

Charles H. Hood Foundation Child Health Research Awards Program

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

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

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 JAGGED1 expression 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

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

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

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

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

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

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

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

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

“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

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 (lIdP), 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

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

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, Autophagy

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

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 from 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 stem cells 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

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

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.