the two areas of focus, and its potential for a significant impact on improving child health, children's clinical outcomes, *or* health care access, affordability, and quality.

2.3.   Research Project (up to 3 pages, *including* the lay summary, *excluding* bibliography).
       Include the following sections: background, methods, innovation, implications for child health.

2.4. Biosketch -- applicant must use the current NIH Biosketch form.

<mark>**Email the internal competition application as a single PDF by Tuesday, March 27, 2018**</mark>
to: melanie.smith@yale.edu (cc: OSP@yale.edu)

FOR FURTHER INFORMATION, CONTACT:
Melanie.Smith@Yale.edu ▪ Funding Resource Center ▪ Office of Sponsored Projects ▪ Yale University ▪ 203-785-4978
Last update: March 9, 2018

2018 Hood Major Grant Recipient

Guoping Feng, Ph.D.
Poitras Professor of Neuroscience
McGovern Institute, Brain and Cognitive Sciences
Massachusetts Institute of Technology

Development of Gene Therapy Approaches for Neurodevelopmental Disorders

Key Words: Neurodevelopmental disorders, Autism, Gene therapy, Genome editing, Shank3, Synapse

Neurodevelopmental disorders such as autism spectrum disorders (ASD) and intellectual disability are some of the most debilitating brain disorders and we currently have no effective treatment for them. Although the genetic factors underlying these disorders are complex, many rare single-gene mutations have been causatively linked to severe ASD and intellectual disability (monogenic disorders). Because most genes have multiple functions in different brain regions and during different developmental stages, developing effective pharmacological treatments for these disorders has proven daunting. The most effective treatment may come from correcting the genetic defect itself through gene therapy. For most monogenic disorders, only one of the two copies of the gene is disrupted and thus upregulation of the expression of the normal copy to compensate for the loss of the disrupted copy is a promising approach for gene therapy. We aim to develop and test two different drug-inducible CRISPR/dCas9-transcription activator (CRISPR/dCas9-TA) approaches for precise control of the Shank3 gene activation. Shank3 is a postsynaptic scaffolding protein critical for the development and function of synapses. Heterozygous deletions and mutations of the Shank3 gene in humans lead to severe neurodevelopmental disorder and ASD. In the first approach, we will develop a system that uses a drug to stabilize the Cas9-transcription activator for precise control of Shank3 gene expression. In the second approach, we will develop a drug-induced dimerization of the split Cas9 system to control Cas9-transcription activator activity on Shank3 expression. We will use Shank3 heterozygous mutant mice (Shank3$^{+/-}$) as our ASD model to test the system. Initially, we will test and calibrate the system in cultured Shank3$^{+/-}$ neurons. After we achieve precise control of gene activation in cultured neurons, we will then test the system in Shank3$^{+/-}$ mice using a blood-brain barrier-penetrating AAV virus to systemically deliver the CRISPR/Cas9-transcription activator system to the brain. In addition to testing the precise control of Shank3 expression in these mice, we will also test whether the system can reverse synaptic and behavioral defects in Shank3$^{+/-}$ mice. If successful, this could be a viable approach to be developed into gene therapy in humans in the future. Importantly, this system can be easily applied to many other neurodevelopmental disorders by simply changing the CRISPR guide RNA sequences to match the gene of interest.

Frederick Alt, Ph.D.

Senior Investigator; Charles A. Janeway Professor of Pediatrics, BCH; Professor of Genetics, HMS; Investigator, HHMI
Program in Cellular and Molecular Medicine
Boston Children's Hospital

Role of Recurrent DNA Break Cluster Genes in Brain Development and Disease

Key Words: High throughput DNA break detection, Recurrent DNA break clusters (RDCs), Neural genes, Neural stem and progenitor cells, Replication stress, Neuropsychiatric disorders, Medulloblastoma, Brain diversity

Our work suggested recurrent DNA double-stranded (DSBs) in developing neural stem and progenitor cells (NSPCs) may predispose to genomic variations associated with medulloblastomas (MB), the major pediatric brain cancer. To search for such DSBs, we developed and applied a sensitive genome-wide DSB-detection method to discover recurrent DSB clusters ("RDCs") in mouse NSPC genomes. All RDCs were in genes, of which most have roles neural cell communication and/or are implicated in neuropsychiatric diseases and cancer. Based on robust preliminary data, we propose three inter-related specific aims that will elucidate mechanisms of RDC generation, extend studies to human neural progenitors, and evaluate contributions of RDCs to recurrent genomic rearrangements in MB. In Aim 1 we will evaluate roles of transcription and replication stress in NSPC RDC formation, elucidate relationships between RDC chromosome domain structure with RDC DSB generation/resolution and gene expression, and develop a new approach to assay NSPC RDC formation in vivo to better elucidate developmental and disease implications. Aim 2 studies will elucidate human RDC genes, which is necessary to directly assess their relevance to genomic alterations found in neuropsychiatric diseases and cancer. We will use our recently developed chromosome-specific targeting approach to identify and characterize human NSPC RDCs. Aim 3 will exploit a mouse MB model developed in our lab in which tumors harbor recurrent chromosomal rearrangements similar to those found in an aggressive class of human MBs to test the hypothesis that RDC DSBs may contribute to the generation of recurrent genomic variations found in MBs. To achieve this goal, we are collaborating with Peter Lichter (DKFZ, Heidelberg, Germany) to perform whole genome sequencing of mouse MBs from our model. If correlations between recurrent MB genomic breakpoints and RDC locations are established, we will test implicated RDC function with an approach adapted from our MB model.

2017 Hood Major Grant Recipient

Stella Kourembanas, M.D.
Chief, Division of Newborn Medicine
Boston Children's Hospital

Preterm Birth: Lung Complications and Stem Cells

Bronchopulmonary dysplasia (BPD) is the most common complication of prematurity characterized by a 'reprogramming' of lung growth with reduced alveoli, fewer blood vessels, and abnormal lung function. BPD has significant long-term pulmonary morbidities, including pulmonary hypertension (PH), airway hyperreactivity, abnormal pulmonary function test results, and, in some cases, emphysematous changes that persist into adulthood. Mesenchymal stem cells (MSCs) are recognized as potential cell-based therapy for diseases of the lung. These multipotent cells exhibit beneficial effects through anti-inflammatory, immunomodulatory, prosurvival and anti-fibrotic mechanisms that are not clearly defined. We showed that bone marrow-derived MSCs or their cell-free conditioned media (CM) prevent and reverse experimental lung injury in the neonatal mouse model of BPD. We isolated exosomes from human MSC-CM, termed MEX, and showed that they can prevent lung inflammation and PH in experimental models. A single dose of MEX given after 2 weeks of hyperoxic injury in neonatal mice abrogates inflammation and fibrosis and significantly improves alveolization. Exosomes are small vesicular structures produced by all cells that contain a distinct cargo which not only represents the cell of origin but is differentially-enriched in specific nucleic acid or lipid species. They serve as an important cell-to-cell communication mechanism. Our working hypothesis is that MEX restore lung homeostasis through immunomodulatory pathways, and enable lungspecific progenitor cells to repair lung injury. We will combine a multifaceted experimental design that includes studies on immune cell responses, human lung-on-a-chip microfluidic system, and human lung MSCs from infants with BPD to test the central hypothesis. Our specific aims are to (1) define the immunomodulatory properties of MEX and their effect on lung pathophysiology; and (2) elucidate the therapeutic efficacy of MEX in vivo on the neonatal hyperoxia model of BPD. Our goal is to develop the most optimal, well-characterized, and functional MSC exosome preparation for human application.

2017 Hood Major Grant Recipient

Jonathan Davis, M.D.
Chief, Division of Newborn Medicine
Tufts Medical Center

Establishing Risk in Neonatal Abstinence Syndrome

The use of opioids and other psychoactive drugs during pregnancy is a major public health problem. Neonatal abstinence syndrome (NAS) affects up to 80% of infants exposed to opioids in utero, although its expression is highly variable. The current approach is to treat all opioid exposed infants as being at risk for NAS, using standard assessment tools followed by treatment when needed. These tools are 40 years old, highly subjective, and associated with significant inter-observer variability. "Low risk infants" remain in the hospital too long while "high risk infants" have delays in starting treatment. Since genetic factors appear to be important in NAS, it would be highly significant if clinical, demographic and genetic factors could be combined to more rapidly identify infants at risk of developing NAS. It is also essential to develop a better assessment tool for NAS. Our overarching hypothesis is that opioid-exposed infants will have better outcomes when we employ: 1) a prediction instrument using clinical, demographic, and genetic factors; 2) a simplified scoring system for the evaluation and treatment of NAS. Aim 1 will analyze clinical, demographic (n=1000) and genetic (n=680) data from mother-infant dyads enrolled in ongoing NAS studies. Aim 2 will then develop comprehensive risk assessment models to predict the development/severity of NAS and guide earlier prenatal/postnatal treatment. For Aim 3, we have already analyzed over 40,000 NAS scores and developed a shortened and more representative assessment tool. We will now validate this approach in a separate NAS cohort. Our long term goals are the development of a prediction tool to establish risk of significant NAS and a better assessment tool to guide earlier identification and treatment. While some candidate variables are already known, others require confirmation or remain to be discovered. These studies should enhance our understanding of NAS and significantly improve outcome.

- **James Noonan, Ph.D.**
  Associate Professor, Department of Genetics
  Yale University

"Discovering gene regulatory networks in early human brain development that contribute to autism spectrum disorder"

Key Words: Autism Spectrum Disorder, Gene regulation, Early cortical development, Chromatin, Genome engineering, Regulatory networks

Autism spectrum disorder (ASD) originates during early brain development and imposes a lifelong burden on affected individuals and their families. Insight into the specific molecular processes and cell types perturbed in ASD remains limited, which has hindered the design of effective treatments. However, recent studies have begun to reveal genetic factors contributing to ASD risk, providing an avenue to understand its biological basis. These efforts have identified multiple ASD risk genes with deleterious, heterozygous de novo mutations in affected individuals. Many of these genes encode chromatin modifiers and converge in gene co-expression networks in mid-fetal human cortex. These findings suggest that disruption of gene regulatory networks in the prenatal human brain contributes to autism pathology. The ASD risk gene with the strongest association yet detected in whole exome surveys is the chromodomain helicase CHD8. The goal of this proposal is to identify the target networks of ASD risk genes controlled by CHD8, thereby revealing common regulatory pathways underlying ASD progression. In preliminary studies, we have mapped genes regulated by CHD8 during mouse and human neurodevelopment. We found that other ASD risk genes are overrepresented among CHD8 targets, and that ASD risk genes were dysregulated by loss of CHD8. We will build on this work by elucidating regulatory networks for multiple ASD-associated chromatin modifiers in three cell types of the developing cortex that have been implicated in ASD: neuronal stem cells, deep layer excitatory projection neurons, and upper layer excitatory projection neurons. In complementary studies, we will use mouse models to determine the cell-type specific effects of CHD8 loss-of-function on regulatory networks during cortical development. Together, our findings will reveal common regulatory pathways underlying ASD, providing fundamental biological insights into the origins of the disorder.

- **Trudy Morrison, Ph.D.**
  Professor, Department of Microbiology & Physiological Systems
  University of Massachusetts Medical School

  "Protection of Neonates from Respiratory Syncytial Virus Infection"

  Key Words: Respiratory syncytial virus, Maternal immunization, Neonates, Vaccine

  Respiratory syncytial virus (RSV) is the primary cause of acute lower respiratory tract infections in infants and young children worldwide, accounting for 33.8 million infections in 2005 alone. No licensed vaccine exists and, furthermore, direct vaccination of infants is likely ineffective, due to the immaturity of their immune system, and is potentially unsafe. Our long-term goal is to protect newborns from RSV infections through the passive transfer of maternal neutralizing antibodies in utero to the fetus after maternal immunization. A complicating issue with maternal immunization is that most people have experienced RSV infections during their lifetimes. But these infections do not result in maternal antibodies that can protect neonates. Thus any vaccine must induce in mothers high levels of neutralizing antibodies in the presence of pre-existing, but very poorly neutralizing, anti-RSV antibodies. We have developed a novel RSV vaccine candidate unlike any previously tested. Our candidate is a virus-like particle (VLP) containing a stabilized pre-fusion form of the RSV F protein (Pre-F), the biologically active conformation of the molecule that is most effective in stimulation of neutralizing antibodies.

  It is our hypothesis that immunization of mothers during pregnancy with the Pre-F containing VLPs will significantly boost their serum neutralizing antibodies responses, even in mothers with a past history of RSV infection. Using cotton rats as surrogates for human populations, we propose four specific aims to test this hypothesis.

  Specific Aim 1: Assess the role of preexisting immunity in induction of neutralizing antibodies by Pre-F VLP Immunization
  Specific Aim 2: Measure the transfer of maternal anti-RSV neutralizing antibodies, after Pre-F VLP vaccination, to neonates
  Specific Aim 3: Evaluate protection of offspring of VLP immunized mothers from RSV challenge
  Specific Aim 4: Assess potential for enhanced respiratory disease (lung pathology) in RSV infected offspring of VLP immunized mothers

- **David Goodman, M.D.**
  Professor of Pediatrics and Health Policy
  Geisel School of Medicine at Dartmouth

"The Epidemiology and Efficiency of Neonatal Intensive Care"

Key Words: Neonatal care, Epidemiology, Outcomes research, Effectiveness research, Quality

Neonatal intensive care has been highly successful at reducing newborn mortality and morbidity, but the quality of care, outcomes, and efficiency has been poorly documented and is poorly understood. In particular, improving the value of care has been elusive, in the absence of outcomes-adjusted efficiency measures of specific neonatal intensive care units (NICUs). We propose the first population-based study of newborn and neonatal intensive care for the total live birth cohorts of four states (ME, VT, NH, MA) and for Blue Cross Blue Shield (BCBS) insured singleton newborns in Texas (N= ~134,000 per year x 4 yrs. or ~536,000 newborns). The proposed analyses will examine overall and regional variation in newborn care, focusing on the illness-adjusted (e.g. birth weight and other perinatal risk factors and diagnoses) use of intensive care (i.e. defined as Levels II, III, IV care) by different newborn conditions and associated utilization and health outcomes. Patient and provider factors associated with the variation in NICU use will also be studied to reveal potential opportunities for improvement in care. The specific aims are:

1. To measure the probability of NICU admissions by newborn characteristics (e.g. birth weight) across states and neonatal intensive care regions.

2. To measure the association of risk adjusted NICU admissions with provider/community characteristics, including measures of capacity (i.e. NICU beds and neonatologists).

3. For those newborns admitted to NICUs, to measure regional and hospital variation in risk-adjusted utilization of NICU services (e.g. length of stay, level of care, imaging, allowed charges) and outcomes including (e.g. inpatient mortality, readmissions, and ER) and their association with provider/community characteristics.

The long term goal of this work is to stimulate further inquiry into the care provided to newborns, and to eventually develop better systems of public reporting and improvement that will improve care and moderate costs.

- **Joel Richter, Ph.D.**
  Professor, Program in Molecular Medicine
  University of Massachusetts Medical School

"Altering the Translational Landscape to Treat the Fragile X Syndrome"

Key Words: Fragile X, Autism, Translational Control

The broad long-term objectives of this proposal are to use the translational landscape to identify new therapeutic targets to treat the Fragile X Syndrome. Fragile X, an autism spectrum disorder, is caused by the loss of the FMRP, an RNA binding protein that represses translation in the brain and other tissues. In the absence of FMRP, translation is excessive and this causes the syndrome. Using Fragile X model mice, we have rebalanced translation in the brain by ablating the gene for CPEB, which codes for an activator of translation. Thus, in FMRP/CPEB double knockout (KO) mice, protein synthesis and all characteristics of Fragile X are rescued to normal or near-normal levels. In mice lacking FMRP, ribosomes elongate polypeptides at a precipitate rate, which is returned to normal when CPEB is also absent. Based on these observations, we propose two specific aims: 1) to use ribosome runoff and ribosome profiling in brain tissue to identify the mRNAs whose translation is rescued in FMRP/CPEB double knockout mice, and 2) to use patient-derived Fragile X induced pluripotent stem cell neurons containing or lacking CPEB to determine whether the same mRNAs are rescued. The proteins encoded by these rescued mRNAs are potential therapeutic targets to treat Fragile X.