Charles H. Hood Foundation Child Health Research Awards Program

July 2015 Award Recipients

- **Sudha Arunachalam, Ph.D.**
  Assistant Professor
  Department of Speech, Language and Hearing Sciences
  Boston University

"Improving Child-Caregiver Interactions for Young Children with Autism"

Key Words: Autism Spectrum Disorder, Language Learning

The goal of this project is to improve the language skills of young children with autism spectrum disorder by helping their caregivers speak to them in a way that increases their comprehension and their learning. Caregivers play a critical role in interventions for preschoolers with autism, but currently little is known about how caregivers should speak to children to best support their language development. The current project aims to increase our understanding of what features of caregiver speech children with autism understand best in order to inform such interventions.

We use an innovative new experimental paradigm in which the caregiver and child play a game together on a tablet. We use an eye-tracker to measure the child's eye gaze as he or she listens to the caregiver's speech. This paradigm allows us, for the first time, to see how children with autism understand their own caregiver's speech in real-time. We use the results of this investigation to determine positive features of the caregiver's speech, and to help caregivers use more of these positive features when speaking to their child.

- **Marcelo Dietrich, M.D.**
  Assistant Professor
  Department of Comparative Medicine and Neurobiology
  Yale School of Medicine

"Hypothalamic Circuits Underlying Brain Development during Childhood"

Key Words: Hypothalamus, energy balance, brain development, behavior, AGRP neurons, mouse models

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Development of the nervous system is critical for a healthy life and proper insertion of the individual in society. Many disorders, including metabolic (e.g., obesity) and neuropsychiatric (e.g., depression, autism) diseases appear to have neurodevelopmental components involved in their etiology. The mechanisms that impact brain development during childhood are unknown and discoveries in the field have the potential to change our view on these medical conditions. We hypothesize that brain development is determined by the metabolic state of the animals.

Based on our previous findings, we postulate that during childhood, hunger-promoting hypothalamic neurons (named Agrp) that integrate information about the metabolic state of the animal control the development and maturation of other brain regions. To test these assumptions, we will investigate the following aims: (1) Test whether manipulation of Agrp neuronal activity during "childhood" influences brain development and behavior; (2) determine the role of the mediators of Agrp neuronal function, GABA, Agrp and NPY, on brain development outcomes. Here, we will use mice as a model organism to test these aims. The development and elucidation of the above aims will shed new light on the role of energy balance regulating hypothalamic circuits on brain development and behavior.

This is a new and innovative line of research that we have pioneered and will open a new dimension to the understanding of brain development in response to environmental changes. These basic experimental studies have the potential to guide the development of new therapeutic approaches to diseases that result of a previously unappreciated change in brain development during childhood.

- **Hiroyuki Inuzuka, Ph.D.**
  Assistant Professor of Pathology, Harvard Medical School
  Beth Israel Deaconess Medical Center

"Targeting Fbw7 for the Treatment of Pediatric T-cell Acute Lymphoblastic Leukemia (T-ALL)"

Key Words: T-cell Acute Lymphoblastic Leukemia (T-ALL), Fbw7, Tumor Suppressor, Acetylation, Ubiquitination, Mediator Complex (MED)

The long-term goals of this research proposal are to uncover the molecular mechanisms by which the SCF(Fbw7) E3 ubiquitin ligase complex is implicated in pathogenesis of the most common pediatric cancer, T-cell acute lymphoblastic leukemia (T-ALL) and to develop a new therapeutic intervention for this disease. Critically important to the translational impact of our proposed studies, tumor suppressor Fbw7 is deleted or mutated with high frequency in human T-ALL, approximately 30% of T-ALL patients. Fbw7 is a substrate recognition subunit of SCF(Fbw7) E3 ubiquitin ligase complex that is involved in numerous cellular processes by promoting the degradation of critical oncogenic proteins.

Our group has made significant contributions to the understanding of the critical role of Fbw7 in T-ALL development and progression by defining Mcl-1 as a downstream substrate of Fbw7. Although deficient Fbw7 function has been implicated in T-ALL, the exact molecular mechanisms underlying the anti-cancer activity of Fbw7 and upstream signaling pathways that control Fbw7 stability and activity have not been fully elucidated. Therefore, in this proposal, we plan to 1) Examine the regulation of Fbw7 function by reversible acetylation during T-ALL development: 2) Investigate the molecular mechanisms by which Fbw7 regulates the stability of components of the Mediator complex to influence T-ALL development. The major goal of this proposal is to explore how Fbw7 signaling pathway is regulated by acetylation-dependent mechanisms (Aim 1), how Fbw7 exerts its anti-cancer functions through regulating downstream pathways including the Mediator complex protein MED15 (Aim 2). Results derived from Aim 1 and 2 will provide the rational for the utilization of deacetylase inhibitors to promote stabilization of Fbw7 in T-ALL cells, or the utilization of TGF beta specific inhibitors in T-ALL that carry mutated or deleted Fbw7, which would overexpress MED15 due to loss of Fbw7 activity, leading to increased TGF beta signaling activity.

- **Kristin Moffitt, M.D.**
  Assistant Professor of Pediatrics, Harvard Medical School
  Division of Infectious Diseases
  Boston Children's Hospital

"Host and Bacterial Factors in Staphylococcus aureus Skin Infections in Autosomal dDminant-Hyper IgE Syndrome"

Key Words: Staphylococcus aureus, STAT3, hyper IgE Syndrome, Job's syndrome, IL-17A, TH17 cells, staphylococcal skin infection

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Staphylococcus aureus is one of the most frequent causes of bacterial infection in children and lack of understanding of critical protective immune responses hinders staphylococcal vaccine development. Patients with autosomal dominant Hyper IgE Syndrome (AD-HIES), also known as Job's syndrome, suffer recurrent S. aureus skin and soft tissue infections (SSSTI). The genetic basis of this primary immunodeficiency includes mutations within stat3, the gene encoding Signal Transducer and Activator of Transcription 3 (STAT3). Differentiation of TH17 cells is STAT3-dependent, and affected patients lack TH17 cells. AD-HIES patients also have defective memory antibody responses. The relative contribution of TH17 cells and memory antibody responses to immunity to SSSTI remains unclear. Furthermore, the interplay between defective STAT3-dependent adaptive immune responses and bacterial virulence factors that facilitate SSSTI in AD-HIES is not well defined.

Using a mouse model of AD-HIES, we will evaluate both host and bacterial gene expression during SSSTI. Using RNA extracted from staphylococcal abscesses in WT and AD-HIES mice, we will evaluate the transcriptome of the host immune response during SSSTI to better elucidate the effector immune responses that are defective in AD-HIES animals during infection. We will also use mice with the same stat3 mutation isolated to either the B-cell or T-cell lineage to evaluate the respective roles of humoral and cellular adaptive immune responses in SSSTI. Also from abscess RNA, we will evaluate the staphylococcal transcriptome to identify bacterial factors that are differentially expressed under varying host immune pressures. Identification of staphylococcal genes whose expression is significantly increased during infection in AD-HIES mice compared to WT mice may inform a deeper understanding of staphylococcal factors that are critical to establish infection. Finally, comparison of the staphylococcal expression profiles in this work with those from abscesses of healthy pediatric patients will inform efforts in staphylococcal vaccine development.

- **Benjamin Shore, M.D., M.P.H.**
  Assistant Professor of Orthopaedic Surgery, Harvard Medical School
  Department of Orthopaedic Surgery
  Boston Children's Hospital

"Responsiveness of the Pediatric Evaluation of Disability Inventory Computer Adaptive Test in Children with Cerebral Palsy"

Key Words: Cerebral palsy, PEDI-CAT, Computer adaptive testing

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Cerebral palsy (CP) is the most common cause of chronic childhood disability in the United States. The Pediatric Evaluation Disability Inventory- Computer Adaptive Test (PEDI-CAT) is a new clinical assessment for children and youth (0-20 years) with functional disability, including those with limitations in mobility requiring walking aids or wheelchairs. The PEDI-CAT using a computer adaptive platform, exhibits a simple form of artificial intelligence, which selects questions that are directly tailored to an individual, thus shortening the test to achieve desired precision.

This prospective study will focus on children with CP undergoing orthopedic and neurosurgical surgery. We will compare the responsiveness of the PEDI-CAT to standard legacy outcome measures. Specifically we will estimate the minimal detectable change (MDC) and identify what the minimum clinically important difference (MCID) is for each domain of the PEDI-CAT. Finally we will identify which children according to the Gross Motor Function Classification Level (GMFCS) experience the greatest benefit in functional mobility from orthopedic/neurosurgical surgery.

The "holy-grail" of outcomes-based research in CP is to develop a common comprehensive functional outcome instrument that is quick to administer, precise, and user friendly with the potential to enable clinicians to serially monitor the impact of medical, surgical and rehabilitation interventions over a range of cognitive and functional disability level. The validation of the responsiveness of the PEDI-CAT has the opportunity to change the way we measure and interpret functional changes after orthopedic surgery in children with CP.

January 2015 Award Recipients

- **Alejandro Balazs, Ph.D.**
  Assistant Professor of Medicine
  Ragon Institute of MGH, MIT and Harvard

  "Evolutionary Dynamics of Pediatric HIV Infection Undergoing Antibody-Mediated Selective Pressure"

  Key Words: Pediatric HIV, Mother-to-Child Transmission, HIV, Vectored ImmunoTherapy, Broadly Neutralizing Antibodies, Viral Suppression, Escape Mutation, Humanized Mouse Models

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Antibodies provide one of the most important natural defenses against viral pathogens. However, in the case of HIV, the natural antibody response is usually unable to generate neutralizing antibodies capable of preventing or controlling infection. Despite this limitation, rare broadly neutralizing antibodies (bNAbs) that can neutralize a majority of the known HIV strains and clades have been isolated from infected humans. We have developed an approach that is capable of directing life-long expression of these antibodies from muscle following a single injection of a modified adeno-associated viral vector, and have shown that is sufficient to protect humanized mice from infection by a variety of HIV strains.

Recent studies have demonstrated that bNAbs in sufficiently high concentrations have the potential to treat models of active HIV infection in humanized mice and non-human primates. However, little is understood regarding how individual antibodies suppress viremia without selecting for escape variants. Previous experiments in non-human primates have suggested a role for antibody-dependent cellular cytotoxicity (ADCC) in preventing infection, but it is uncertain to what extent ADCC influences control of an established infection or destruction of the viral reservoir. We propose to investigate the evolutionary dynamics of antibody-mediated suppression of HIV strains that successfully transmitted between mother and child in vivo.

To achieve this goal, we will first create a new model of clinical HIV infection in humanized mice using viral isolates from infected children. We will use this model to determine the ability of bNAbs, both individually and in combinations, to control these infections and study the evolutionary reaction of these swarms to antibody selective pressure through deep sequencing.

- **Daniel Bauer, M.D., Ph.D.**
  Assistant Professor in Pediatrics, *Harvard Medical School*
  Staff Physician in Pediatric Hematology/Oncology, *Boston Children's Hospital*

  "Genome Editing of BCL11A for Beta-Thalassemia"

  Key Words: Genome Editing, CRISPR/Cas9, BCL11A, Fetal Hemoglobin, Beta-Thalassemia, Hemoglobinopathy

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The beta-thalassemias are a heterogenous group of inherited disorders characterized by inadequate production of the beta-globin constituent of hemoglobin. Despite regular blood transfusion and chelation, patients suffer from substantial morbidity and mortality. Re-induction of fetal hemoglobin (HbF) would counteract the fundamental molecular defect and could constitute curative therapy. Recently we identified an adult-stage erythroid enhancer of BCL11A as a critical determinant of HbF level subject to common genetic variation associated with beta-thalassemia disease severity. We predict that disrupting this genetic element would ameliorate beta-thalassemia by mimicking protective genetic variation. Therapeutic application of genome editing technology allows the precise modification of genetic sequences in human cells. This modality represents a promising novel treatment strategy for genetic diseases, particularly for blood disorders given the ease of autologous hematopoietic stem cell (HSC) harvest, manipulation, and transplant. In this proposal we describe how we will: identify minimal sequences necessary for BCL11A erythroid enhancer function; compare technologies for genome editing in primary human HSCs; and determine the safety and efficacy of such edited cells in reconstituting hematopoiesis. These experiments will enable the development of clinical trials of therapeutic genome editing for the beta-thalassemias.

- **Jason McLellan, Ph.D.**
  Assistant Professor of Biochemistry
  Geisel School of Medicine at Dartmouth

  "Molecular Mechanisms of Small Molecule RSV Fusion Inhibitors"

  Key Words: Respiratory syncytial virus, Infectious disease, Bronchiolitis, Pneumonia, Fusion inhibitors, X-ray crystallography

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Respiratory syncytial virus (RSV) is a highly contagious paramyxovirus that is the leading cause of pneumonia and bronchiolitis in children less than one year of age. A safe and effective vaccine is not available, and passive prophylaxis with the monoclonal antibody palivizumab is expensive and only modestly effective, restricting its use to high-risk infants in developed countries. Thus, there is an urgent need for alternative strategies to prevent RSV infection. Small molecules that block the function of the RSV fusion glycoprotein (RSV F) have shown promise in clinical trials, however, the mode of binding and mechanism of action for these compounds remain unknown. Such knowledge would greatly facilitate the rational development of more potent compounds with better efficacy in humans.

Our long-term goal is to translate structural and mechanistic information on RSV glycoproteins into preventive and therapeutic strategies. The objective of this project is to determine, at a molecular level, where small molecules bind RSV F and how they prevent fusion of the viral and cellular membranes. Based on our preliminary data, our hypothesis is that these molecules bind inside the cavity of the prefusion conformation of RSV F and stabilize it, preventing its conversion to the postfusion state. In Aim 1, we will use our recently engineered prefusion-stabilized RSV F proteins in isothermal titration calorimetry experiments and X-ray crystallographic studies to determine the stoichiometry of binding and the binding sites of the small molecules. In Aim 2, we will develop in vitro and cell-based triggering assays to test our hypothesis that the small molecules stabilize and prevent triggering of RSV F. Collectively, the results from these studies will pave the way for the rational design of improved fusion inhibitors that will block RSV infection and reduce the disease burden of this major childhood pathogen.

• **Margie Skeer, Sc.D.**
Assistant Professor of Public Health and Community Medicine
Tufts University School of Medicine

"Understanding the Protective Mechanisms of Family Dinners: Psychometric Testing and Evaluation of the Family Dinner Index"

Key Words: Family dinner, Youth risk, Obesity, Psychometric testing

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Empirical evidence suggests that youth who frequently eat meals with their parents are less likely to engage in risk behaviors or to be overweight or obese. However, the majority of these studies only measure family meal frequency, not other aspects of family meals that may be protective. We hypothesize that, aside from frequency, family meals may have positive effects through various mechanisms related to characteristics such as meal duration, attendance, use of technology at meals, level of conversation, and feelings about mealtime, among others.

To address this gap in the literature, the investigative team developed parent- and child-versions of the Family Dinner Index (FDI), which is the first measure to examine family dinners from a multidimensional perspective. The purpose of this study is to finalize and subsequently test the parent- and child-versions of the FDI (FDI-P and FDI-C, respectively) in order to assess reliability and validity. The first aim is to conduct cognitive interviews with ten parent-child dyads (n=20) to systematically analyze potential sources of response error on the items. Data from the cognitive interviews will be used to finalize the measures in preparation for psychometric testing. The second aim is to psychometrically test the FDI-P and FDI-C among a sample of 200 parent-child dyads (n=400) in the greater Boston area. For this aim, we will: 1) evaluate reliability of the items, including item correlation and variation; 2) reduce the items to create the optimal length for the FDI-P and FDI-C; 3) create a scoring structure for the measures; and 4) test the associations between family dinners and youth outcomes to determine criterion validity. The long-term objectives of this project are to use these standardized, validated measures in longitudinal epidemiologic research on risk- and weight-related outcomes among youth, which will ultimately be used to develop innovative preventive interventions related to family dinners.

- **Courtney Yuen, Ph.D.**
  Instructor in Medicine
  Brigham and Women's Hospital

  "Efficacy and Safety of a Simplified Treatment to Prevent Multidrug-resistant Tuberculosis in Children"

  Key Words: Tuberculosis, Multidrug-resistant tuberculosis, Prophylaxis

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Every year, 8 million children worldwide are infected with the bacteria that cause tuberculosis. Once infected, children are at elevated risk for developing severe and fatal forms of tuberculosis. Prophylactic treatment in infected children can avert tuberculosis disease and death. Effective and safe prophylactic treatment is available for children infected with bacteria susceptible to the most common antituberculosis drugs. However, evidence is sparse to guide prophylaxis of children exposed to multidrug-resistant tuberculosis (MDR-TB), which is more difficult to treat and equally fatal.

The proposed project is a cluster-randomized trial to assess the efficacy and safety of a simplified prophylactic regimen of levofloxacin for 6 months compared to a recently reported three-drug, 6-month regimen. The specific aims are (1) to determine whether the single-drug regimen is as effective as the three-drug regimen, (2) to characterize adverse events among children receiving levofloxacin-based prophylactic treatment, and (3) to measure pharmacokinetic parameters of levofloxacin in children.

The study will be conducted in Lima, Peru. We will enroll 400 children who are: (1) under 15 years of age, (2) living in the households of adults with infectious, fluoroquinolone-susceptible MDR-TB, and (3) not themselves sick with tuberculosis. Subjects will be randomized by household to either a single-drug or a three-drug prophylactic regimen. Subjects will undergo clinical evaluation monthly for 1 year to determine progression to tuberculosis disease and assess adverse events. Blood samples will be collected to determine peak serum drug concentrations of levofloxacin.

If the single-drug regimen is as effective as the three-drug regimen, it would present a less burdensome prophylaxis option for children exposed to MDR-TB. This study will also add to the limited knowledge base about adverse events in children receiving fluoroquinolone-containing prophylactic regimens and about the pharmacokinetics of levofloxacin in children receiving either prophylaxis or treatment for tuberculosis disease.

July 2014 Award Recipients

- **Eric Folker, Ph.D.**
  Assistant Professor of Biology
  Boston College

  "The Role of Nuclear Positioning in Centronuclear Myopathies"

  Key Words: Muscle, Nuclear position, Centronuclear myopathy

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Centronuclear myopathies (CNM) are a group of muscle diseases that are typically diagnosed between birth and adolescence. The most prominent cellular pathology associated with this disease is the alignment of nuclei in the center of each muscle cell. This is in stark contrast to the typical arrangement of nuclei in which they are evenly spaced at the periphery of the muscle cell. Yet, despite this prominent pathology which gives the disease its name, the role of aberrant nuclear positioning in disease progression has been largely unexplored. This likely stems from the identification of the genes that are mutated in CNM patients which all regulate T-tubule formation. Because T-tubules are essential for muscle contraction, the role of CNM-linked genes in T-tubule formation has garnered much interest. However, movement of nuclei in muscle proceeds T-tubule formation. Therefore, it is essential to understand whether CNM-linked genes impact nuclear movement to fully understand how they cause muscle disease.

Specific Aim 1 will investigate the role of CNM-linked genes in regulating nuclear position during muscle development. We use the model organism Drosophila melanogaster because the cellular structure of the muscles is very similar between flies and humans. However Drosophila offers superior genetic tools and is amenable to high resolution microscopy to identify cellular defects.

Specific Aim 2 will identify the mechanisms by which CNM-linked genes regulate nuclear movement in muscle. We will determine whether they act within known or novel pathways. Furthermore, we will investigate how these mechanisms relate to other muscle diseases

Overall, this project will provide crucial understanding of the cellular pathologies that underlie CNM. This data is essential for future therapeutic development as it is impossible to combat the muscle weakness when the underlying cause is not clear.

- **Yick Fong, Ph.D.**
  Assistant Professor of Medicine
  Brigham and Women's Hospital

  "The Role of the Dyskerin Complex in Stem Cell-specific Transcriptional Control and the Pathogenesis of Dyskeratosis Congenita"

  Key Words: Embryonic stem cell, Induced pluripotent stem cells, Pluripotency, Transcription, Coactivators, Dyskeratosis congenita

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Dyskeratosis congenita (DC) is a rare but fatal human genetic disorder that impairs stem cell function. Symptoms include mucocutaneous abnormalities, bone marrow failure and predisposition to cancer. The onset of some of these symptoms usually appears between the ages of 5 and 10 years, although diagnosis has occurred in infants. Mutations in the dyskerin (DKC1) complex, a small nucleolar ribonucleoprotein complex critical for telomerase and ribosome biogenesis, have been linked to DC. Thus, it is generally believed that depletion of stem cells associated with DC is primarily due to telomerase dysfunction and ribosome deficiency. However, using an unbiased in vitro transcription assay to isolate protein factors from embryonic stem cell (ESC) extracts that support stem cell-specific gene activation, I uncovered a previously unrecognized function of the DKC1 complex in stimulating the transcription of key pluripotency genes targeted by core stem cell-specific activators OCT4 and SOX2. I showed that the DKC1 complex is recruited to the regulatory regions of OCT4/SOX2-target genes in both mouse and human ESCs. Importantly, the binding of DKC1 to these genes are likely functional because depletion of DKC1 in mouse ESCs reduced the expression levels of core pluripotency genes Oct4, Sox2, Nanog, and blocked somatic cell reprogramming in mouse embryonic fibroblasts. Based on these findings, I hypothesize that disruption of the transcriptional network controlling stem cell self-renewal by DKC1 mutations could be responsible for some of the clinical phenotypes of DC. The goal of this proposal is to determine the full range of transcriptional targets of the DKC1 complex in a genome-wide scale in ESCs. I also aim to determine the impact of DC mutations on RNA binding repertoire and coactivator activity of the DKC1 complex. These studies could enhance our understanding of the pathogenesis of DC at the transcriptional level and reveal new therapeutic approaches.

• **Dong Kong, Ph.D.**
Assistant Professor of Neuroscience
Tufts University School of Medicine

"Mitochondria, Cognitive Defects, and Child Obesity"

Key Words: Mitochondria, Synaptic plasticity, Hippocampal neurons, Cognition, Knockout mouse, AAV, High-calorie diet, Child obesity

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Child obesity, a serious medical condition with various complications, affects 17% of children and adolescents in the United States, and the percentage is continuing to rise. Although child obesity causes much less physical harms compared with adult-onset obesity, accumulating evidence has suggested that it is tightly related to cognitive dysfunctions, which is particularly problematic since childhood is the crucial developmental stage for the maturation of brain structures and related functions. In line with the observations in human, high-fat, high-calorie diet (HFD) feeding in rodents was reported to impair both hippocampal structures and physiology, in particular, its glutamatergic neurotransmission. The related mechanistic studies, unfortunately, are still in their infancy. The prevention and treatment for the cognitive decay in child obesity, therefore, are still lacking.

In a recent effort, we identified a mitochondrial protein, which is abundantly expressed in the hippocampus, modulates neuronal glutamatergic transmission, and is highly responsive to HFD feeding. Based on multiple lines of evidence and our preliminary data, we hypothesize that this protein, and its related mitochondrial dysregulation, play a causal role in the HFD-induced impairment of synaptic plasticity in the hippocampal neurons, and are responsible for the decline in cognition. We propose to employ manifold genetic and optic approaches to assess this. We will use mouse and viral genetic tools to knockout or overexpress this protein specifically in the hippocampal neurons. We will first investigate the alterations in the synaptic transmission and neuronal activity (Aim1). We will then analyze whether such alterations can be translated into the regulation of hippocampus-related cognitive behaviors and whether this protein mediates HFD-related perturbation (Aim2). We will also use advanced molecular and imaging tools to interrogate the mechanisms underlying these changes. These proposed studies will likely provide us mechanistic framework and establish novel therapy targets for cognitive defects in child obesity.

- **Duncan Maru, M.D., Ph.D.**
  Instructor in Medicine, Global Health Equity
  Brigham and Women's Hospital

  "Integrating Pediatric Care Delivery in Rural Healthcare Systems"

  Key Words: mHealth, Implementation Science, Surveillance, Health Systems Strengthening

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A central challenge in the delivery of evidence-based interventions to promote under-five child survival is the coordination of care, from frontline health workers, to primary care clinics, to district hospitals, to specialty providers. Additionally, children who survive or avoid once-fatal diseases such as congenital and rheumatic heart diseases, prematurity, neurodevelopmental conditions, and disabilities sustained from traumatic injuries, are increasingly living well into adolescence, young adulthood, and beyond. Healthcare delivery systems in resource-limited settings, however, are ill-equipped to manage such patients' care. Mobile technologies, coupled with effective management strategies, may enhance implementation and coordination of evidence-based interventions. Here, we propose a stepped-wedge, randomized trial evaluating a mobile health care coordination and quality improvement intervention within two rural district healthcare systems in Nepal. Twenty clusters across the two sites will be involved in the study, totaling population of approximately 72,000 of which 13,000 are children under the age of five. The intervention focuses on two primary strategies to improve district healthcare delivery: structured quality improvement at primary clinics and mobile phone-based care coordination. These activities are coordinated via hospital-based clinicians and village-based frontline health workers.

Specific Aim One: To assess, via a stepped-wedge, cluster randomized controlled trial, the effectiveness of a mobile health care coordination, Chronic Care Model, and structured quality improvement intervention on reducing under-five mortality as the primary outcome. The unit of randomization is a government-defined village cluster.

Specific Aim Two: To assess the impact of the Chronic Care Model intervention on the median follow-up rate across all chronic diseases followed. Secondary outcomes include median medication adherence, global symptoms score, self-rated health, disability score, and inpatient days in hospital.

- **Jessica Savage, M.D., M.H.S.**
  Instructor in Medicine, Department of Rheumatology, Immunology, Allergy
  Brigham and Women's Hospital

  "Environmental Antimicrobial Chemicals and Allergic Disease"

  Key Words: Triclosan, Parabens, Personal care products, Asthma, Allergy, Sensitization, Microbiome

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  Allergic diseases (e.g. asthma, food allergy, eczema, hay fever) are the most common diseases of childhood, and their prevalence has nearly doubled in the last 20-30 years in the United States for unclear reasons. In light of the growing evidence that disruption of the human microbiota-the mutualistic and commensal organisms that live within us- is associated with allergic disease, it is notable that the increase in allergy followed the widespread introduction of personal care products such as hand soap and body lotion containing the antimicrobial chemicals triclosan and parabens, which were invented over 40 years ago. Our previous work demonstrated a novel association between these antimicrobial chemicals and allergic disease among children ages 6-18. In this proposal we will further determine the association between antimicrobial chemicals and allergy in younger children, and their effects on the child intestinal microbiome, a likely intermediate in their association with allergy. In Aim 1 we will determine the cross-sectional association between urinary levels of triclosan and parabens and allergic disease and sensitization in 450 3-year-old children participating in the follow-up phase of the Vitamin D Antenatal Asthma Reduction Trial (VDAART). In Aim 2, we will determine the association between these chemicals and intestinal microbial diversity and function in VDAART children. Because our study is ancillary to VDAART, an NIH funded clinical trial that is already collecting clinical and laboratory outcomes relevant to this proposal, this study represents an efficient use of resources to determine if purposeful exposure to antimicrobial chemicals through personal care products contributes to the development of allergy. This study will determine the exposures and mediators that contribute to allergy and has the potential to lead to simple and effective strategies to prevent allergic disease and slow the current alarming rise in prevalence of childhood allergy.

January 2014 Award Recipients

- **Andrea Ciaranello, M.D., M.P.H.**
  Assistant Professor of Medicine
  Department of Medicine, Infectious Disease
  Massachusetts General Hospital

"Novel Approaches to Improve Medication Adherence among HIV-infected Adolescents: the CEPAC-Adolescent Model"

Key Words: Adolescent, HIV, Adherence, Modeling, Cost-effectiveness

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Effective medications to prevent vertical HIV transmission and prolong the lives of HIV-infected infants have led to a shift in the US pediatric HIV epidemic: perinatally HIV-infected adolescents (PHIVA) now represent the majority of HIV-infected children in the US. Adherence to antiretroviral therapy (ART) is critical to suppressing HIV viral load, improving immune function, and avoiding opportunistic infections and death. Medication adherence is difficult for adolescents with many chronic conditions, but may be particularly challenging for PHIVA. Current adherence interventions have had limited success; investigators are considering novel approaches, such as financial incentives for suppressed HIV viral loads and nanoparticle-based long-acting ART, which, if successfully engineered, could replace daily pills with monthly injections.

As data emerge about novel interventions, computer-based clinical policy models can project their likely clinical outcomes and costs, informing both implementation and future research. The Cost-effectiveness of Preventing AIDS Complications (CEPAC)-Pediatric model is a detailed simulation of HIV disease in young African children. Working with adolescent HIV experts, we now propose to develop a new model for adolescents in the US, populated with data from prospective, multicenter cohort studies. We will use the model to evaluate the potential clinical impact, cost, and cost-effectiveness of current and novel adherence interventions among PHIVA:

1. Current practice: individualized combinations of counseling, SMS reminders, peer support groups, home visits, and directly-observed therapy.
2. Financial incentives: quarterly payments for successfully suppressed HIV viral loads.
3. Long-acting ART: monthly injectable ART, replacing oral therapy.

Our research team has developed innovative models of perinatal HIV in Africa that have influenced international HIV guidelines. The new CEPAC-Adolescent model will expand this work to the growing population of PHIVA in the US, inform researchers and clinicians as they design future studies and implementation, and lay a foundation for future studies of optimal care for HIV-infected adolescents.

- **Vandana Gupta, Ph.D.**
  Instructor in Pediatrics
  Department of Medicine
  Boston Children's Hospital

  "Elucidation of Molecular Pathways and Therapeutic Developments in Nemaline Myopathy"

  Key Words: Congenital Myopathy, Nemaline myopathy, Skeletal muscle, Impaired locomotion, High throughput drug screens

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Nemaline Myopathy characterized by muscle weakness and impaired locomotion, form a group of heredity diseases affecting children. Clinically, they form a heterogeneous group of myopathies ranging from severe congenital from with death from respiratory failure during first year of life to a mild-childhood onset myopathy with survival in to adulthood. Applicant has recently identified mutations in a kelch protein as the genetic cause of nemaline myopathy. This proposal is aimed at supporting the career development of Dr. Vandana Gupta as she explores the molecular mechanisms of this novel kelch protein in muscle diseases and develop animal models. In skeletal muscle diseases, strong efforts are currently being devoted to develop therapies in congenital myopathies. However, lack of suitable targets has been a major hindrance in development of successful treatments. Therefore, high throughput screening using FDA approved chemicals will help in the identification of candidate drugs as well as insight on the pathways perturbed in nemaline myopathy. Specific Aim 1 will investigate the role of this kelch protein in ubiquitination pathways in normal and diseased skeletal muscle. Findings from this will be applied to investigate the pathways perturbed in nemaline myopathy patients. Specific Aim 2 will focus on developing zebrafish models and performing high throughput screens using FDA approved chemicals and lead optimizing.

Dr. Gupta's long term aim as an independent investigator is to study the genetic causes of human neuromuscular disorders and their molecular basis to devise treatment strategies using model organisms. The support provided with this award will help her to complete her training and with provide her with a launch pad to obtain data for a successful R01 application to fund her future work.

- **Jun Jiang, M.D., Ph.D.**
  Instructor of Cell and Developmental Biology
  University of Massachusetts Medical School

  “Trisomy Correction in Down's syndrome Mouse Models”

Key Words: Down's syndrome, Trisomy correction, Mouse models, Transplantation, Neural progenitors

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Down's syndrome is the leading genetic cause of intellectual disabilities in humans, occurring in 1 out of 700 live births. The millions of Down's syndrome patients across the world also face multiple other health issues, including congenital cardiac defects, high incidence of Early-onset Alzheimer disease, and hematopoietic disorders. Given that Down's syndrome is caused by an extra copy of chromosome 21 that involves over-dosage of 400 genes across a whole chromosome, it precludes any possibility of a genetic therapy. Our lab has long studied the natural dosage compensation mechanism for X chromosome inactivation. To "dosage compensate" X-linked genes between females and males, the X-linked XIST gene produces a large non-coding RNA that silences one of the two X chromosomes in female cells. We have recently demonstrated the proof of principle that the imbalanced expression of hundreds of genes across an extra chromosome 21 can be de facto corrected in Down's syndrome patient stem cells, by the targeted addition of a single gene, XIST. Remarkably, deficits in proliferation and neural rosette formation are rapidly reversed upon silencing one chromosome 21. Successful trisomy silencing in vitro surmounts the major first step towards potential development of "chromosome therapy". This significant finding was published in Nature and received worldwide attention. The milestone that we have achieved opens new avenues for Down's syndrome translational research. The ultimate goal of this research is to bring Down's syndrome into future genetic therapy. Here, we request the Hood fund to support development of trisomy corrected mouse models of Down syndrome and test human neural stem cells therapy in Down's syndrome mouse brain. If we can further demonstrate an in vivo rescue of Down's syndrome phenotype in mouse models as proposed, findings from this project would promote future efforts to translate "chromosome therapy" to human Down's syndrome patients.

- **Rene Maehr, Ph.D.**
  Assistant Professor of Medicine
  Program in Molecular Medicine
  University of Massachusetts Medical School

  "Development of a Pluripotent Stem Cell-based Approach to Model DiGeorge Syndrome"

  Key Words: DiGeorge Syndrome, Pluripotent Stem Cells, Disease Modeling, Cell Replacement Therapy, Directed Differentiation, Functional Assay

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DiGeorge syndrome (DGS) occurs at a high frequency in newborns and is characterized by congenital heart disease, hypoparathyroidism and thymus hypoplasia. Complete absence of the thymus and ensuing immunodeficiency are observed in a subset of patients and have been linked to a defect during pharyngeal foregut development. Transplantation of thymic tissue is used to treat the immunodeficiency but is hampered by the limited availability of adequate donor tissue. Advances in the generation of disease-specific human pluripotent stem cells (PSCs) have led to opportunities for disease modeling and ultimately for the development of autologous cell replacement therapies. The next step is to develop protocols for differentiating stem cells into DGS relevant cell types.

Recent improvements have been made in differentiating PSCs toward human thymic epithelial progenitor-like cells. However, the differentiation efficiencies are low and the functionality of obtained cell populations is not equivalent to fetal counterparts. We hypothesize that pharyngeal foregut endoderm derived from human PSCs can efficiently differentiate into DGS-relevant pharyngeal derivatives, such as thymic epithelial cells, when the proper molecular context is provided. We propose to use novel reporter mouse models to identify specific transcription factors that control the development of thymic epithelial progenitors. The knowledge that we gain about transcription factor networks in these mouse cells will be used to develop methods for differentiating human PSCs toward thymic epithelial cells. In addition, we will explore the functionality of human PSC-derivatives in a functional assay system that will be used to monitor T lymphocyte development.

This study will provide key insight into pharyngeal development and stem cell biology. At the completion of this project, we expect to have made significant progress in generating a disease model to study the underlying mechanism of DGS, and will bring the field one step closer to generating a stem cell-based therapy for DGS.

- **Victor Navarro, Ph.D.**
  Instructor in Medicine
  Department of Medicine, Division of Endocrinology
  Brigham and Women's Hospital

  "The Role of Tachykinins in the Central Control of Sexual Maturation: Implications for the Timing of Puberty"

  Key Words: Puberty, Tachykinins, Kisspeptin

Pubertal maturation is a developmental process regulated by multiple central and peripheral factors. The identification of factors involved in the neuroendocrine control of puberty onset is continuously increasing; however, the exact mechanism determining the timing of sexual maturation still remains elusive. Recently, neurokinin B (NKB) has emerged as a crucial regulator of pubertal maturation in humans. NKB belongs to a family of closely related peptides, the tachykinins, which also includes substance P (SP) and neurokinin A (NKA). However, the role – if any – of SP and NKA in puberty is unknown. Our preliminary studies in female mice have demonstrated that the activation of SP receptors (NK1R) induces clear stimulatory LH and FSH responses and that chronic injection of NK1R agonists advances puberty. Therefore, I hypothesize that SP, and possibly NKA, plays a role in the initiation of GnRH release during puberty. The main goal of this proposal is to characterize the roles of SP and NKA in the central control of reproduction as potential mechanisms to overcome disorders of puberty onset resulting from central disruptions of tachykinin tone, using the mouse as my experimental model. Specifically, the aims of this proposal are to: 1) characterize the pattern of expression of SP and NKA mRNA (both encoded by the Tac1 gene) and their effects on GnRH and gonadotropin release across postnatal development; 2) determine whether the actions of SP and NKA on GnRH and gonadotropin release are dependent on Kiss1 neurons (known gate-keepers of puberty onset); 3) evaluate the timing of puberty and reproductive phenotype in animals devoid of SP and NKA (Tac1KO). Overall, the successful completion of this proposal will improve our understanding of the mechanisms governing puberty onset and perhaps offer new strategies to treat pubertal disorders in children.

July 2013 Award Recipients

- **Suneet Agarwal, M.D., Ph.D.**
  Assistant Professor in Pediatrics
  Boston Children's Hospital

  "Manipulating Heteroplasmy in Mitochondrial Genetic Diseases"

  Key Words: Bone marrow failure, Genetics, Mitochondrial DNA, Heteroplasmy, Genome editing, Induced pluripotent stem cells

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The broad, long-term objectives of this project are to improve our understanding of the role of mitochondrial function in cellular differentiation in normal and pathological states, and to develop strategies to correct mitochondrial genetic defects. A variety of childhood diseases are caused by dysfunction of the mitochondria. In congenital mitochondrial DNA (mtDNA) disorders, a mixture of normal and mutated mtDNA, termed heteroplasmy, exists at varying levels in different tissues, which fluctuates throughout life and may correlate with disease. For instance in the mtDNA disease Pearson syndrome (PS), children are born with severe blood problems, pancreatic insufficiency, and metabolic acidosis. If children survive, they may go on to develop myopathy and neurological diseases. For PS and other mtDNA disorders, there are limited therapies and no cures. I hypothesize that there are thresholds of heteroplasmy, above which the burden of mutant mtDNA causes tissue-specific functional impairment, and below which mitochondrial dysfunction is tolerated. I further hypothesize that there are genetic factors that influence changes in heteroplasmy in stem cells. Under this award we will test these hypotheses using the following approaches. In Specific Aim 1, I will define the threshold percentage of normal mitochondrial DNA that is required for adequate hematopoiesis and other tissue-specific function using patient samples and in vitro directed differentiation of patient-specific induced pluripotent stem (iPS) cells. In Specific Aim 2, I will analyze genetic differences between different iPS clones to understand the factors that drive changes in mtDNA heteroplasmy in cells. In Specific Aim 3, I will develop methods to shift heteroplasmy by targeted degradation of deleted or normal mtDNA using genome-editing tools. Through these studies, I aim to determine the pathological threshold of mitochondrial dysfunction in hematopoiesis, and translate a strategy to selectively deplete mutant mtDNA to a level below this threshold, for therapeutic use.

- **Heather Burris, M.D., M.P.H.**
  Instructor in Pediatrics
  Department of Neonatology
  Beth Israel Deaconess Medical Center

  "The Epigenetics of the Cervix, Linking the Environment to Spontaneous Preterm Delivery"

  Key Words: Preterm birth, Epigenetics, DNA methylation, Gene expression, MicroRNA, Cervix

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Preterm birth remains an enormous public health issue affecting over 450,000 infants annually in the US. While investigators have recognized preterm risk factors including smoking, black race, prior preterm delivery and short cervix, the prediction of exactly which women will deliver early remains elusive. Identification of at-risk women enables obstetricians to intervene during pregnancy with cerclage placement or progesterone therapy. Current interventions are targeted at women with short cervical length, but not all women who deliver early are identified this way. Improvement in the accuracy of risk prediction remains a critical area of research aimed at preterm birth prevention.

Epigenetics refers to differences in gene expression in the absence of genetic sequence variation. DNA methylation and microRNA (miRNA) profiles are two epigenetic mechanisms affecting transcription and translation respectively. Our preliminary data suggest that during pregnancy, cervical DNA methylation of the prostaglandin receptor 2 gene (PTGER2) and of retrotransposon (LINE-1) are associated with both smoke exposure and the length of gestation. We have demonstrated feasibility of obtaining miRNA profiles from cervical swabs.

We aim to test the hypothesis that DNA methylation and miRNAs profiles of the cervix, a key target tissue during pregnancy, can predict who will deliver spontaneously early. We will obtain cervical swabs from 800 pregnant women presenting for routine transvaginal ultrasounds at 18-20 weeks' gestation (performed in all pregnancies at our institution) and will analyze DNA methylation and gene expression (mRNA) of PTGER2 and LINE-1 in 40 cases and 160 matched controls. We will use state-of-the-art technology to detect 800 miRNAs and determine whether profiles differ in women who deliver preterm vs. term.

This study has the potential not only to improve our understanding of epigenetic mechanisms contributing to preterm birth, but to lead to discovery of novel biomarkers to identify women who could benefit from preventative interventions.

- **Lindsay DiStefano, Ph.D.**
  Assistant Professor, Department of Kinesiology
  University of Connecticut

  "Pediatric Lower Extremity Injury Prevention: Monitoring Changes Over Time"

  Key Words: Injury Prevention, Youth Sport, Anterior Cruciate Ligament

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Youth sport-related injury affects millions of children each year resulting in significant health care costs and potential for devastating long-term consequences, such as the early development of osteoarthritis. Exercise-based injury prevention programs performed as a sport warm-up activity can improve neuromuscular control and reduce the rate of lower extremity injuries, including injuries to the anterior cruciate ligament (ACL). The majority of previous research on injury prevention programs has been in adolescent or adult populations. Early intervention with children prior to high school may be critical for improving long-term adoption of injury prevention in sport and preventing future injury. There is a gap in knowledge about how to implement injury prevention programs in children younger than 14 years old with regards to the dosage and type of program needed to modify neuromuscular risk factors for injury.

The long-term objective of this project is to identify the most effective and efficient injury prevention program for children to promote early implementation and widespread adoption of injury prevention programs in youth sports. Specific Aim 1 will compare the effectiveness of a simplified injury prevention program to a traditional program on modifying neuromuscular risk factors for lower extremity injury. Specific Aim 2 will assess the retention of improvements in neuromuscular control over time to evaluate how often injury prevention programs need to be implemented. We propose a cluster-randomized trial of 30 youth soccer teams with approximately 400 participants. Teams will be randomized to receive either the Basic or Traditional injury prevention program prior to soccer activities for 8-weeks, or be in the control group. Measures of neuromuscular control (e.g. movement technique and balance ability) will be assessed immediately before and after completing the intervention period. Two retention assessments will be made two and four months following the intervention period.

- **Stephanie Eisenbarth, M.D., Ph.D.**
  Assistant Professor in Laboratory Medicine
  Yale School of Medicine

  "Targeting Dendritic Cells to Block Allergen Sensitization in Asthma"

  Key Words: Asthma, Dendritic Cells, Allergy, T cells

Asthma is driven by an exaggerated type 2 helper T cell (Th2) response to environmental allergens that results in bronchoconstriction, airway inflammation and over time, lung remodeling and fibrosis. Immunosuppression and symptomatic treatments have improved disease outcomes but there is little in our armamentarium to prevent, stop or reverse the initial sensitization to allergens. During early childhood (around 6 years of age), many children "outgrow" asthma and remain symptom-free, typically for life. The mechanism of this reversal is unclear but suggests that a unique window exists during which pathological sensitization can be reversed. New approaches to alter the early immune events resulting in T cell sensitization are needed and promotion of T cell tolerance or even induction of regulatory T cells would be a major advance in allergen-specific therapy.

Work over the past 15 years has determined that a particular type of antigen-presenting cell, an activated dendritic cell (DC), regulates the balance between Th2 activation versus tolerance to allergens. DCs collect antigen from the lung and once they receive a licensing signal, primarily from innate immune receptors, they mature and migrate to the lymph node to present these antigens to naïve T cells. We recently discovered that one member of the NOD-like receptor (NLR) family of innate immune receptors is required for migration of a specific subset of mature DCs from the lung during inflammatory responses. NLRP10 dictates whether lung CD11b-expressing DCs, particularly potent in helper T cell priming, migrate from the lung to draining lymph nodes during antigen exposure. The goal of this proposal is to determine if loss of NLRP10 in pulmonary dendritic cells promotes T cell tolerance during exposure to antigens in the lung with the ultimate goal of targeting this pathway in the early stages of asthma to shift the balance between allergen sensitization and tolerance.

- **Sarbattama Sen, M.D.**

Assistant Professor of Pediatrics
The Mother Infant Research Institute
Tufts Medical Center
Current Institution: *Brigham and Women's Hospital*

"BMI-Based Prenatal Vitamins to Ameliorate Oxidative Stress in Obese Pregnancy"

Key Words: Obesity, Prenatal Vitamins, Oxidative Stress

The proposed research project is a novel intervention aimed at ameliorating oxidative stress and inflammation in obese pregnancy with a personalized, BMI-based dosage of prenatal antioxidant vitamins. Oxidative stress and inflammation have been closely linked with adverse pregnancy outcomes such as fetal malformations, preeclampsia and preterm delivery. Dr. Sen previously identified the importance of oxidative stress in the causative pathway of intergenerational obesity in an animal model and found that supplementation of obese animals with an antioxidant cocktail improved offspring outcome. In addition, her pilot data show that oxidative stress is markedly increased, and antioxidant defenses are markedly depleted, in obese pregnant women.

The central hypothesis of this study is that obese pregnancy is characterized by an oxidant/antioxidant imbalance, which increases inflammation and adversely impacts maternal health and neonatal outcome. Restoring oxidant/antioxidant balance with a body mass index-based prenatal micronutrient supplement will decrease oxidative stress and inflammation and improve both maternal and neonatal outcomes in obese pregnancy.

This hypothesis will be tested in a randomized controlled trial of obese women. 170 women will be recruited, some early in pregnancy and some of whom are planning pregnancy, to yield an analytic sample of 83 mother/infant pairs. Participants will be randomized to a standard prenatal vitamin or a BMI-based prenatal vitamin, with BMI-based doses of vitamins C, E, B6 and folate. The primary outcome of the study will be levels of antioxidant vitamins and specific markers of inflammation and oxidative stress; secondary outcomes will be improvement in maternal and neonatal clinical status.

Given its overwhelming current and future impact, innovative approaches to diminish the effect of maternal obesity are of paramount public health importance. This intervention could provide a safe, feasible and effective method to limit the effects of obesity on subsequent generations.

January 2013 Award Recipients

- **Yee-Ming Chan, M.D., Ph.D.**
  Postdoctoral Research Fellow
  Boston Children's Hospital

  "Kisspeptin as a Novel Tool for the Evaluation of Delayed Puberty"

  Key Words: Delayed Puberty, Adolescence, Reproductive Hormones

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The evaluation of the adolescent with delayed puberty is a vexing diagnostic challenge for pediatricians and pediatric endocrinologists. Delayed puberty is most often due to constitutional delay of puberty (CDP), a self-limited condition in which puberty starts late but then proceeds normally. In contrast, some adolescents with delayed puberty have isolated GnRH deficiency (IGD), a permanent disorder caused by the inability to produce or respond to the master reproductive hormone GnRH (gonadotropin-releasing hormone). Currently, the two conditions can only be distinguished retrospectively, by waiting to see if an adolescent eventually enters puberty spontaneously. The lack of a prospective method to diagnose IGD or CDP delays the definitive treatment of IGD, thereby prolonging psychosocial distress and increasing the risk of osteoporosis due to sex-hormone deficiency. Conversely, the specter of the diagnosis of IGD contributes to the anxiety of adolescents with CDP who, until they finally enter puberty spontaneously, are left to wonder whether they have a permanent medical condition.

The hormone kisspeptin was recently found to be a direct and specific stimulus for the secretion of GnRH. Over the past 2 years, the PI of this proposal has administered kisspeptin to healthy men, healthy women, and adult patients with IGD as the first-available in vivo probe of GnRH neuronal function.

This proposal extends these human studies to pediatric populations to explore the first clinical application of kisspeptin: distinguishing IGD from CDP. Through an FDA- and IRB-approved protocol, this study will administer kisspeptin to adolescents with delayed puberty to kisspeptin. The responses of adolescents who eventually enter puberty (i.e., those with CDP) will compared to those who never enter puberty spontaneously (i.e., those with IGD). Thus, this study will establish the use of the "kisspeptin-stimulation test" as a much-needed and long-sought diagnostic tool in the evaluation of delayed puberty.

- **Chunling Lu, Ph.D.**
  Assistant Professor of Global Health and Social Medicine
  Brigham and Women's Hospital

  "The Effectiveness of Community Financing Approach in Improving Child Nutrition Status in Rwanda"

  Key Words: Child Nutrition Status in Rwanda, Mutuelles, Community-Based Financing scheme, Nutritional Care

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Poor nutrition in early childhood is associated with high risk of disease and death in childhood, poor health as an adult, reduced adult income, and perpetuating the poverty cycle. More than one-third of children under age five in developing countries, estimated at 200 million, have stunted growth. In sub-Saharan Africa, the prevalence of stunting, an indicator of chronic malnutrition, has been rising over the past decade.

Current nutrition interventions for under-five children in developing countries include providing education, food and micronutrients, and hygiene and treatment services to children. Evidence suggests that the most effective interventions are those with regular financial support via integration into existing local health systems. Designing a payment scheme that could provide sufficient funding for sustaining nutrition programs and promoting access to nutritional care is crucial for reducing child malnutrition in the resource-poor settings. This study investigates the role of Mutuelles, a community-based health insurance program in Rwanda, in promoting production and utilization of nutritional and medical care for under-five children and improving under-five children's nutrition status.

Using nationally-representative population-based surveys the Rwanda Demographic and Health Survey and the National Health Facility Survey, the proposed project has two specific aims: (1) to examine the relationship between the Mutuelles risk pooling fund received by health facilities and the level of their production of nutrition and child care, and (2) to assess the effect of Mutuelles coverage on under-five child's utilization of nutrition and medical care and their nutritional status. The first study is conducted at the health facility level using health care production model. The second study is conducted at the individual level using a multilevel modeling strategy including characteristics of the child, the mother, the household, and the district health systems. Panel data regression models are also used to explore the relationship at the provincial level.

- **Thomas Maresca, Ph.D.**
  Assistant Professor
  University of Massachusetts Amherst

  "Detecting and Correcting Errors During Cell Division"

  Key Words: Cell Division, Aneuploidy, Down Syndrome, Error Correction, Kinetochores, Spindle

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Errors in chromosome segregation result in a pathological cellular state called aneuploidy that leads to a range of birth defects including Down syndrome. Accurate cell division depends on chromosomes becoming bioriented, a configuration where each sister chromatid is attached to microtubules from opposing spindle poles. Properly bioriented attachments are stabilized by forces that generate tension across the kinetochore, which is the protein complex that links chromosomes to microtubules. However, erroneous attachments are common during cell division and must be corrected to avoid aneuploidy. Error correction requires the selective destabilization of kinetochore-microtubule (kt-MT) interactions on improperly attached chromosomes. Current understanding of the molecular mechanisms responsible for destabilizing incorrect kt-MT attachments is far from complete. Our long-term goal is to understand the mechano-molecular fundamentals of cell division and, in doing so, to identify cellular processes that can be targeted by therapies to reduce the incidence of aneuploidy. The objective of this proposal, and a first step towards achieving our long-term goal, is to characterize novel aspects of error correction by utilizing live-cell assays in D. melanogaster tissue culture cells that combine molecular engineering with high- and super-resolution microscopy approaches. Specific aim 1 will define the contribution of tension-dependent structural changes within kinetochores, called intrakinetochore stretch, to the regulation of error correction. The goal of aim 2 is to identify a novel error correction pathway that we hypothesize is mediated by pole-based kinase gradients. We anticipate that successful completion of the proposal will significantly advance our understanding of the critical process of error correction and identify conserved cellular mechanisms that could be therapeutically targeted treat and prevent a range of diseases that impact children.

- **Michael Talkowski, Ph.D.**
  Assistant in Genetics, Instructor in Neurology
  Massachusetts General Hospital

  "Prospective Study of Genomic Aberrations during Prenatal Gestation"

  Key Words: Genomics, Transcriptomics, Prenatal Diagnostics, DNA Sequencing, RNA Sequencing, Genetic Testing, Fetal Cell Free DNA

Congenital abnormalities affect 3% of all newborns and are devastating to those afflicted and their caregivers, often throughout the lifespan. The current state-of-the-art for the prenatal and neonatal clinical detection of chromosomal abnormalities, which are the largest known cause of birth defects, feature two low-resolution methods that are insensitive to the discovery of a wide portion of the pathogenic variant spectrum. Not coincidentally, up to 85% of individuals referred for developmental defects do not receive a clinically meaningful result from conventional genetic testing. This proposal represents a multi-disciplinary approach to understand the genetic cause of congenital abnormalities and transform the current medical standard for their detection. It will feature an innovative diagnostic component, a cutting-edge molecular genetic component, and a high-risk component dedicated to technology development. These studies will leverage novel genomic approaches developed by the PI in preliminary studies. Our hypothesis is that genomic variation that is cryptic to current cytogenetic techniques can be delineated early in prenatal development, and the consequences of these events on gene expression can be prospectively evaluated, dramatically improving predictive outcomes and the potential for early medical intervention. In Aim 1 we will perform prenatal DNA sequencing from amniocytes of 25 fetuses with a congenital anomaly detected by fetal ultrasound and a cytogenetically detected chromosomal abnormality. In Aim 2, we will Determine the global transcriptional impact of chromatin disruption during fetal development by RNA sequencing of all subjects from Aim 1 at the time of amniocentesis and at birth. In parallel, Aim 3 will involve deep sequencing of fetal-cell free DNA circulating in maternal blood on a small subset of five subjects to calibrate the sensitivity of this non-invasive method of prenatal delineation of chromosomal abnormalities.
These parallel approaches will seek to radically alter the course of genetic testing for congenital anomalies and introduce convergent genomic profiling to transform conventional genetic testing.

- **Stephen Van Hooser, Ph.D.**
  Assistant Professor of Biology
  Brandeis University

  "The Impact of Genes and Experience on the Development of Brain Circuits"

  Key Words: Developmental Diseases, Genetic and Environmental Interactions, Neuroscience

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During development, cortical circuits are assembled through processes that are specified genetically and those that require sensory experience. Interactions between genes and environment have been implicated in several developmental diseases like autism, fragile X syndrome, epilepsy, and attention deficit disorder. Further, abnormal sensory experience has a profound, permanent impact on neural circuit construction, such as in the disease amblyopia. There are two important obstacles to understanding the basic mechanisms underlying these diseases: a) scientists know little about which genes mediate experience-dependent construction of brain circuits; and b) scientists have lacked the ability to directly observe the influence of genes and experience on brain circuits in the living animal. Here, we propose to examine the interaction of genes and sensory experience in the developing mouse visual cortex. The first gene we will examine is the small GTPase Rem2; previous work in vitro has shown that altering Rem2 expression dramatically alters dendritic branching and synapse formation. Further Rem2 expression is modulated by activity/experience, which further amplifies changes in dendritic branching. We will examine the influence of Rem2 in the intact animal using an advanced 2-photon imaging system that is capable of monitoring changes in dendritic spines and stimulus response properties over a period of several hours. Thus, this project will be able to tease apart the interaction of genes and experience and shed light on how these interactions go awry in developmental diseases.

July 2012 Award Recipients

- **Jonathan Comer, Ph.D.**
  Research Assistant Professor, Department of Psychology
  Center for Anxiety and Related Disorders
  Boston University

  "A Controlled Trial of Telemethods to Expand the Availability of Parent-Child Interaction Therapy for Disruptive Preschoolers"

  Key Words: Telemedicine, Pediatric Mental Health Care, Disruptive Behavior Disorders, Preschoolers, Underserved Youth, Parent-Child Interaction Therapy

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Childhood disruptive behavior disorders are highly prevalent, emerge in early childhood, exhibit considerable stability, and are associated with profound disability. These disorders confer sizable risk for later life psychopathology, delinquency, school drop-out, family dysfunction, substance use, criminality, and reduced health-related quality of life. Early onset is associated with more intractable course and comorbid pathology and subsequent life dysfunction.

Early intervention is critical, but broad access to effective treatment is hindered by geographic disparities in children's mental health care. For example, research provides robust support for the efficacy of Parent-Child Interaction Therapy for the treatment of early child disruptive behavior disorders, but significant gaps persist between treatment in experimental settings and services available in the community. The present study will evaluate the efficacy of an Internet-delivered format of Parent-Child Interaction Therapy (I-PCIT), relative to a waitlist control condition, in a randomized controlled trial of 50 young children (3-5 years) with oppositional defiant disorder and/or conduct disorder from across the state of Massachusetts. Independent evaluations will be conducted at baseline, mid-treatment, post-treatment, and 6 month follow-up. Analyses will examine improvements in child diagnoses and symptoms, parenting practices, and family functioning and quality of life. Secondary analyses will examine mediators of treatment response.

Establishing the efficacy of an Internet-based format for the delivery of evidence-based parent management is a critical step in the evaluation of new technologies and their potential for advancing children's mental health care. Drawing on teleconferencing technology, such a format affords real-time interactions for the provision of care traditionally delivered in person, regardless of a family's geographic proximity to an expert mental health facility. Moreover, drawing on technological innovation to deliver interventions directly to families in their natural settings may extend the ecological validity of treatments, as treatments are delivered in the very contexts in which child problems occur.

- **Roger A. Edwards, Sc.D.**
  Assistant Professor, School of Pharmacy and Department of Health Sciences
  Bouvé College of Health Sciences
  Northeastern University

"Use of a Computer Agent to Promote and Support Breastfeeding"

Key Words: Breastfeeding, Computer Agent, Health education

Breastfeeding is one of the most notably underutilized health behaviors that have lifelong implications. No other health behavior, besides exercise, has such a documented benefit in so many health conditions. Yet, only 14.8% of U.S. infants are breastfed exclusively for six months (American Academy of Pediatrics recommendation), despite the mounting evidence for the health risks of not breastfeeding. Improving rates of breastfeeding will have a significant public health impact today and in the years ahead.

Evidence has shown that mothers are not fulfilling their desired infant feeding goals. Initiation and continuation of breastfeeding can be difficult due to inadequate healthcare professional and social support. Mothers often receive conflicting and incorrect advice, and assistance that is either invasive or insufficient.

Given the efficacy of face-to-face consultation and its limits, a promising approach for effectively conveying health information is the use of interactive computer animated agents that simulate face-to-face conversation with a provider. We have developed and pilot-tested a tablet laptop-based computer-animated conversational agent (Computer Agent) designed to improve breastfeeding rates in mothers interested in breastfeeding. Results demonstrated the feasibility of the use of the Computer Agent and affirmed the design of the randomized controlled trial (RCT). Mothers randomly assigned to receive additional support and information through the Computer Agent also had greater intentions to breastfeed after exposure to the Agent.

We would like to expand use of the Computer Agent by making it available via the Internet during the prenatal, perinatal, and postnatal periods. We will conduct an RCT to assess the impact of the Internet-based intervention on mothers' initiation and duration of exclusive breastfeeding for six months after birth, along with subjects' attitudes towards breastfeeding and their confidence in breastfeeding.

• **Arvin Garg, M.D., M.P.H.**
Assistant Professor of Pediatrics
Boston Medical Center

"Do Basic Unmet Material Needs and Social Safety Nets Influence Child Maltreatment Risk? A Nested Case-Control Study"

Key Words: Child Maltreatment, Basic Unmet Material Needs, Social Safety Net Programs, Poverty

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Child maltreatment is a significant public health problem that has harmful consequences for children. Poverty--by virtue of the undue stressors it places on parents-- is the single greatest risk factor for maltreatment. To date, however, studies have not been able to elucidate specific modifiable sequelae of poverty that predispose parents to maltreat their children. Basic unmet material needs--food, childcare, and housing-- are common stressors for impoverished families and represent the targets of specific federal entitlement programs (e.g., social safety nets); thus, in theory, they are modifiable. Although the National Research Council called for the reduction of multiple vulnerabilities as a goal for maltreatment research almost two decades ago, it still remains unknown whether basic unmet needs and receipt of social safety nets influence maltreatment risk.

The long-term goal of this proposed study is to reduce the number of impoverished children who are maltreated. We propose a nested case-control study of 160 low-income mother-infant dyads (40 cases with reported maltreatment, 120 matched controls) drawn from Dr. Garg's current NICHD-funded R00 cluster RCT. This parent study provides the infrastructure to access a large, diverse sample of healthy parent-child dyads from Boston's most impoverished neighborhoods, and to utilize prospectively collected data on basic unmet needs and receipt of social safety nets. The specific aims of the proposed study are to: (1) determine the relationship between basic unmet needs (food, housing, childcare) and child maltreatment; and (2) determine whether social safety net programs (WIC, food stamps, housing and childcare vouchers) protect against maltreatment.

Findings from this study have important implications for maltreatment prevention research, child health, and social policies. This proposed study will inform the development of a R01 grant to investigate the impact of a multi-faceted primary care-based intervention that addresses basic unmet material needs on reducing maltreatment among impoverished children.

• **M. Kyle Hadden, Ph.D.**
Assistant Professor of Medicinal Chemistry
Department of Pharmaceutical Sciences
University of Connecticut

"Vitamin D3 Analogues as Hedgehog Pathway Inhibitors"

Key Words: Vitamin D3, Hedgehog Signaling, Vitamin D Receptor, Medulloblastoma

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Medulloblastomas (MBs) are tumors most often derived from the central region of the cerebellum and are the most common malignant central nervous system tumor in children. The hedgehog (Hh) pathway is a developmental signaling pathway essential for directing growth and tissue differentiation in the central nervous system during embryonic development. Several distinct lines of evidence suggest a causative link between dysregulation of Hh signaling and MB formation in humans. A small molecule inhibitor of Hh signaling, GDC-0449, has proven clinically effective for the treatment of Hh-dependent MB; however, resistance to GDC-0449 treatment has already been observed in an MB patient, highlighting the continued need for developing improved Hh pathway inhibitors as anti-MB agents.

Recent studies have demonstrated that vitamin D3 (VD3), a precursor to the hormonally active form of vitamin D (calcitriol), inhibits the Hh pathway and exhibits anti-cancer activity in vitro and in vivo. Current and ongoing research in the Hadden lab focusing on the development of VD3 analogues as Hh inhibitors has identified several classes of compounds that are potent and selective inhibitors of Hh signaling.

The overall objective of the studies described in this proposal is to continue the development of VD3 analogues as Hh pathway inhibitors. With this central goal in mind, the following Specific Aims will be undertaken. (1) synthesize and characterize vitamin D3 analogues with modifications to the southern (Aim 1) or northern (Aim 2) region of VD3 and (2) evaluate the in vitro Hh inhibitory activity of these analogues (Aim 3). It is anticipated that the successful completion of these studies will provide several VD3 analogues as potent, selective Hh pathway inhibitors that will be subsequently studied in mouse models of MB.

• **Janghoo Lim, Ph.D.**
Assistant Professor, Department of Genetics
Program in Cellular Neuroscience, Neurodegeneration and Repair
Yale University

"Molecular Pathogenesis Studies of Childhood Neurological Disorders; Rett and Angelman Syndromes"

Key Words: Rett Syndrome, Angelman Syndrome, Autism Spectrum Disorder, Neurological Disorder

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Rett (RTT) and Angelman (AS) syndromes are devastating childhood neurological disorders classified as part of the autism spectrum disorders that affect about three percent of children in the United States. RTT and AS are genetically distinct but phenotypically similar diseases with many overlapping neurological phenotypes including intellectual disabilities and abnormal autistic behaviors. The causative genes/mutations for both disorders have been identified and studied with the goal of developing a cure. However, to date there is little effective therapy for these diseases, underlining the need to indentify an effective protein that can be used as a drug target to treat these debilitating disorders. This can be accomplished if we are better able to understand the pathogenic mechanisms and pursue molecular interventions on that basis.

Our long-term research goals are to better understand the pathogenic mechanisms underlying RTT and AS, and ultimately to translate the findings into the development of therapeutics. The fact that RTT and AS display many similar clinical features such as cognitive deficits and autistic behaviors raises the question of whether they impact similar genetic or molecular pathways. Based on previous studies and data presented in this proposal, we hypothesize that the overlapping neurological phenotypes of RTT and AS are due to shared molecular functions between MeCP2 and E6AP (the gene products causative of RTT and AS, respectively). In this proposal, we will test this idea via the following specific aims: (1) test whether alterations in Ube3a expression can modulate the MECP2-induced phenotypes, or vice versa, and (2) determine the molecular mechanisms underlying the genetic interaction between MeCP2 and E6AP. We propose a multidisciplinary approach that combines biochemistry, cell biology, behavior, and molecular genetics with in vivo animal models. Our proposed research will provide insight into therapeutic interventions for RTT, AS, MECP2 duplication syndrome, and related child brain disorders.

January 2012 Award Recipients

- **Irina Bezsonova, M.Sc., Ph.D.**
  Assistant Professor in Residence
  Department of Molecular, Microbial and Structural Biology
  University of Connecticut Health Center

  "The Role of Usp7 in Pediatric Neuroblastoma"

  Key Words: Cancer, Pediatric Neuroblastoma, Usp7, HAUSP, p53, Ubiquitination

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The p53 signaling pathway is a complex multi-component network central to cancer biology. Over 50% of all tumors have mutated tumor suppressor p53. However, in Neuroblastoma (NB) - the most common solid malignancy in childhood responsible for more pediatric cancer deaths than any other pediatric tumor, the p53 gene is rarely mutated; instead, proteins responsible for maintaining an appropriate level of p53 in a cell are affected. In NB tumors the level of p53 in the nucleus is severely down-regulated due to malfunction of de-ubiquitinating enzyme Usp7, responsible for rescuing p53 from degradation.

Despite significance of Usp7 in cancer development its structural organization and many aspects of its function are poorly understood. While the two N-terminal domains of USP7 and their interactions with binding partners have been characterized neither structure nor functional significance of the large C-terminal region (C-Usp7) are known. Here we propose to investigate the C-Usp7 both structurally and functionally using NMR spectroscopy and X-ray crystallography in conjunction with ubiquitination/deubiquitination assays.

Our specific aims are (1) to identify structured domains in the C-terminal region of Usp7 and determine their 3D structures both individually and in tandem, and (2) to probe potential auto-regulatory intra-molecular interactions between the C-terminal domains of Usp7 and its catalytic domain, investigate the effect of putative interactions on Usp7 activity and structurally characterize these interactions. In a long term, the resulting atomic resolution structures and biochemical analyses of Usp7 activity will provide a basis for design of drugs suitable for treatment of pediatric Neuroblastoma.

- **Elisa Boscolo, Ph.D.**
  Instructor, Vascular Biology Program
  Children's Hospital Boston

  "NOTCH Signaling during Pathological Blood Vessel Formation and Maturation"

  Key Words: Infantile Hemangioma, Vascular Progenitor Cells, Pericytes

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Infantile hemangioma (IH) is a vascular tumor that occurs in 5-10% of infants of European descent. A defining feature of this tumor is its dramatic growth and development into a disorganized mass of blood vessels that can cause disfigurement and tissue/organ disruption. We recently developed the first IH mouse model based on injection of patient-derived hemangioma stem cells (HemSC). HemSC can fully recapitulate the tumor life cycle as they differentiate into endothelial cells and pericytes.

In IH and in the murine model, numerous pericytes surround the blood vessels. We aim to investigate the origin and the role of these numerous pericytes and we propose that NOTCH signaling is involved in the HemSC-to-pericyte differentiation. Our plan is to focus on JAGGED1 because we and others recently reported JAGGED1 overexpression in proliferating IH. Thereby we are interested in understanding JAGGED1 role in the pathogenesis of IH. Furthermore, our working hypothesis that JAGGED1 interacts with NOTCH3 to induce pericyte differentiation, is supported by recent literature that shows defects in smooth muscle cell maturation in NOTCH3 deficient mice and reports NOTCH3 implication in the perivascular differentiation.

In Aim 1 our studies will be conducted by downregulating the JAGGED1expression in the endothelium and NOTCH3 in the HemSC by short interference RNA-mediated silencing and with NOTCH signaling inhibitors, such as DAPT, as a strategy to inhibit HemSC-to-pericyte differentiation and thereby blood vessel formation in IH.
In Aim2 we propose to investigate the regulation of JAGGED1--NOTCH3 signaling in a model of physiological vasculogenesis. We will study the pericyte differentiation of bone marrow mesenchymal progenitor cells (bmMPC) when combined with cord blood Endothelial Progenitor Cells (cbEPC) in Matrigel. The comparison between a pathological and a normal vasculogenesis model will enable us to understand how to fine-tune this signaling to avoid excess of blood vessel growth.

- **Katie McLaughlin, Ph.D.**
  Instructor of Pediatrics
  Children's Hospital Boston

  "Neurobiological Mechanisms Linking Adverse Childhood Experiences to Adolescent Mental Disorders"

  Key Words: Childhood Adversity, Child Trauma, Brain Development, Prefrontal Cortex, Amygdala, Ventral Striatum, Psychopathology

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Adverse environmental experiences, including maltreatment and violence, are associated strongly with mental disorders in children and adolescents. The current proposal tests a neurobiological model linking childhood adversity (CA) exposure to adolescent mental disorders. This model posits a central role of heightened amygdala and ventral striatum reactivity to environmental stimuli and poor prefrontal cortex control over these sub-cortical structures as mechanisms linking CAs to mental disorders.

I propose that CA exposure leads to a cascade of neurobiological changes that culminate in heightened emotional reactivity, a tendency to experience intense negative emotional reactions and a dysregulated physiological response to environmental events paired with poor ability to modulate these emotional experiences. Emotional reactivity results from asymmetrical resting activation of the prefrontal cortex--with greater activation in the right relative to the left hemisphere--elevated amygdala reactivity to environmental stimuli, and failure of the prefrontal cortex to adequately inhibit this activation, heightening risk for internalizing disorders. I also propose that CA exposure increases reward sensitivity and impairs the cognitive control system that directs reward motivation towards socially appropriate stimuli, resulting in failure to delay gratification and inhibit short-term rewarding behaviors in the service of longer-term goals.

Poor executive functioning results from over-activation of the ventral striatum to reward and poor inhibition of this activation by the prefrontal cortex, elevating risk for externalizing disorders. These predictions will be tested by acquiring resting electroencephalogram (EEG) and structural and functional magnetic resonance imaging (MRI) from an existing cohort of adolescents with and without exposure to CAs. fMRI tasks are designed to activate the amygdala and/or the ventral striatum as well as specific regions of the prefrontal cortex that regulate activation in these structures. Findings will be used to inform the development of interventions targeting children exposed to adversity and violence, a critical public health priority.

- **Valerie Schumacher, Ph.D.**
  Instructor in Pediatrics
  Children's Hospital Boston

  "A Role for Staufen 2-Containing RNA Granules in Chronic Kidney Disease"

  Key Words: Chronic Kidney Disease, End Stage Renal Disease, Podocyte, RNA Granules, Staufen 2

Chronic kidney disease in children leads to irreversible kidney damage and lifelong dialysis or kidney transplant. This represents a tremendous burden for patients and their families, and high costs to the health care system. The final common pathway in chronic kidney disease from many causes is damage to the complex cytoarchitecture of podocytes, a key cell type in the filtering apparatus (glomerulus) of the kidney. The current treatments for children with chronic kidney disease are not based on an understanding of the molecular basis of the disease, and in many instances these treatments fail to prevent the onset of End Stage Renal Disease (ESRD) and the requirement for dialysis or kidney transplant.

My laboratory has taken a novel and unique approach that is designed to understand and ultimately improve the regenerative capability of podocytes, and thus prevent or treat chronic kidney disease. Towards this goal we have developed preliminary data to demonstrate that podocytes assemble and localize cytoplasmic ribonucleoprotein particles (RNPs), or RNA granules to store translationally silent mRNAs within their long cytoplasmic extensions (foot processes) for local translation, in a similar manner as another polarized cell type, the neuron. This grant proposes to continue our studies on the role of RNA granules as an adaptive mechanism involved in maintaining foot process architecture and thus preventing ESRD. The major focus will be on the role of the RNA binding protein Staufen 2 in regulating the cytoskeletal assembly of podocyte foot processes both under physiological conditions as well as in response to injury. To address this task, a complementary approach will be taken including immortalized podocyte cell lines and conditional Staufen 2 knockout mice. A greater molecular understanding of this physiologic pathway may eventually lead to new therapies to prevent ESRD.

• **Zhu Wang, Ph.D.**
Assistant Professor, Department of Pediatrics
Connecticut Children's Medical Center

"Statistical Methods for Postoperative Morbidity after Cardiac Surgery in Children"

Key Words: Cardiac Surgery, Morbidity, Intensive Care Unit, Length of Stay, Complication, Variable Selection, Clustered Data, Missing Data

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This study proposes new statistical methods for predicting postoperative morbidity outcomes after cardiac surgery in children. Due to a continuing fall of mortality rate for pediatric cardiology surgery, morbidity data such as number of complications and length of stay (LOS) should be used to assess increasingly important early outcomes for patients and the health care system. These variables are "count" data in which the observations can take only the non-negative integer values. Often the number of zeros in the sample can't be accommodated properly by a simple model, leading to zero-augmented data. In high-dimensional settings with many predictors, the existing methods are insufficient for variable selection.

The project is motivated by statistical issues involved in a NIH sponsored study. In a multi-center prospective study, 311 children between 1 month and 18 years old undergoing cardiac surgery were enrolled between 2007 and 2009. The study collected preoperative, intraoperative, and postoperative risk factors, along with biomarkers for complications. We are primarily interested in identifying factors for predicting intensive care unit LOS and complications.

We will study novel methods which can conduct coefficient estimation and select variables simultaneously, and extend to multi-center study analysis and scenarios with missing data. The specific aims of the proposal are: 1) To develop variable selection methods for predicting zero-augmented data with high-dimensional predictors. 2) To develop these variable selection methods in the unique setting of multi-center study analysis. 3) To develop these variable selection methods for application in the common situation of missing data.

July 2011 Award Recipients

- **Jeffrey Dvorin, M.D., Ph.D.**
  Assistant Professor of Pediatrics and Associate Physician in Medicine
  Department of Medicine, Division of Infectious Diseases
  Children's Hospital Boston

  "Functional Characterization of an Essential Protein Kinase in the Malaria Parasite Plasmodium Falciparum"

  Key Words: Malaria, Plasmodium Falciparum, Parasite Egress, Kinase

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Malaria is a major global cause of morbidity and mortality, with >200 million cases and almost one million deaths every year. Most of the deaths from malaria occur in children under five years of age and are caused by infection with Plasmodium falciparum. Therefore, malaria remains an important pediatric infectious disease. Clinical malaria results from the asexual replication of the parasite in human red blood cells. A molecular understanding of the life cycle of P. falciparum will facilitate the rational design of new therapies. The parasite relies on efficient invasion into and egress out of human red blood cells during the blood stage of its life cycle. I have identified a plant-like calcium-dependent protein kinase PfCDPK5 that is crucial for P. falciparum egress. When the level of this protein is decreased, the parasites remain trapped inside an infected red blood cell. I hypothesize that PfCDPK5 mediates an essential step in the parasite life cycle through phosphorylation of effector substrate proteins in response to a calcium-based egress trigger.

The goal of the proposed research is to provide a molecular characterization of PfCDPK5 function. We will utilize an inducible expression system in the parasite and advanced genetic and cell biologic techniques. The goal will be achieved through two specific aims. In the first, the localization, trafficking, and regulation of the kinase will be defined using both transgenic parasites and in vitro kinase reactions. In the second specific aim, the substrate(s) of PfCDPK5 will be identified using a candidate gene approach and an unbiased proteomic approach.

The long-term objective of this project is to discover the critical PfCDPK5-dependent signaling pathway required for efficient parasite egress out of human red blood cells. Results from these studies will be an essential component of an NIH R01 grant application.

- **Julie Goodwin, M.D.**
  Assistant Professor of Pediatrics
  Yale University School of Medicine

  "The Role of the Endothelial Glucocorticoid Receptor in the Development of Atherosclerosis"

  Key Words: Mouse Model, Atherosclerosis, Endothelium, Glucocorticoid Receptor

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  Cardiovascular morbidity and mortality and, in particular, atherosclerosis are sequelae of multiple conditions which are becoming more common as the population ages and children grow more obese. Manipulation of glucocorticoid metabolism is being investigated as a potential therapy for atherosclerosis. We hypothesize that the endothelial glucocorticoid receptor is a critical mediator of atherosclerosis likely through impairment in local cortisol and eNOS metabolism, which promotes a pro-inflammatory state. Using a mouse model with a tissue-specific deletion of the glucocorticoid receptor in the vascular endothelium we propose to examine the role of this receptor in the development and maintenance of atherosclerosis, a role that has previously not been investigated.

  The first aim of this project is to characterize the in vivo cardiovascular phenotype of control and knockout mice after timed feeding of an atherogenic diet. Using a variety of tissue staining methods we plan to evaluate the content and extent of atherosclerotic lesions in the aorta and brachiocephalic arteries of animals from both genotypes. Characterization of the phenotype will also include measurement of serum corticosterone and lipid levels in both groups as well as analysis of aortic homogenates from each genotype for specific atherogenic genes.

  The second aim of this project is to investigate the signaling pathways and gene expression affected by the absence of the endothelial glucocorticoid receptor in an in vitro cell culture model. Using an siRNA approach to knock down the glucocorticoid receptor in mouse lung endothelial cells isolated from our mice, we plan to evaluate apoptosis in response to incubation with oxLDL, adhesion molecule expression after treatment with TNF-alpha, and pro- and anti-atherogenic gene expression by PCR array in response to oxLDL. Examination of the PI3K/Akt/eNOS pathway as it influences apoptosis will also be studied.

- **John Harris, M.D., Ph.D.**
  Assistant Professor of Medicine
  University of Massachusetts Medical School

  "Targeting the IFN-Gamma Signaling Pathway for the Treatment of Vitiligo"

  Key Words: Vitiligo, Skin, Autoimmunity, IFN-Gamma, Cytokines, Chemokines

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Our goal is to dissect the role of the IFN-gamma pathway in vitiligo pathogenesis in order to develop new, targeted treatments for disease. Vitiligo is a disfiguring autoimmune disease of the skin that results in depigmentation. It affects 1-2% of the population and, similar to type 1 diabetes, 50% of patients first develop the disease as children. It is psychologically devastating because it is so visible and difficult to treat, and children are particularly susceptible to social stigmatization. Vitiligo is caused by autoreactive, melanocyte-specific CD8+ T cells, which migrate from the blood into the skin and directly destroy melanocytes, the pigment-producing cells of the skin.

IFN-gamma-related genes are specifically expressed in the skin of humans and mice with vitiligo. Therefore, we hypothesize that modulating the IFN-gamma pathway will effectively treat disease. To test this hypothesis, we developed a mouse model of vitiligo that closely resembles human disease. Using this model, we found that IFN-gamma and IFN-gamma-dependent chemokines are expressed within affected skin, are critical for depigmentation, and are required for autoreactive T cell accumulation within the skin.

We will further dissect the role of IFN-gamma in the pathogenesis of vitiligo, and use this understanding to develop new treatments. We will use our unique mouse model to address the following specific aims:

1) Determine which cell types in the skin contribute to vitiligo pathogenesis through IFN-gamma signaling, either by deleting the cell population entirely when possible, or selectively eliminating STAT1 expression (essential for IFN-gamma signaling) in each cell type in the skin.

2) Identify the role of IFN-gamma-dependent chemokines in T cell migration to, and through, the skin by analyzing disease and migration patterns in chemokine and chemokine receptor-deficient mice.

3) Test antagonists of chemokine signaling as novel treatments for vitiligo, including chemokine neutralizing antibodies and chemical inhibitors of their receptor.

- **Dimitrios Iliopoulos, Ph.D.**
  Assistant Professor
  Department of Cancer Immunology and AIDS
  Dana-Farber Cancer Institute

  "Identification of Novel Molecular Circuits in Pediatric Ulcerative Colitis"

  Key Words: Pediatric Ulcerative Colitis, microRNAs, Inflammation, Circuits

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  Inflammatory Bowel Diseases (IBDs), including Crohn's disease and ulcerative colitis (UC), are chronic inflammatory diseases of the digestive track. It has been estimated that 1.4 million individuals in the United States have been diagnosed with IBD and around 10% of them are children and adolescents under the age of 17. However, most of the scientific information about the molecular basis of IBD has been obtained by studying adult patients and there is no evidence about the genetic components involved in pediatric IBD pathogenesis. Recently, microRNAs have been found to be involved in the pathobiology of several human diseases, however their role in pediatric IBDs is still unknown. According to our preliminary data, microRNAs are deregulated in pediatric IBD and specifically the microRNA miR-124 is highly down-regulated in pediatric UC patients relative to healthy controls. Furthermore, we have identified that miR-124 regulates directly STAT3 inflammatory signaling pathway in colonic epithelial cells. The central goal of this proposal is to reveal the role and function of miR-124/STAT3 molecular circuit in the pathogenesis of pediatric UC and identify if perturbation of this circuit has any therapeutic effects. Specifically, we will elucidate the significance of miR-124/STAT3 interaction in tissues derived from UC animal models and pediatric UC patients. In addition, we plan to identify genes relevant to pediatric UC that are regulated by miR124/STAT3 inflammatory circuit by whole-genome analysis. Furthermore, we will test if perturbation of the miR-124/STAT3 circuit would suppress the development of ulcerative colitis in mice. Overall, the proposed work not only will enhance our understanding how microRNAs are involved in pediatric UC pathogenesis in the molecular level, but also will reveal the potential effects of miR-124 overexpression to suppress UC development.

- **David Skurnik, M.D., Ph.D.**
  Instructor in Medicine
  Brigham and Women's Hospital

  "Broad-Spectrum Immunoprophylaxis and Therapy for Bacterial Meningitis in Neonates"

  Key Words: Bacterial Meningitis, Immunoprophylaxis, Neonates

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  Neonatal bacterial meningitis continues to be a serious disease with an unchanging rate of adverse outcomes of 20-60%. The 3 major pathogens in developed countries are: Group B streptococcus (GBS), Escherichia coli K1 and Listeria monocytogenes (LM). Due to the emergence of antibiotic resistant strains, morbidity and mortality rates may significantly increase. Unexpectedly, we have found these three pathogens all produce the conserved bacterial surface polysaccharide poly-N-acetyl-glucosamine (PNAG). This molecule is involved in biofilm formation by multiple bacterial species and has been shown to be a target antigen for antibody-mediated protection in models of S. aureus and E. coli systemic infections and S. aureus skin infections. The goal of the proposed research is to use antibodies to PNAG for prevention and treatment of bacterial infections in neonates. Specifically, we plan to use polyclonal and a fully human monoclonal antibody against PNAG in a model of GI infection followed by systemic translocation using neonatal mice to evaluate antibody efficacy in preventing systemic and brain (meningitis) infection caused by K1 E. coli, GBS and L. monocytogenes. Although the latter organism is primarily an intracellular pathogen against which T-cell mediated immunity is effective, the discovery that it produces surface PNAG may provide for antibody-mediated protection by blocking bacterial translocation into the brain. The mechanisms of protection mediated by these antibodies will be tested through their bactericidal and opsonophagocytic activities as well as their ability to block binding, entry and translocation across monolayers of human brain microvascular endothelial cells. Studies in C3b-deficient new-born mice will quantify the contribution of complement-dependent antibody-mediated killing and inhibition of cellular translocation to protective efficacy against systemic spread and meningitis. These aims will determine if passive transfer of either hyper-immune IVIgG or the fully human MAb to PNAG into neonates might protect against the major pathogens causing neonatal meningitis.

January 2011 Award Recipients

- **Renee Boynton-Jarrett, M.D., Sc.D.**
  Assistant Professor
  Boston University School of Medicine

  "A Nested Case-Control Study of Genetic and Psychosocial Determinants of Early Puberty"

  Key Words: Puberty, Girls, Genetics, Psychosocial stress, Gene-environment interactions

  Declining age at onset of puberty, widening differences by race/ethnicity, and significant health and social consequences of early puberty are of growing clinical and public health concern. Yet the major determinants of early puberty remain poorly understood. Compelling research supports an association between childhood adversities and early puberty. Alterations in the hypothalamic-pituitary-adrenal and -gonadal axes due to chronic psychosocial stress may influence functioning of organ systems responsible for sexual maturation. There are few epidemiologic and no genetic studies of pubertal onset among U.S. minority populations, although African American girls carry the greatest burden of risk for early puberty. The overarching hypothesis guiding this proposal is that psychosocial stress during critical developmental periods contributes to early puberty, such associations may be modified by individual genetic variations, and this may partially explain racial/ethnic disparities in risk for early puberty.

  We propose a nested case-control study of 200 African American girls (60 cases with early puberty and 140 age-matched controls) from the Boston Birth Cohort (BBC), a prospective, predominantly low-income population, currently following ~1,800 children. Precise measurement of early puberty, defined as physician-assessed Tanner stage >/=2 prior to age 8, will be aided by robust hormonal assays, bone age and peak height velocity assessment. Specific aims of the proposed research are to: (1) determine the relation between psychosocial stress in early life and the onset of early puberty; and (2) investigate whether genetic variants associated with pubertal timing or stress reactivity modulate the impact of psychosocial stress on risk for early puberty.

  Findings from this study will help identify important psychosocial and genetic determinants of early puberty in a population of African American girls who experience a disproportionate burden of risk, and inform clinical and public health interventions to prevent early puberty and mitigate its health and social consequences.

- **Jing Chen, Ph.D.**
  Instructor in Ophthalmology, HMS
  Children's Hospital Boston

  "Targeting Wnt Pathway for Retinopathy of Prematurity"

  Key Words: Retinopathy, Prematurity, Retina, Vasculature, Wnt, Lrp5

  Retinopathy of prematurity (ROP) is the leading cause of blindness in children, with life-long impact on their vision. A fundamental problem in ROP is lack of blood vessel growth in the retina after premature birth. This poor vascularization causes ischemia and hypoxia in the retina, which then stimulates subsequent abnormal and sight-threatening vessel proliferation. Current ablation surgery is only partially effective and identification of less-invasive therapies is much more desirable. The long term goal of this project is to identify such therapies through better understanding of the molecular pathways involved in the pathogenesis of ROP. Here we propose an attractive strategy to tackle ROP through investigation of Wnt signaling pathways, mutations of which are implicated in severe forms of ROP as well as several rare hereditary eye diseases with similar pathology as ROP. We hypothesize that Wnt signaling through interaction between retinal neurons, vessels and inflammatory cells affects retinal blood vessel growth in ROP, and selectively targeting components in this pathway can lead to novel treatment options for the disease. We will test this hypothesis with two aims. In Aim 1, we will assess phenotypically retinal vasculature in a well-established oxygen-induced mouse model of ROP using mice with mutations in Wnt signaling, and analyze expression of genes regulated by Wnt to impact ROP. In Aim 2, we will assess whether specific pharmacologic modulation of components in Wnt pathway with selective ligands or inhibitors, or genetic manipulation with siRNA or cationic DNA over-expression, can prevent vessel loss and pathologic vessel formation as well as neuronal degeneration in mouse ROP.

• **Steven Hatch, M.D.**
Center for ID and Vaccine Research
University of Massachusetts Medical School

"Maternally Derived Anti-Dengue Antibodies and the Risk of DHF in Infants"

Key Words: Dengue infection, Dengue hemorrhagic fever (DHF), Antibody dependent enhancement (ADE), Secondary infection, Infants, Neutralizing antibodies

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This study proposes to directly test the hypothesis that antibody-dependent enhancement (ADE) is the critical factor in the development of dengue hemorrhagic fever (DHF) in infants. DHF occurs in two distinct clinical settings: a) in children and adults with secondary infection, and b) in infants with primary dengue infection born to dengue-immune mothers. The ADE hypothesis proposes that pre-existing serotype cross-reactive non-neutralizing anti-dengue antibodies bind the heterotypic dengue virus during secondary infection and enhance its uptake into immune cells, leading to increased viral load and DHF. This model suggests that DHF in dengue-infected infants is caused by the enhancing effect of waning maternal anti-dengue antibodies, thereby increasing the infant's risk of DHF.

The effect of maternal immunity on DHF in infants has been studied exclusively in Southeast Asia. However, the maternal dengue seroprevalence approaches 100% in this part of the world. As a consequence, the ADE model of infant DHF cannot truly be tested in Southeast Asia, because all infants possess anti-dengue antibodies at birth. In the Western Hemisphere, by contrast, women may have experienced either a single dengue infection, more than one dengue infection, or no dengue infection at all. The ability to include dengue-naïve mothers as controls allows for the ADE hypothesis to be tested directly in a clinical study.

The following proposes a case-control study designed to evaluate the influence of maternal dengue seroprevalence on the risk of DHF in infants. The specific aims (and broad methods) of this project are as follows:

#1: Compare rates of dengue seroprevalence of mothers from two groups of infants: infants with DHF and those with symptomatic DENV infection but without DHF.

#2: Test ADE in vitro using the serum from the mothers of both groups of DENV-infected infants (as a surrogate for ADE activity of pre-illness infant sera).

#3: Evaluate the relationship between ADE activity and estimated peak viremia levels.

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

  "The Effect of Age on Outcome after Traumatic Brain Injury in Apolipoprotein E4 Carriers"

  Key Words: Traumatic Brain Injury, APOE4, Beta Amyloid

  The best known genetic risk factor for poor outcome after traumatic brain injury (TBI) in adults is the apolipoprotein E4 (APOE4) allele of the APOE gene. There is conflicting evidence as to whether APOE4 is a risk factor for poor outcome after TBI in children. The detrimental effect of APOE4 on outcome after TBI in adult carriers is thought to be in part due to impaired metabolism of the beta amyloid protein, which is also implicated in the pathogenesis of Alzheimer's Disease (AD). In Alzheimer's Disease, beta amyloid has been shown to accumulate in the synapse leading to synapse loss and dysfunction. Beta amyloid also accumulates after TBI and a recent landmark study suggests that inhibition of beta amyloid formation after TBI improves functional and histopathological outcomes in aged mice. The possibility that beta amyloid may influence outcome after TBI in children has not been reported in experimental or human TBI. This is a particularly relevant issue in pediatric TBI because there is some evidence to suggest that beta amyloid is less toxic to the immature brain. Beta amyloid toxicity after TBI may also not be as relevant to APOE4 carriers injured in childhood, as beta amyloid metabolism in APOE4 carriers appears to be significantly better in young versus aged mice. These age-dependent alterations in beta amyloid toxicity and metabolism in experimental models may explain why the role of APOE4 in outcomes in childhood TBI remains unclear with reported effects that have ranged from detrimental to none to protective. The purpose of this application is to characterize the interaction between age at injury and APOE4 in terms of synaptic beta amyloid, histopathology, and functional outcome after TBI and to explore whether therapeutic interventions targeting beta amyloid are appropriate across the spectrum of childhood.

- **Alexander Soukas, M.D., Ph.D.**
  Instructor in Medicine
  Massachusetts General Hospital

  "Obesity and Diabetes Genetics in C. elegans and mice"

  Key Words: Obesity, Diabetes, Genetics

  Obesity is an enormous public health problem and is increasing in prevalence in childhood and adolescence. Obesity early in life is highly associated with diabetes, coronary heart disease, stroke, hypertension, dyslipidemia, and cancer, major causes of morbidity and mortality. Complex human genetics underlie the development of obesity. However, because human genetics cannot inform the mechanism of action of disease genes, facile model systems are needed to explore genes involved in human diseases. C. elegans genetics, genomics and genetic conservation with humans provides a means to explore genetic pathways regulating metabolism at a level of the whole organism at a pace and depth not possible in mammalian systems. We propose a combination of C. elegans and mouse genetics to elucidate the gene network underlying fat mass regulation. C. elegans will be used 1) to study disease mechanisms for human obesity and diabetes disease genes emerging from genome-wide association studies (GWAS) and to 2) study the gene network through which target of rapamycin complex 2 (TORC2) regulates metabolism, fat mass, and growth. We previously identified mutations in C. elegans TORC2, a highly conserved kinase complex, that cause high body fat mass, short lifespan, and slow growth. In C. elegans and humans alike, TORC2 is a key regulator of genes involved in metabolism, diabetes, obesity, and cancer, and is thus a powerful therapeutic target. Genetic, genomic, informatic, and biochemical strategies will be used to explore the mechanisms by which human GWAS genes and TORC2 regulate metabolism. Further, to investigate the role of TORC2 in mammalian obesity and diabetes, targeted deletion of TORC2 in mouse liver will be conducted. Finally, genetic findings made in C. elegans will be validated in vivo in mice. The ultimate goal is to identify conserved regulators of metabolism that will illuminate disease mechanisms of human obesity and diabetes.

July 2010 Award Recipients

- **Abraham Brass, M.D., Ph.D.**
  Instructor in Medicine
  Massachusetts General Hospital

  Currently: Assistant Professor, Microbiology and Physiology Systems Department
  University of Massachusetts Medical School

  "Understanding Intrinsic Immunity: Investigation of IFITM3's Inhibition of Influenza A Virus Infection"

  Key Words: influenza A Virus, Pediatric Influenza, Viral Host Interactions, Intrinsic Immunity, Interferon, Restriction Factor

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  Influenza epidemics exact a formidable toll on world health and disproportionately effect the very young and old. At present, the emergence of a novel influenza A H1N1 viral strain has created a pandemic, producing illness in over 200 countries. To find host-cell modifiers of influenza A H1N1 viral infection, we completed a large scale genetic screen and detected several proteins which are important in decreasing influenza A virus infection, including a role for Interferon-inducible trans-membrane protein 3 (IFITM3). The loss of IFITM3 resulted in elevated viral replication in multiple cell lines tested, and proved to be critical for IFN-induced viral resistance, accounting for 40% to 70% of IFN's protective ability. IFITM3 belongs to a family of four closely related proteins in humans, and five proteins in mice. This application aims to elucidate the role of the IFITM proteins in the host response to viral infection. Successfully achieving the aims of this proposal will provide an in depth knowledge of the actions of IFITM3 and will inform us more fully about our intrinsic immune response to viruses. In our first aim we seek to define the mechanism of IFITM3-mediated inhibition of viral infection using functional, structural and image-based studies. The experiments in aim 1 will improve our understanding of how the IFITM3 stops viruses, and therefore may suggest new ways to prevent or treat influenza. Our second aim is designed to detect protein interactions involving IFITM3 and rigorously test the functional relevance of these connections. We expect these studies to identify new effectors of cell intrinsic immunity and also deepen our understanding of the actions of IFITM3. Fulfilling these two aims will further the project's long term goal of understanding how our cells defend themselves against viral invasion and may provide new tools and strategies for stopping infections.

• **Thomas Murray, M.D., Ph.D.**
Associate Research Scientist of Pediatrics and Laboratory Medicine
Yale University

"The Role of Lactate Metabolism in Pseudomonas Aeruginosa Biofilm Formation"

Key Words: Pseudomonas Aeruginosa, Lactate, Biofilm, Cystic Fibrosis

Chronic pulmonary infection with Pseudomonas aeruginosa causes recurrent hospitalization and mortality in children with cystic fibrosis (CF). These chronic infections are difficult to eradicate because P.aeruginosa forms organized biofilms encased in a protective, extracellular matrix composed of exopolysaccharide. Altering the nutritional environment can change the biofilm structure, increasing the susceptibility of P.aeruginosa biofilms to antibiotics. Our long term goal is to identify novel therapeutic targets to treat chronic infection in children with CF, either by manipulating an environmental factor required for biofilm formation or by disrupting bacterial pathways that trigger biofilm formation. Evidence from other bacteria shows extracellular lactate, a potential energy source for P.aeruginosa elevated in the bronchoalveolar lavage fluid from CF patients, is an important determinant of biofilm formation and in vivo colonization. We have identified a novel mutation in a predicted P.aeruginosa lactate permease (lldP), which alters biofilm formation.

We hypothesize that lactate metabolism is one factor that determines P.aeruginosa biofilm formation and represents a potential therapeutic target. The specific aims of this study are to: 1) Determine the mechanism by which lactate metabolism influences P.aeruginosa biofilm formation. We will measure lactate uptake and metabolism, examine biofilm formation, and measure exopolysaccharide synthesis in wild type P.aeruginosa and in mutant strains lacking either the lactate permease or related lactate oxidase. These experiments will be conducted with varied extracellular lactate levels to understand how changing extracellular lactate alters biofilm formation. 2) Determine whether the in vitro defects in biofilm formation due to altered lactate uptake are important for mammalian lung infection. Using a novel CF murine model of lipopolysaccharide induced chronic inflammation, wild type and CF mice will be infected with wild type P.aeruginosa or a strain lacking the lactate permease and the lungs examined for the presence of bacteria and inflammation.

- **Lise Nigrovic, M.D., M.P.H.**
  Assistant Professor
  Children's Hospital Boston

  "Development and Pilot Testing of a Computerized Decision Rule for Children with Minor Blunt Head Trauma"

  Key Words: Clinician Decision Rule Implementation, Automated Decision Support, Blunt Head Trauma, Radiation Risk

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We propose to develop and pilot test a computerized decision support tool for the care of children with blunt head trauma in the emergency department (ED) environment. We will measure our ability to deliver the decision support tool for the care of children with minor blunt head trauma at the point of decision making and order entry for cranial computed tomography (CT).

While minor head trauma is a common reason for ED evaluation of children, the prevalence of clinically important traumatic brain injury requiring intervention is very low. Utilization of cranial CT for evaluation of children after minor head trauma has been steadily increasing over the past decade. An emergent CT, however, is not without substantial risks. The most important risk is the long-term induction of lethal malignancy resulting from the radiation exposure associated with CT scans.

The Pediatric Emergency Care Applied Research Network recently published a validated clinical decision rule for the care of children with blunt head trauma utilizing a prospective cohort of almost 45,000 children with blunt head trauma evaluated in the ED. The rule identifies children at very low risk of clinically important traumatic brain injury who may not need acute neuro-imaging. We propose to develop and then pilot test a computerized decision support tool for the published PECARN head trauma rule. We will deliver this decision support at the time of cranial CT decision making. In addition, we expect that the knowledge gained from this study will inform a subsequent larger multi-center implementation study.

- **In-Hyun Park, Ph.D.**
  Assistant Professor
  Yale University

  "Investigation of Functional Myogenic Progenitors from Human ES and iPS Cells for Duchenne Muscular Dystrophy"

  Key Words: Reprogramming, iPS Cells, hES Cells, DMD, Myogenic Progenitors

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  Duchenne muscular dystrophy (DMD) is severe recessive X-linked disorder, and one of the most prevalent pediatric genetic diseases (1 in 3,500 newborn males). Mutations in dystrophin, a major component of the cytoskeletons of muscular fibers, are the underlying cause of DMD, resulting in structural instability within cardiac and skeletal muscle, and accelerates turnover of myogenic stem cell pools. Since the discovery of dystrophin as an underlying gene of DMD, gene and cell therapy were attempted to treat or cure DMD. Lentiviral vectors or adeno- or adeno-associated vectors expressing mini-dystrophin or exon-skipping oligomers showed a limited success in rescuing dystrophic phenotype. Clinical application of physiological myoblasts, satellite cells, showed no adverse but also no effective treatment on DMD patients. Recently preclinical success of mesoangioblastic pericytes in ameliorating muscular dystrophic symptom opens a promising opportunity for systemically transplantable cell-based therapy for DMD. What is critical is to obtain cells histocompatible for patients. Expression of four defined factors (Oct4, Sox2, Klf4, Myc) reprograms somatic cells to become induced pluripotent stem (iPS) cells that potentially allows obtaining autologous myogenic progenitors for DMD patients. Our long-term research goal is to derive patient specific systemically transplantable myogenic progenitors from pluripotent stem cells, as a novel cellular source for DMD patients. From our preliminary investigation, we isolated cells with myogenic potential (MPCs, myogenic progenitor cells) differentiated from human pluripotent stem cells (hPSC). hPSC-MPC showed the heterogeneity of cell populations and we seek to enrich myogenic population that can be transplanted systemically. Following specific aims are proposed; 1) to functionally determine the cell surface phenotype of hPSC-MPCs, and 2) to apply genetic approach to improve the systemic delivery of the hPSC-MPCs into target muscle. Success of our proposed research will provide a robust novel cell source for DMD treatment.

- **Christian Schlieker, Ph.D.**
  Assistant Professor of Molecular Biophysics and Biochemistry
  Yale University

  "Investigating Nuclear Envelopathies from the Perspective of Protein Quality Control"

  Key Words: Nuclear Envelopathies, Laminopathies, Protein Misfolding Diseases, Proteotoxicity, Protein Quality Control, Nuclear Envelope, Protein Aggregation, Authophagy

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  Nuclear envelopathies are a diverse group of congenital diseases that are caused by mutations affecting proteins in the nuclear envelope or lamina. Emery Dreifuss muscular dystrophy and progeria syndromes are not only amongst the most severe forms, they also have a very early onset and therefore affect children severely. Both are caused by mutations in the Lamin A gene. These Lamin A alleles act as dominant negatives and often form protein deposits when overexpressed. However, no phenotype is observed upon genetic ablation of Lamin A in animal models. We therefore hypothesize that envelopathy-associated alleles act at least in part through proteotoxicity, i.e. by a gain of function mechanism that leads to a poisoning of the protein quality control system. How proteins in the nuclear periphery are turned over or repaired is largely unknown, and the mechanisms that serve to remove nuclear protein aggregates are equally elusive.

  Our goal is to unravel the cellular mechanisms that regulate protein homeostasis in the nuclear periphery, and to elucidate the role that these pathways play in muscular dystrophies, premature aging and related envelopathies that affect children. To accomplish our goal, we will establish novel model substrates to study protein toxicity and turnover in relation to nuclear envelopathies. Furthermore, we will exploit viral proteins known to manipulate the nuclear envelope as a novel approach to identify cellular factors involved in protein turnover and aggregate removal from the nucleus.

  The results obtained from these studies will provide the first molecular insights into the constituents responsible for turnover of protein aggregates in the nucleus. The cellular factors identified in this study will greatly enhance our understanding of nuclear envelopathies and will also serve as a critical step toward the development of therapeutic interventions to improve and possibly extend the life expectancy of children afflicted with these diseases.

January 2010 Award Recipients

- **William Anderson, Ph.D., M.D.**
  Instructor of Surgery
  Brigham and Women's Hospital

  "The Impact of Interictal Spike Events on Visual Object Recognition"

  Key Words: Epilepsy, Cognitive Testing, Interictal Spike, Visual Processing

  The goal of this proposal is to investigate the link between interictal epileptiform activity and cognitive performance in children. Our aims involve first developing a reliable, automated, intracranial interictal spike detection algorithm. This will involve decomposing an incoming signal, which in this case is an intracranial electroencephalogram, into its wavelet coefficients, and then instituting an algorithm which detects features of interest. The algorithm will be designed to run online so as to be able to detect interictal spikes in real time on patients undergoing invasive monitoring for resective surgery. The second aim is to investigate whether interictal spikes effect cognitive performance, which in this context will involve tasks related to visual object recognition. The detection of a spike by our automated algorithm, will trigger one of two delay-match-to-sample tasks. These tasks will first involve the presentation of a noisy image, followed by a probe image taken from a pre-assigned database of images. Our results will be controlled against two other experimental conditions which present images not ostensibly time-locked to interictal spike detection. If successful, this proposal will represent a significant contribution to the body of evidence supporting the detrimental effects of interictal activity on cognition.

- **David Guertin, Ph.D.**
  Assistant Professor
  University of Massachusetts Medical School

  "Nutrient Sensing Pathways in Muscle Regeneration"

  Key Words: mTOR, mTORC1, mTORC2, Raptor, Rictor, Rapamycin, PI3K, PTEN, Muscle Regeneration, Satellite Cells, Stem Cells, SMPs, Skeletal Muscle Precursors, Muscular Dystrophy, Rhabdomyosarcoma

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The broad, long-term goal of this project is to identify signaling pathways that can be manipulated to grow stem cells efficiently in culture. Our current focus is on the nutrient and growth factor sensing mTOR pathway and its role in regulating skeletal muscle stem cell function. In preliminary studies, we find that mTOR may regulate the self-renewal and differentiation of skeletal muscle precursor cells (SMPs), which are thought to represent the skeletal muscle stem cell pool. SMPs prospectively isolated form adult skeletal muscle can be transplanted and engrafted into recipient mice, thus providing a potential source of transplantable stem cells for treating muscle degenerative diseases. Fully understanding the mechanisms controlling SMP proliferation, self-renewal and differentiation is critical to making this reality.

Our objective in this proposal is to comprehensively define how mTOR signaling regulates skeletal muscle precursor cells with the hope of improving our ability to propagate these cells for therapeutic purposes. To achieve this, we are using mouse genetics to manipulate mTOR signaling in SMPs in vivo, then isolating and purifying the cells to determine the mechanism of how mTOR controls proliferation, differentiation, and muscle regeneration.

In Specific Aim 1, we use gain-of-function genetics to determine which SMP cell functions are driven by mTOR activity, while in Specific Aim 2 we use loss-of-function genetics to define the requirements for mTOR in muscle regeneration. Muscle stemcells have the potential to treat muscle degenerative diseases and may be the stem cell of origin in rhabdomyosarcoma, thus our studies will have broad impact towards understanding and treating multiple childhood diseases.

- **Adam Lacy-Hulbert, Ph.D.**
  Assistant Professor
  Massachusetts General Hospital

  "Role of Alpha(v) Integrins in Establishment of Intestinal Immunity"

  Key Words: Crohn's Disease, Colitis, Inflammation Immunity

  Our long term research interests are in the regulation of immune responses, particularly in the intestine and other mucosal sites. We recently discovered a new mouse model of Inflammatory Bowel Disease (IBD) caused by genetic deletion of single adhesion molecule, alpha(v) integrin. Our understanding to date is that alpha(v) is required for the immune system to generate specific T cell populations that serve to down regulate immune responses to normal gut components (such as benign bacteria and food antigens) and also provide immune defense against disease-causing bacteria. Furthermore, we have found that this process occurs early in development in the mouse, before the age of weaning and colonization of the intestine by bacteria.

  In this grant, we propose to understand how the intestinal immune system of young alpha(v) knockout mice differs from that control mice, and which of those differences go on to cause colitis in later life. We also aim to find out when in development these critical steps occur.

  Successful completion of this work will lead to a greater understanding of the mechanisms by which immune regulation occurs in the intestine and will hopefully guide future treatment and prevention of childhood IBD

- **Paul Lerou, M.D.**
  Instructor in Pediatrics
  Children's Hospital Boston

  "p53 Regulation in Human Pluripotent Stem Cells"

  Key Words: Embryonic Stem Cell, Induced Pluripotency Stem Cell, p53, Cell Cycle

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  Human pluripotent stem (hPS) cells can be derived from human embryos or by reprogramming somatic cells via over-expressing defined pluripotency factors. These cells have enormous therapeutic potential as a source of cellular replacement therapy and can serve as a platform for in vitro study of disease and screening of therapeutic agents. The cell cycle of hPS cells differs significantly from that of somatic cells: nearly absent G1 phase, hyperphosphorylated retinoblastoma protein, constitutive cyclin E/A-CDK2 activity, and altered p53 activity. In somatic cells, such molecular alterations result in genomic instability and tumorigenesis, yet ES cells maintain genomic stability and retain the capacity to differentiate and contribute to normal organismal development. Recent data has shown that disabling p53 significantly increases the efficiency of reprogramming somatic cells to pluripotency, however, the impact on genomic stability and development potential of the resultant iPS cells is unclear. We hypothesize that although p53 regulation is altered in hPS, p53 protein plays an important role in maintaining genomic stability and the pluripotent state.

  Aim1: Characterize the components of the p53-signaling network in human pluripotent stem cells. Although the p53 network has been extensively characterized in both somatic and cancer cells, this is not the case for hPS cells. RNA interference and well-characterized compounds will be used to interrogate this network in normally proliferating hPS cells and in response to DNA damage.

  Aim 2: Use fixed and live-cell imaging techniques to characterize p53 dynamics. We have optimized culture conditions to image single hPS cells using immunofluorescence. We will perform quantitative image analysis to characterize p53 dynamics. We will also build a p53-fluorescent fusion protein reporter into hPS cell lines to perform quantitative live-cell imaging.

  Our studies will translate into a better understanding of pluripotency and reprogramming thereby helping to realize the therapeutic potential of human pluripotent stem cells.

• **Jamie Maguire, Ph.D.**
Assistant Professor
Tufts University School of Medicine

"Impact of Maternal Depression on Offspring Development"

Key Words: Child Development, Postpartum Depression, Stress, Emotional Development, Cognitive Development

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Postpartum depression is associated with deficits in child development. These studies have largely relied on correlations found in human studies due to the lack of useful animal models of postpartum depression. We have recently characterized a mouse model which exhibits abnormal postpartum behaviors, including depression-like behaviors, restricted to the postpartum period. We will utilize this model to test the hypothesis that maternal depression underlies the deficits in offspring development. We will examine anxiety, depression, and cognitive behaviors in offspring born to control mice and those born to mice exhibiting postpartum depression. In addition, we will perform cross-fostering experiments to determine if maternal depression directly influences child development. To determine how maternal depression may be transferred to the offspring, we will test the hypothesis that stress-induced steroid hormones negatively impact offspring development. Stress is a predicting factor for postpartum depression and elevated levels of stress-associated steroid hormones are associated with postpartum depression. To investigate if stress hormones may be passed from the mother to the offspring and mediate the negative impacts of postpartum depression on child development, the levels of the stress-related steroid hormone, corticosterone, will be compared between control mice and mice exhibiting postpartum depression. In addition, corticosterone levels will be measured in the offspring born to control mothers or mothers exhibiting postpartum depression. The impact of maternal stress on offspring behavior will be assessed in mice born to control mothers and mothers subjected to chronic ultramild stress. Children born to mothers with postpartum depression have deficits in emotional and cognitive development, increased incidence of violent crime, depression, drug abuse, and suicide. Insight into how these negative aspects are transferred from mother to offspring will be relevant to all these negative issues regarding child development.