

## Charles H. Hood Foundation

### July 2009 Award Recipients

**Jason Harris, M.D., M.P.H.**

*Assistant Professor, Department of Pediatrics*

*Massachusetts General Hospital*

**“Cellular Immune Responses to Vibrio Cholerae Infection”**

**Key Words:** *Cholera, Vibrio Cholerae, Human Immune Response, Pediatrics, Cholera Vaccines*

#### **Scientific Abstract**

Vibrio cholerae causes 3 to 5 million cases of secretory diarrhea and over 100,000 deaths annually. The majority of these deaths occur among children between the ages of 2 to 10 years. Although infection with V. cholerae results in durable immunity, current vaccines are very ineffective at protecting against cholera in children. An understanding of T-cell responses to natural cholera and vaccination may lead to improvements in cholera vaccines.

This proposal builds on an existing collaboration between the candidate and scientists in Bangladesh to study the human immune response to V. cholerae infection and vaccines. The application proposes a two-year study to characterize cellular immune responses to Vibrio cholerae infection, with two specific aims: (1) To compare the characteristics of CD4+ T-cell responses to V. cholerae antigens in cholera patients and vaccinees; and (2) To evaluate the association between initial CD4+ T-cell responses and the subsequent duration of B cell immunologic memory over one year after natural V. cholerae infection or vaccination. By understanding differences in the CD4+ T-cell response to natural V. cholerae infection and vaccines, our goal is to identify mechanisms by which long term protective immunity to cholera develops, and use this to design better cholera vaccines.

The candidate is trained in pediatrics and has demonstrated commitment to a career in clinical research in the area of pediatric infectious diseases. This award would permit the candidate to develop a research program in cellular immune responses to V. cholerae; separate from the currently funded scientific objectives and interests of his recent NIH career development award mentors. This will allow the candidate to develop as an independent biomedical researcher, and data generated under the proposed program will enable the candidate to apply for independent funding for future studies of immunologic responses to cholera and other enteric infections of children.

**Zhengyu Liu, Ph.D.**

*Instructor, Department of Medicine*

*Massachusetts General Hospital*

**“Novel Potential Treatment for Juvenile Diabetes”**

**Key Words:** *Juvenile Diabetes, Beta Cell, Alpha Cell, GLP-1, SDF-1, PC1/3*

**Scientific Abstract**

About one of every 400 to 600 children and adolescents in the United States has or will have juvenile diabetes. Juvenile diabetes results from destruction of the insulin-producing beta cells of the pancreatic islets by the autoimmune system. An understanding of the factors and the cellular mechanisms that control beta cell growth and survival may lead to new treatments for diabetes. The architecture of human islets is remarkable in that the alpha cells are mixed in with the beta cells so that more than 70% of beta cells are in heterotypic contact with alpha cells. We propose to demonstrate a novel role for alpha cells in the islets as helper cells for and possibly progenitor cells of beta cells. We will show a novel role for alpha cells – to switch to the production of Glucagon-like peptide-1 (GLP-1) as a beta cell regeneration factor during times of beta cell stress and injury, such as occurs in juvenile diabetes. We recently described the expression of the chemokine stromal cell-derived factor-1 (SDF-1), and its receptor CXCR4 in pancreatic beta cells. We identified an important role of the SDF-1 /CXCR4 axis in pancreas development and in the growth and survival of beta cells. We also found that SDF-1 production and secretion is drastically induced by beta cell injury. This proposal will address the role of SDF-1 produced from injured beta cells in stimulating reprogramming of alpha cells and production of GLP-1, which in turn enhances beta cell regeneration. We also expect to see that the SDF-1 /CXCR4 axis, along with GLP-1 /GLP-1R axis, exerts cytoprotective and proliferative effects on beta cells by activation Wnt signaling, resulting in the attenuation of diabetes in mice. The understanding the mechanisms leading to beta cell regeneration is essential for the development of novel treatments for juvenile diabetes.

**Jill Maron, M.D., M.P.H.**

*Assistant Professor, Department of Pediatrics*

*Division of Medicine, Floating Hospital for Children at Tufts Medical Center*

**“Neonatal Salivary Genomic Profiles to Assess Gastrointestinal Development and Disease”**

**Key Words:** *Neonatal, Saliva, Genomic, Necrotizing Enterocolitis, Development, Non-Invasive*

### **Scientific Abstract**

This discovery-driven translational research project will use non-invasive neonatal salivary genomic profiles to generate important and novel genomic and proteomic data of the developing premature infant. The two specific aims of this research project are 1.) test the hypothesis that neonatal salivary genomic expression profiles will provide novel and informative data regarding the premature infant's developing gastrointestinal tract and assess their readiness to feed through the detection of mucosal, mesenchymal and neurodevelopmental gene transcripts, and 2.) test the hypothesis that neonatal salivary genomic expression profiles obtained during the acute and convalescent stages of necrotizing enterocolitis (NEC) will provide informative and insightful data regarding the pathophysiology of the disease. To achieve these aims, saliva will be collected from neonates prior to the initiation of feeds, through the advancement of feeds, and during the introduction of oral feeding. Additional samples will be taken from a small subset of infants who subsequently develop clinical, radiographical and/or surgical evidence of NEC. Salivary RNA will be extracted, amplified, and analyzed on whole genomic microarrays. Advanced bioinformatic comparisons and analyses will be performed on serial salivary samples from the same infant over time to discover the time course and appearance of developmental transcripts necessary for proper digestive motility and successful oral feeding. Additional comparisons will be made between those infants who did and who did not develop NEC to elucidate pathological processes occurring in premature neonates in the setting of this disease. This study will provide a comprehensive analysis of neonatal genomic and proteomic pathways and networks, allowing us to discover normal premature development, identify key biomarkers that may predict infants at risk for developing NEC, aid in our understanding of NEC, and identify gene targets for future therapy.

**Lisa Minter, Ph.D.**

*Research Assistant Professor*

*Department of Veterinary and Animal Sciences*

*University of Massachusetts Amherst*

**“Targeting NF- $\kappa$ B Signaling in a Murine Model of Acquired Aplastic Anemia”**

**Key Words:** *Aplastic Anemia, Bone Marrow Failure, Autoimmunity, NF- $\kappa$ B, TH1*

### **Scientific Abstract**

Severe acquired Aplastic Anemia (sAA) is a rare but devastating bone marrow failure syndrome. More devastating than its symptoms are the facts that it disproportionately affects children and adolescents, and even children who fully respond to treatment are hounded by the specter of relapse, or progression of their disease to myelodysplastic syndrome or leukemia in later years. It is characterized by loss of hematopoietic stem cells (HSC) from the bone marrow (BM) and pancytopenia in the peripheral blood. Research within the last decade has helped to elucidate its pathophysiology as an

aberrant, autoimmune response. T helper type-1 (TH1) cells are required for normal immune function but, under aberrant conditions, they can perpetuate the inflammatory destruction of autoimmune diseases such as multiple sclerosis, Crohn's disease, and rheumatoid arthritis. Accumulating data suggest sAA is a TH1-mediated autoimmune condition. The NF-kappaB/Rel proteins are tightly-controlled transcription factors regulating a wide variety of cellular processes. We have shown NF-kappaB works in concert with another transcription factor, Notch, to regulate interferon gamma (IFNg) expression, a signature pro-inflammatory cytokine produced by TH1 cells and which is increased in patients with sAA. We hypothesize that NF-kappaB activation facilitates TH1-mediated destruction of hematopoietic stem cells during immune-mediated BM failure, and that inhibiting the action of this protein complex will lessen the severity of disease. We will test this hypothesis using a small molecule inhibitor of NF-kappaB then validate our results using transgenic animals that are deficient in NF-kappaB signaling. Finally, we will assess the effect of the inhibitor on the expression of a panel of candidate genes, known to be dysregulated in patients with sAA. Data from these studies may reveal novel targets for developing adjuvant therapies in the treatment of severe acquired Aplastic Anemia.

**Lars Mueller, M.D.**

*Instructor of Pediatrics*

*Department of Hematology/Oncology*

*Children's Hospital Boston*

**"Validation of Disease-specific Fanconi Anemia Induced Pluripotent Stem (iPS) Cells a Tool for Studying Bone Marrow Failure and Enabling Regenerative Medicine"**

**Key Words:** *Fanconi Anemia, Induced Pluripotent Stem Cells, iPS Cells, Embryonic Stem Cells, Gene Therapy, Bone Marrow Failure*

### **Scientific Abstract**

Fanconi anemia (FA) is an inherited bone marrow failure and cancer predisposition syndrome that poses significant medical and scientific challenges. On a cellular level, genomic instability and defective DNA repair are major hallmarks of the condition. Clinically, bone marrow failure occurs with a median age of onset of 7 years, ultimately necessitating allogeneic stem cell transplantation (HSCT). Outcomes of unrelated donor HSCT remain poor, warranting the development of novel treatment approaches. Translational research efforts have been impeded by a combination of disease-specific factors, including the inability of mice with targeted deletions of FA genes to faithfully recapitulate the bone marrow failure phenotype of the human disease. In addition, the exhaustion of CD34+ hematopoietic stem and progenitor (HSC/P) cells in patient bone marrow occurs early in the course of the disease, limiting the availability of target cells for somatic gene therapy and biologic studies. The recent development of direct reprogramming as a means of generating induced pluripotent stem (iPS) cells from

somatic cells may circumvent these limitations by providing a new expandable source of hematopoietic stem cells. However, the hematopoietic differentiation potential of disease-specific iPS cells has not been documented thus far and the equivalence of iPS and ES cells in this regard needs to be established. We will compare the hematopoietic differentiation capacity of murine and human iPS and ES cells from a common (complementation group A) and a less common but more severe (complementation group D2) genotype. We hypothesize that human FA iPS cells will provide a unique expandable source of hematopoietic stem and progenitor cells, amenable to disease-specific investigations and regenerative medicine approaches. To examine whether the genomic instability of FA cells represents a disease-specific challenge during direct reprogramming, we will assess the chromosomal integrity of FA iPS cells by karyotype analysis and comparative genomic hybridization.

**Peter Nigrovic, M.D.**

*Instructor in Medicine*

*Division of Rheumatology, Immunology and Allergy*

*Brigham and Women's Hospital*

**"Neutrophil Recruitment in Inflammatory Arthritis"**

**Key Words:** *Neutrophil, Arthritis, Ly6G, Migration*

### **Scientific Abstract**

Juvenile idiopathic arthritis (JIA) affects almost 300,000 children nationally, and in approximately half of cases persists into adulthood. Without adequate treatment, the formation of pannus tissue and infiltrating leukocytes can destroy cartilage and bone, resulting in distorted skeletal growth and permanent joint injury. While juvenile-onset arthritis is clinically heterogeneous, active arthritis is invariably associated with infiltration of neutrophils into the synovial fluid, where they can serve as potent sources of cytokines, chemokines, lipid mediators and proteases.

Despite the ubiquity of the neutrophil in arthritis, the mechanisms supporting the accumulation of these cells in the unique synovial microenvironment are incompletely understood. Here we present preliminary data that the commonly-used neutrophil marker Ly6G is a novel regulator of neutrophil migration into the joint. Ly6G is GPI-linked protein of unknown function expressed predominantly by neutrophils. While antibodies against this antigen are typically used to deplete circulating neutrophils, we have found that low doses of antibody abrogate murine inflammatory arthritis despite preservation of neutrophil number. Preliminary studies of Ly6G demonstrate striking elevation of Ly6G surface expression on neutrophils recruited to inflamed sites, and expression is briskly upregulated on neutrophils exposed to activating stimuli. Confirmatory studies in vitro demonstrate that Ly6G ligation induces profound impairment of neutrophil migration. We propose a set of Aims targeted to define the

biology of Ly6G on murine neutrophils and thereby to better understand a mechanism implicated in the control of neutrophil recruitment to the inflamed joint. The results of these studies may contribute to the identification of a homologous mechanism in human neutrophils that could serve as a novel target for therapy in JIA and other inflammatory diseases affecting children.

### **January 2009 Award Recipients**

#### **Sek Won Kong, M.D.**

*Instructor, Harvard Medical School*

*Department of Cardiology/Informatics Program*

*Children's Hospital Boston*

#### **“Uncovering Molecular Targets of Histone Deacetylase Inhibitors in Cardiac Hypertrophy and Heart Failure”**

**Key Words:** *Heart Failure, Hypertrophy, Chip-Seq, Histone Deacetylase Inhibitor, Treatment*

#### **Scientific Abstract**

Improvements in operative and perioperative care of children with structural congenital heart disease have resulted in dramatic advances in short- and medium-term outcomes. However, heart failure remains a significant cause of morbidity and mortality in them.

In the diseased heart, mechanical and neurohormonal stimuli alter gene expression that ultimately leads to heart failure. Histone acetylation opens the chromatin to allow activation of gene expression, while histone deacetylation represses transcription. Histone acetyltransferase and histone deacetylase (HDAC) are key regulators of the cardiac response to stress, and HDAC inhibitor (HDACi) ameliorates heart failure in animal models. However, the genes targeted by HDACi have not been characterized in heart, and different classes of HDACs seem to have opposite effects on hypertrophy. Suppressing class I HDAC attenuates hypertrophy, while reduced class II HDAC promotes hypertrophy.

We hypothesize: 1. Anti-hypertrophic genes are repressed by HDAC in a murine heart failure model, when hypertrophic genes are actively transcribed. 2. HDACi ameliorates heart failure by de-repressing anti-hypertrophic genes. 3. Different HDACs interact with specific transcription factors to regulate target genes.

Aim 1. We will define the differential pattern of histone acetylation using chromatin immunoprecipitation followed by massively parallel sequencing. Changes in the histone acetylation state of a gene will be correlated to its expression in a murine heart failure model, compared to control. Aim 2. We will treat heart failure mice with a broad

spectrum HDACi and a class I specific HDAC or vehicle, and profile the histone acetylation and gene expression. Aim 3. To identify transcription factors that recruit HDACs in heart failure, we will perform in silico motif discovery using genes obtained from Aims 1 and 2.

Completion of this project will be a significant step in understanding heart failure pathogenesis, and will enhance our ability to develop novel targeted therapies for heart failure.

**Junhao Mao, Ph.D.**

*Assistant Professor*

*Department of Cancer Biology*

*University of Massachusetts Medical School*

**“The Role of the Hedgehog Pathway in Childhood Rhabdomyosarcoma”**

**Key Words:** *Hedgehog signaling, Stem Cells, Rhabdomyosarcoma*

**Scientific Abstract**

The Hedgehog (Hh) signaling pathway, initially identified by its patterning activity in the *Drosophila* embryo, plays many distinct roles in vertebrate development. De-regulation of Hh signaling has also been suggested to be involved in the formation of a wide range of tumors, including rhabdomyosarcoma (RMS), the most common soft-tissue sarcoma in children, which is thought to arise from a skeletal muscle lineage. However, little is known about the mechanism by which the Hedgehog pathway carries out its action on this devastating childhood tumor.

Based on previous studies and our preliminary data, we hypothesize that dysregulated Hh signaling disrupts the tight regulation of self-renewal and transit proliferation of postnatal muscle stem cells, inducing transformation and RMS genesis. Further, RMS may contain a minor population of cancer stem cells, likely derived from muscle stem cells, which undergo self-renewal and generate a variety of heterogeneous differentiated tumor cells. To test these hypotheses, we will first use a combination of genetic and cellular tools to examine the potential oncogenic effects of the Hh pathway on stem cell regulation in RMS. We will also extend the mechanistic study by characterization of Hh-Gli mediated transcriptional networks in RMS in a global fashion. We anticipate that results from the proposed experiments will lead to better understanding of the functional consequence and mechanisms of the Hh pathway in regulating childhood skeletal muscle tumorigenesis, and will shed light on future therapeutic approaches.

**Gromoslaw A. Smolen, Ph.D.**

*Instructor in Medicine, Harvard Medical School*

*Massachusetts General Hospital Cancer Center*

**“Novel Regulators of Epithelial-to-Mesenchymal Transition (EMT)”**

**Key Words:** *Birth Defects, Cell Migration, EMT, Zebrafish, Development, Functional Genomics, Rnai Screen, High-Throughput*

**Scientific Abstract**

A number of congenital birth defects have been traced to underlying defects in cell migration. One of the major mechanisms of cell migration induction is epithelial-to-mesenchymal transition (EMT), by which stationary epithelial cells acquire a migratory mesenchymal character and are able to traverse great distances during embryogenesis. The process of EMT is used repeatedly during embryogenesis in such diverse settings as heart valve and limb muscle formation, as well as palate fusion and neural crest migration. However, the molecular mechanisms regulating EMT are not fully understood. Therefore, a major question remains: What are the molecular mechanisms that inhibit EMT and maintain the epithelial phenotype?

To identify genes involved in EMT regulation we have already performed a high-throughput screen using a lentiviral whole-genome pooled human shRNA library and identified a number of genes whose knockdown confers migratory ability to stationary epithelial cells. Given the success of the initial screen, we propose to further characterize the confirmed hits with the following experimental aims:

- 1. Functionally characterize the candidates in EMT-related assays.** Candidate genes will be evaluated in knockdown and overexpression experiments by performing EMT-related assays in cell culture.
- 2. Functionally characterize the candidates in zebrafish development.** Candidate genes will be tested for *in vivo* EMT involvement during normal vertebrate development.

Collectively, this proposal represents the first genome-scale effort to elucidate the comprehensive set of endogenous EMT regulators. The identification of the molecular circuits that regulate EMT offers the possibility of fundamentally transforming our understanding of mechanisms contributing to birth defects, particularly those whose etiology can be traced to underlying defects in cell migration and epithelial remodeling. Further understanding of such molecular networks will allow for the eventual translation of these advances into sensitive molecular diagnostic tools and novel therapeutic interventions for a number of congenital birth defects.

**Kathleen E. Walsh, M.D., M.Sc.**

*Assistant Professor*

*Department of Pediatrics*

*University of Massachusetts Medical School*

**“Preventing Errors in the Home Care of Children with Cancer”**

**Key Words:** *Outpatient Oncology Care, Patient Safety, Medication Errors, Home Care, Self-Management Support*

### **Scientific Abstract**

**Background:** In our multicenter study of outpatient cancer care, we found that medication errors occurred twice as often in children than adults and that these pediatric errors occurred in the home. The actual rate of home medication errors is likely even higher, as home administration errors are often not captured in the medical record.

**Objectives:** 1) To describe the prevalence of and characterize the types of home medication errors among children with cancer; and 2) To conduct qualitative focus groups using a purposive sample of parents and healthcare providers of children with cancer to develop interventions to prevent home medication errors.

**Methods:** To characterize home medication errors, we propose to conduct 100 home visits to families of children with cancer on chemotherapy treated at UMass Memorial Medical Center and Dana-Farber Cancer Institute. We developed, pilot tested, and published a home visit method which includes four components: (1) observation of medication administration, (2) medication review, (3) in-depth parent interviews, and (4) physician review of possible errors. In 15 pilot visits to families of children with cancer we found 56 errors. These proposed 100 home visits will lead to our identifying the worst – most prevalent, most dangerous – errors, and to our learning where things are going wrong. In order to address our second objective, parent/provider focus groups will use the home visit findings to redesign the home medication use systems following a modified Failure Modes and Effects Analysis and, based on that redesign, will develop interventions to prevent home pediatric oncology errors which we will test in a future study.

**Conclusions:** The proposed two site study will quantify home medication errors in children with cancer and develop interventions to prevent these errors. At its completion, we will be well positioned to perform an evaluation of these interventions at multiple outpatient oncology clinics across the country.

**Ann Chen Wu, M.D., M.P.H.**

*Instructor*

*Department of Ambulatory Care and Prevention*

*Harvard Medical School and Harvard Pilgrim Health Care*

**“Effectiveness of Pharmacogenetic Testing in Asthma”**

**Key Words:** *Asthma, Pharmacogenetics, Pharmacogenomics, Beta2-Agonist, Bronchodilator Response, Predictive Test*

### **Scientific Abstract**

Research on genetic testing holds special promise to improve the health of children. Because individual genetic codes are determined before birth, using effective genetic tests during childhood would maximize their potential benefit compared with waiting until adulthood. In the fast-growing field of pharmacogenetics, surprisingly scant data exist on the effectiveness of pharmacogenetic testing in real-life populations, especially children.

The overall goal of this project is to help the developers and potential users of pharmacogenetic tests formulate and apply them to maximize their benefits in real-life settings. The Specific Aims of this study are to: (1) evaluate the effectiveness of a pharmacogenetic test in predicting beta<sub>2</sub>-agonist response in a health care system and (2) evaluate the projected health benefits, costs, and cost-effectiveness of the pharmacogenetic test for beta<sub>2</sub>-agonist response in Aim 1 under varying assumptions in real-life populations.

The goal of Aim 1 is to evaluate whether a candidate pharmacogenetic test predicts beta<sub>2</sub>-agonist response in a diverse, real-life population of patients with asthma. The general population will be 1000 children and adults with asthma whose genotypic and phenotypic data have been collected and linked as part of the Informatics for Integrating Biology and the Bedside (I<sup>2</sup>B<sup>2</sup>) project, a major Harvard initiative.

To evaluate the benefits and cost-effectiveness of this test, we will use the Pediatric Asthma Policy Model, a computerized decision analytic model that predicts the natural history of asthma. As well as advancing knowledge about the clinical effectiveness and real-life viability of this specific test, this work will serve as a paradigm for future pharmacogenetics research. These studies will offer generalizable lessons on how the effectiveness of pharmacogenetic tests should be evaluated in diverse populations of children and adults and their projected clinical and economic impact can be analyzed to inform policy choices about their adoption in real-life settings.

## July 2008 Award Recipients

### Sumita Bhaduri-McIntosh, M.D., Ph.D.

*Assistant Professor*

*Department of Pediatrics, Infectious Disease*

*Yale University*

### **“The Role of Regulatory T Cells in Immune Evasion during Establishment and Maintenance of Latency by EBV”**

**Key Words:** Epstein-Barr virus, regulatory T cells, immortalization, EBV-lymphoma

#### **Scientific Abstract**

Epstein-Barr Virus (EBV)-lymphomas are aggressive tumors that affect as many as 20% of solid-organ transplant recipients, and occur in immunocompromised individuals, especially in children who are EBV-naïve. Since therapeutic options are fraught with numerous challenges, there is a clear need for additional investigation to explore alternate strategies of intervention. This proposal is focused on identifying critical interactions between T cells and EBV during establishment and maintenance of immortalization of B cells with the downstream goal of developing new approaches to prevent development of EBV-lymphomas. Our preliminary studies and work done by others have led me to hypothesize that EBV subverts T cell responses by stimulating a regulatory T cell (Treg) population during B cell immortalization and lifelong persistence of immortalized B cells and that this subversion involves interactions between CD58 on B cells and CD2 on Treg cells. Therefore the specific aims are:

1. To examine if EBV recruits help from a regulatory T cell population to aid in establishment of and maintenance of immortalized B cells. We will deplete Treg cells from peripheral blood cells or provide them in-trans during ex vivo infection of autologous B cells by EBV and examine their ability to facilitate immortalization of B cells, ability to produce interleukin-2, and ability to inhibit proliferation of effector T cells. We will correlate with phenotypic characteristics, determine their mechanism of action, and whether they are also involved in maintenance of immortalized B cells.
2. To determine the consequences of inhibition of CD58 activity by examining expression of CD23 and IL6, function of Treg cells, and immortalization of B cells. Neutralizing antibody to CD58 and RNA-interference mediated knock-down expression of CD2 will be used to determine the role of CD58 and CD2 in mediating EBV-directed Treg functions that facilitate establishment and maintenance of immortalization of B cells.

**Suzy D.C. Bianco, Ph.D.**

*Instructor in Medicine*

*Department of Medicine and Endocrinology*

*Brigham and Women's Hospital*

**“Altered GPR54 signaling as the basis of Pubertal Disorders”**

**Key words:** *GPR54 signaling, GPR54 desensitization, kisspeptin signaling, Puberty onset, precocious puberty, delayed puberty*

### **Scientific Abstract**

The goal of this project is to determine how the timing of puberty onset is regulated by GPR54, a G-protein coupled receptor (GPCR), and its ligand, kisspeptin; which have been recently identified as the upstream regulators of GnRH secretion. While the mechanisms that regulate pubertal onset are not known, it is known that inactivating mutations in GPR54 cause failure to undergo puberty. In contrast, our preliminary results show that an autosomal dominant GPR54 amino acid substitution (Arg386Pro) identified in a girl with central precocious puberty (a disorder with disproportionately high female incidence) increases GPR54 responsiveness. They also show that GPR54 is desensitized and internalized in response to continuous kisspeptin stimulation; and that the Arg386Pro mutation delays GPR54 desensitization. Thus, we *hypothesize* that the timing of signaling and desensitization of GPR54 plays a critical role in controlling puberty. In addition, the amino acid substitutions in GPR54 may affect its responsiveness by interfering with signaling or desensitization, thereby contributing to the clinical presentation of pubertal disorders such as precocious or delayed puberty. Although GPCR desensitization is generally strongly regulated, no data have been published on GPR54 desensitization. The *short term* goal of this project is to define the mechanisms underlying GPR54 desensitization, in order to understand how genetic mutations of this receptor affect the timing of pubertal onset. Specifically, the aims of this proposal are to: (i) Define the mechanisms of GPR54 desensitization and internalization and (ii) Define how amino acid substitutions in GPR54 found in patients with pubertal disorders affect these mechanisms. A thorough understanding of GPR54 signaling may uncover the basis of gender differences in normal and abnormal pubertal development, as well as reveal a new array of potential targets of pharmacological manipulation for the treatment and prevention of abnormal pubertal development.

**Hetal Kocinsky, M.D.**

*Associate Research Scientist*

*Department of Pediatrics*

*Yale School of Medicine*

**“Regulation of Proximal Tubule NHE3 by Phosphatases”**

**Key Words:** Na<sup>+</sup>/H<sup>+</sup> exchanger 3, hypertension, protein phosphatase 1, protein phosphatase 2A

**Scientific Abstract**

Pediatric hypertension is an increasing problem amongst children with an estimated prevalence between 4.5 and 23%. Through its tight regulation of volume and salt balance, the proximal tubule of the kidney and NHE3 play an essential role in blood pressure homeostasis. NHE3 is a Na<sup>+</sup>/H<sup>+</sup> exchanger found on the apical membrane of proximal tubule cells, and its activity is tightly controlled by multiple physiologic and hormonal factors. Multiple studies have demonstrated that phosphorylation of NHE3 by various kinases modulates its activity. However, minimal investigative attention has been devoted to the mechanisms and regulatory roles of NHE3 dephosphorylation. The specific aims of this proposal focus on the role of phosphatases in the regulation and dephosphorylation of NHE3. Our preliminary data provides strong evidence that NHE3 is dephosphorylated by one or more phosphatases. This proposal aims to 1) determine if PP1 and/or PP2A are the specific phosphatases involved in the dephosphorylation and regulation of NHE3, and 2) identify which amino acids of NHE3 are dephosphorylated by PP1 and/or PP2A. For the first aim, the applicant will assay NHE3 activity and phosphorylation in the presence of multiple phosphatase inhibitors, evaluate for a potential physical interaction between NHE3 and PP1 or PP2A, and characterize cell lines with siRNA knockdown of selected PP1 and PP2A catalytic subunits. For the second aim, phosphospecific antibodies will be used to study two known NHE3 phosphorylation sites (serines 552 and 605), site-directed mutagenesis will be used to study other known or putative NHE3 phosphorylation sites, and high performance liquid chromatography and mass spectrometry techniques will be utilized to identify novel sites of NHE3 phosphorylation. These studies will deepen our knowledge of NHE3 regulation, thereby enhancing our knowledge of blood pressure physiology and pathophysiology, and potentially contributing to the development of new treatment strategies for hypertension.

**Michael Silverstein, M.D., M.P.H.**

*Assistant Professor of Pediatrics*

*Department of Pediatrics*

*Boston Medical Center*

**“Empowering Low–Income Depressed Mothers with Preterm Infants:  
A Randomized Controlled Trial”**

**Key words:** *maternal depression, prematurity, poverty, neonatal intensive care unit, empowerment interventions, adherence*

**Scientific Abstract**

Premature infants are born at biological risk for poor health and developmental outcomes; premature infants of low–income families face additional social risks known to exacerbate these outcomes. The Institute of Medicine recognized this important public health problem in its 2006 report, *Preterm Birth*, which argued for the need to improve the quality of NICU follow–up care for preterm infants. The underpinning of this proposal is that unaddressed maternal depression – common among families of premature infants – contributes to suboptimal adherence to follow–up services, and (both through this mechanism and directly) adversely impacts children’s health and development.

Theory–based empowerment strategies have demonstrated effectiveness for improving the mood and functioning of depressed adults, and for improving adherence to treatment. They therefore represent a family of feasible interventions to improve the outcomes of this vulnerable NICU population. We propose a randomized controlled trial of one such strategy – Problem Solving Education (PSE) – involving 150 low–income mothers at risk for depression, who have premature infants ( $\leq 33$  weeks gestational age) in two Boston NICUs. We aim to:

1. Determine the impact of PSE on the following six–month outcomes:
  - a. caregiver depressive symptoms and functioning;
  - b. adherence to child health supervision and immunization schedules, vision screening, and early intervention evaluation.
2. Obtain empiric estimates of study parameters to inform the planning of a subsequent RCT of the intervention on longer–term outcomes, including within–group standard deviation of continuous outcome measures, proportion of control group subjects to experience discrete outcomes, and correlation of outcomes measured repeatedly.

Approximately 100,000 children are born prematurely to low–income families each year. Parent–directed PSE aims to improve outcomes for these children through the prevention and/or attenuation of maternal depressive symptoms, as well as through family activation and promotion of adherence to follow–up care. If successful, PSE could

also provide the cornerstone of a more generalizable empowerment strategy for families of children with chronic medical conditions.

**Yong Xiong, Ph.D.**

*Assistant Professor*

*Department of Molecular Biophysics and Biochemistry*

*Yale University*

**“Molecular Dissection of the Fanconi Anemia Pathway of DNA Damage Response”**

**Key words:** *Fanconi Anemia, cancer, DNA damage response*

**Scientific Abstract**

The childhood disease Fanconi anemia (FA) is characterized by mutations in proteins in a novel DNA damage response network that includes the breast and ovarian tumor suppressors FANCD1 and FANCN (also known as BRCA2 and PALB2). Two other FA proteins, FANCD2 and FANCI mediate the recruitment of the tumor suppressors to damaged chromatin for DNA damage removal. However, the exact biochemical and structural basis for the interactions have not been determined.

Our long-term goal is to establish the underlying principals of the crosstalk between the FA pathway and the DNA repair machinery. In this proposal, we aim to characterize FANCD2 and FANCI and their interactions with FANCD1 and FANCN through biochemical studies by identifying their interaction complexes, define their functional attributes, and exploring crystallization conditions to determine their crystal structures. To achieve these objectives, we will use gel mobility shift assays and affinity pull-down assays to define the DNA binding properties of FANCI and to test for functional synergy in DNA binding between FANCD2 and FANCI. We will also characterize the FANCN interaction with DNA substrates and determine the functional relevance of these interactions. In addition, we will dissect the functional domains in FANCD2, FANCI, and FANCN, and examine patient-derived truncation alleles to identify domains necessary for functional interactions. Furthermore, we aim to produce stable FA protein constructs by mutation, truncation and co-expression of complexes and screen for crystallization. X-ray crystallographic techniques will be used for structure determination.

Results obtained from our studies may lead to improved diagnosis and treatment outcomes for this devastating childhood disease, in particular, for the prevention of cancer progression in surviving FA patients.

## January 2008 Award Recipients

### Nadine Gaab, Ph.D.

*Assistant Professor of Pediatrics, Harvard Medical School*

*Department of Medicine*

*Children's Hospital Boston*

### **"Neural Pre-Markers of Developmental Dyslexia in Children prior to Reading Onset"**

**Key words:** *developmental dyslexia, functional magnetic resonance imaging, morphological/functional differences children, neural pre-markers, reading, language, early identification*

### **Scientific Abstract**

Developmental dyslexia (DD), which may affect 5–17% of all children, is a learning disability characterized by difficulties with accurate and/or fluent word recognition and poor decoding performance. Several studies have shown differences in the structural neuroanatomy and functional networks of reading related processes between school age children with and without a diagnosis of DD. However, there is no study to date that examined whether pre-reading children with a family history of DD already show alterations of their functional and structural neuroanatomy and whether these alterations predict reading performance after reading onset.

This study aims to identify early neural markers in pre-reading children (age 4–6) with and without a family history of DD. In phase I, children's language/cognitive abilities will be examined. Furthermore, functional networks of reading related processes such as phonological processing will be compared between pre-reading children with and without a family history of DD using functional magnetic resonance imaging.

Additionally, structural brain measures (e.g., gray matter volume) will be compared between these children. All children will be re-invited after they learned to read (one year later; phase II), and their language and cognitive abilities will be reexamined. This proposal hypothesizes that children's brain measures obtained in phase I prior to reading onset will predict reading outcome after reading onset (phase II).

The proposed research has crucial clinical, psychological and social implications. Studies have shown that children with learning disabilities are less likely than their peers to complete high school and to enroll in programs of higher education, and more likely to enter the juvenile justice system. The identification of a child at risk for DD prior to reading onset is essential for the development of novel, and the improvement of existing early intervention programs, as well as the development of social networks for parents and children with a risk for DD.

**Bernhard Kühn, M.D.**

*Instructor in Pediatrics*

*Assistant in Cardiology*

*Department of Cardiology*

*Children's Hospital Boston*

**"Mechanisms of Cardiomyocyte Proliferation"**

**Key words:** *cardiomyocyte, proliferation, cell cycle, cell division, myocardial regeneration, heart failure, congenital heart disease*

**Scientific Abstract**

Congenital heart disease, the most common birth defect, is frequently associated with heart failure due to defective myocardium. Although cardiogenic progenitor cells may maintain myocardial turnover, they do not give rise to a robust regenerative response. Alternative approaches to regenerate myocardium are needed and would revolutionize how we treat heart failure in the future. The long-term goal of this research is to provide innovative strategies for the treatment of heart failure in the setting of congenital heart disease. Our preliminary studies indicate that neuregulin1 (NRG1) induces cardiomyocyte proliferation by activating membranous receptor tyrosine kinases consisting of the ErbB2 and ErbB4 subunits. NRG1-activated ErbB2 and ErbB4 then stimulate the intracellular phosphatidylinositol-3-OH kinase (PI3K) pathway. **We hypothesize that the NRG1/ErbB2/ErbB4 complex controls post-natal cardiomyocyte proliferation.** The Specific Aims are:

**SA 1: Characterize the cellular mechanisms of NRG1-induced cardiomyocyte proliferation.**

We hypothesize that NRG1 induces cardiomyocyte proliferation. We will address these questions:

- a.) Does NRG1 induce proliferation of differentiated cardiomyocytes?
- b.) Do all cardiomyocytes have equal proliferative potential?
- c.) Do cardiomyocytes dedifferentiate when they divide?

**SA 2: Characterize the molecular mechanisms of NRG1-induced cardiomyocyte proliferation.**

We hypothesize that specific membranous and intracellular signaling modules are required for NRG1-induced proliferation of differentiated cardiomyocytes. We will address these questions:

- a.) Are ErbB2 and ErbB4 required for NRG1-induced cardiomyocyte proliferation?
- b.) Is the PI3K pathway required for NRG1-induced cardiomyocyte proliferation?

The objective of the proposed work is to characterize how NRG1, ErbB2, and ErbB4 control cardiomyocyte proliferation. NRG1, ErbB2, ErbB4, and the PI3K pathway may provide new targets for innovative therapies for heart failure.

**Brian Tseng, M.D., Ph.D.**

*Assistant Professor*

*Department of Neurology, Division of Pediatric Neurology*

*Massachusetts General Hospital*

**"Creatine Pathways: Novel Treatments for Duchenne Muscular Dystrophy"**

**Key words:** *Duchenne muscular dystrophy, mdx mice, creatine, GAMT, AGAT*

**Scientific Abstract**

A widely held mystery in the field of pediatric muscular dystrophy has been, "Why is the mdx mouse **not** crippled?" It lacks the same dystrophin protein as boys with DMD yet it can live and run well. For the past 6 years, I have been investigating the mdx mouse with microarray and bioinformatics tools to identify upregulated genes in muscles of the mdx mouse that are not in the boys with DMD. I have found that the mdx mouse upregulates both GAMT (guanidinoacetate methyltransferase) and AGAT (arginine:glycine amidinotransferase) while boys with DMD downregulate them. These 2 genes encode the key enzymes required to synthesize creatine. Our preliminary data strongly suggest that switching off GAMT in the double knockout GAMT<sup>-/-</sup>:mdx mouse does make the mdx mice more crippled. Based on these studies, we hypothesize that the endogenous synthesis of creatine could be advantageous in resisting early metabolic crisis in dystrophin-deficient muscle cells.

We are trying to better understand why the mouse would turn on such important genes while the boys with DMD turn them off. With bioinformatics perspective, we find the 5' end 2 kilobase core promoter region of GAMT of mouse is markedly different compared to human and could account for the species-difference. With muscular dystrophy mouse models, we will test in-vivo putative GAMT promoter agonists (prednisone, thyroid hormone and growth hormone) plus antagonists to see if the GAMT levels and phenotype can be altered. Furthermore, using promoter expression cell-based assays and high-throughput screening tools in the MGH MIND core facility, we seek to identify small molecule compounds/drugs that in-vitro can upregulate or de-repress GAMT in human muscle cells.

Understanding the molecular mechanisms by which GAMT is regulated in human versus mouse could provide a new treatment direction for human DMD, the most common and lethal pediatric neuromuscular disorder.

**Lauren Wise, Sc.D., M.Sc.**

*Assistant Professor of Epidemiology*

*Boston University School of Public Health*

*Epidemiologist*

*Slone Epidemiology Center at Boston University*

**"Pre-Pregnancy Obesity and the Risk of Preterm Birth and Macrosomia: A Prospective Study of African-American Women"**

**Key Words:** *Obesity, Body Fat Distribution, Pregnancy, Preterm Birth, Macrosomia, Minorities, Females*

### **Scientific Abstract**

The prevalence of overweight and obesity is increasing rapidly in the US, especially among women of childbearing age and black women. Obesity causes major changes in maternal metabolism and may be involved in increased energy accumulation by the fetus. In studies of predominantly white populations, obesity has been linked to an increased risk of several adverse pregnancy outcomes including macrosomia. The data on preterm birth are more mixed, with some studies suggesting an increased risk of preterm birth associated with obesity, particularly among nulliparous women.

The prevalence of both preterm birth and macrosomia has been on the increase in the United States. Preterm birth, which occurs twice as commonly among black babies as white babies, and macrosomia are associated with adverse pediatric outcomes, including neurodevelopmental disability, chronic respiratory disease, high blood pressure, and obesity. Little is known about how anthropometric factors influence preterm birth and macrosomia in black women. We will assess the relation of pre-pregnancy body mass index (BMI, kg/m<sup>2</sup>) and body fat distribution (waist circumference and waist-to-hip ratio) with risk of preterm birth (<37 weeks of gestation) and macrosomia (birth weight ≥4000 g) in the Black Women's Health Study, a US prospective cohort study of black women. We also propose to assess whether gestational weight gain and parity modify these associations. Birth outcome data will be derived from mothers ages 21-44 years reporting a singleton birth in 1997, 1999, 2001, or 2003 (N=7,990 births). Preterm birth data have already been validated; infant birth weight will be validated in a random sample of BWHS participants. Generalized estimating equation models will be used to estimate odds ratios and 95% confidence intervals, with adjustment for potential confounders. The proposed study of modifiable anthropometric risk factors for preterm birth and macrosomia offers great potential to reduce adverse birth outcomes in US black women.

## July 2007 Award Recipients

### Laurel Leslie, M.D., M.P.H.

*Assistant Professor*

*Departments of Medicine and Pediatrics*

*Tufts–New England Medical Center*

### **“Medication Use for Behavioral Problems among Youth in Child Welfare”**

**Key Words:** *Child welfare, medication use, child health services research, behavioral health, foster care*

### **Scientific Abstract**

An astonishing number of youth are involved with the U.S. child welfare/child protective services system (‘child welfare’), approximately 3 million per year. These youth have unusually high rates of behavioral problems, at four to nine times that of youth in the general population. The adverse emotional circumstances they experience precipitating their involvement with child welfare also make them particularly susceptible to poor health outcomes in adulthood.

A relatively large body of research now exists describing gaps in the use of outpatient behavioral services for youth with behavior problems in child welfare; this contrasts starkly with the dearth of available studies on medication use. Advocacy groups have voiced concerns regarding potential over-reliance on medication in children in child welfare, and agencies across the country are struggling with limited data and resources to develop policies around the provision of behavioral services, including medication, for youth in their care.

This study proposes to begin to address these gaps with the ultimate goal to improve the identification, provision, and monitoring of behavioral care for these youth. First, we will determine current rates of medication use for behavioral problems in youth in child welfare using data previously collected through the National Survey on Child and Adolescent Well-being Study, the only nationally representative longitudinal study of approximately 5500 youth involved with child welfare. Logistic regression and growth mixture modeling methodologies will be employed to identify point prevalent and longitudinal drug use patterns in youth in NSCAW and to investigate differences in rates across communities. Second, we will use qualitative research methods to examine current child welfare efforts to ensure safe and effective medication use for behavioral problems in the youth in their care, including investigation of current policies and procedures, new initiatives, and unique challenges faced by child welfare agencies, given the populations they serve.

**Eric Morrow, M.D.**

*Instructor in Psychiatry*

*Department of Psychiatry*

*Massachusetts General Hospital*

**“Genetic Investigation of Cognitive Development in Autistic Spectrum Disorders”**

**Key words:** genetics, autism, pervasive developmental disorders, childhood epilepsy, mental retardation, developmental neuroscience, population genetics.

**Scientific Abstract**

Autistic disorder is a severe neurodevelopment condition of increasing public concern. Understanding of the pathophysiology of autism is rudimentary and there are no medical treatments. Because autism is highly heritable, gene discovery is important to building a foundation for improved diagnosis and treatment.

This project’s overriding hypothesis is that autism is highly heterogeneous genetically. Homozygosity mapping is a gene-finding methodology that capitalizes on the increased power to map genes within individual families with consanguinity, and thereby diminishes the problems imposed by heterogeneity across families. This research will investigate two genetically complementary patient populations affected by autism in order to discover autism susceptibility genes. The first patient population represents a special founder population wherein parents are cousins (i.e. consanguineous) and homozygosity and deletion mapping will be conducted. The second patient population represents a large collection of North American pedigrees (>700 families). The principal tool for analysis will be high-density genotyping microarrays.

The project hypothesizes: 1. Homozygosity and deletion mapping will hasten gene discovery in autism as these methods diminish the effects of genetic heterogeneity. 2. Genes identified in special founder populations will have clinical significance in outbred populations. Hypotheses that will be tested through these Specific Aims include: Aim 1: To identify genomic deletions and duplications (thereby contiguous candidate genes) involved in autism in consanguineous pedigrees, using high resolution copy number analysis, and mapping of homozygous deletions with 500K SNP microarrays. Aim 2: To identify autosomal recessive loci and genes involved in familial autism in consanguineous pedigrees using homozygosity mapping. Aim 3: To assess candidate genes identified in Specific Aim 1 and 2 for mutations using resequencing in North American pedigrees.

Resources available include collaborators at the Children’s Hospital Boston Developmental Medicine Center for patient phenotyping, and the Medical and Populations Genetics Program at the Broad Institute of MIT and Harvard.

**Paula Quatromoni, D.Sc., M.S., R.D.**

*Assistant Professor*

*Department of Health Sciences*

*Sargent College of Health and Rehabilitation Services, Boston University*

**“Evaluating IMOVE: An Environmental Intervention to Promote Healthy Eating in Middle-School Children from Massachusetts Communities at High Risk for Childhood Obesity”**

**Key words:** child, obesity, intervention, school, nutrition, healthy eating, environment

### **Scientific Abstract**

An innovative school nutrition program called IMOVE was developed by a Boston-area foodservice industry leader, with implementation funded by the Massachusetts Department of Public Health. IMOVE addresses childhood obesity risks by providing access to affordable and nutritious food choices through school foodservice. A unique opportunity exists to evaluate the program’s impact on healthy eating behaviors in schools, as well as the economic impact on schools and industry. This project proposes a quasi-experimental research design involving four middle schools to evaluate a year-long foodservice intervention in low-income communities of racially mixed children at high risk for obesity. Two schools will be randomly allocated to receive the intervention and two comparison schools in the same communities will be chosen based on similar demographic characteristics. The hypotheses are that the intervention will: 1) increase participation in the school lunch program, 2) improve the availability of healthy foods in school, 3) be sustainable with high rates of participation, 4) be rated positively by participants, 5) promote desired changes in individual food purchase and consumption, and 6) be economically sound for schools and industry. Outcomes include participation in the school lunch program and in the IMOVE program; purchase and sale of cafeteria, competitive, and intervention foods; sensory evaluation (acceptability) of intervention foods; individual food and nutrient intake; body mass index; and program costs. Analyses will compare intervention and comparison schools as well as baseline and follow-up measures. Changes in food purchase patterns will be evaluated in each school and will also be aggregated across school communities to provide evidence of program impact. This evidence-based evaluation of the IMOVE program will have implications for policy, dissemination, and programming to combat the childhood obesity epidemic. A team of experts in nutritional epidemiology, program evaluation, behavioral science, biostatistics, foodservice administration, education, and business will conduct this research.

**Susanne Schlisio, Ph.D.**

*Instructor*

*Department of Medical Oncology*

*Dana-Farber Cancer Institute*

*(currently at the Ludwig Institute for Cancer Research, Stockholm, SWEDEN)*

**“Neuronal Apoptosis by EglN3 and the 1p36 Gene KIF1Bbeta: Implication in Neuroblastoma Development”**

**Key Words:** Prolyl Hydroxylase EglN3, KIF1Bbeta, Neuronal Apoptosis, 1p36, Neural crest progenitors, Neuroblastoma

**Scientific Abstract**

Developmental apoptosis of neuronal precursors is crucially important for determining the final number of terminally differentiated cells. Deregulation of this process is implicated in disease. During neural development cells undergo apoptosis as growth factors such as nerve growth factor (NGF) become limiting. Abnormal NGF signaling has been linked to pediatric nervous system tumors such as neuroblastoma and medulloblastoma. We recently showed that cancer genes such as VHL, NF-1, c-Ret, and succinate dehydrogenase (SDH) complex genes act upon a developmental apoptotic pathway that is activated when NGF becomes limiting for neuronal progenitor cells and which requires the EglN3 prolyl hydroxylase as a downstream effector.

I hypothesize that failure of EglN3-induced developmental apoptosis in neuronal precursors predispose to Neuroblastoma and other neural crest derived tumors and I am therefore seeking to understand the mechanisms by which EglN3 causes cell death. By performing an unbiased, functional, genome-wide screen for genes that suppress EglN3-induced neuronal apoptosis I identified the kinesin KIF1Bbeta as downstream effector of EglN3. KIF1Bbeta is both necessary and sufficient for neuronal apoptosis when NGF becomes limiting. KIF1Bbeta maps to 1p36.2, a region of the genome that is frequently deleted in neural crest derived tumors including neuroblastomas. I am aiming to understand how, mechanistically, EglN3 regulates KIF1Bbeta and how this translates into cell death. Understanding the mechanisms by which EglN3 regulates KIF1Bbeta to cause neuronal cell death might, in time, allow us to identify other proteins linked to developmental apoptosis and pediatric cancers and might also identify novel targets for therapeutic intervention.

**Yasuyoshi Ueki, M.D., Ph.D.**

*Instructor*

*Department of Developmental Biology*

*Harvard School of Dental Medicine*

**“Role of SH3BP2 as a Novel Regulator of TNF–alpha Production and Osteoclastogenesis**

**Key words:** SH3BP2, cherubism, macrophage, TNF–alpha, inflammation, osteoclast, bone loss, M–CSF, RANKL

### **Scientific Abstract**

The goal of this proposal is to find the molecular mechanisms associated with SH3BP2 that are essential for tumor necrosis factor (TNF)–alpha production in macrophages and for osteoclastogenesis. We recently discovered that the signaling adapter protein, SH3BP2, is a previously unrecognized regulator of TNF–alpha generation in macrophages and of osteoclast differentiation. This progress in understanding the regulation of myeloid cell activation resulted from studies of the pediatric disorder, “cherubism”.

Cherubism is a heritable and disfiguring craniofacial disorder characterized by the accumulation of inflammatory fibrous tissue with excessive bone degradation in the jaws. Using a genetic approach, we discovered several missense mutations in SH3BP2 in cherubism patients. To investigate how mutant SH3BP2 causes cherubism, we created a mouse model using the most common of these mutations. Homozygous cherubism mice exhibited systemic macrophage inflammation and M–CSF–induced high TNF–alpha production in macrophages. The result was severe inflammatory bone loss and joint destruction typically seen in patients suffering from rheumatoid arthritis. Bone marrow cells of both hetero– and homozygous mice showed increased sensitivity to RANKL and, therefore, enhanced osteoclastogenesis.

To explore the role of SH3BP2 in TNF–alpha production and osteoclast differentiation more precisely, we propose to characterize SH3BP2 interacting proteins in macrophages and osteoclasts using mass spectrometry. A second goal is to determine whether SH3BP2 plays a role in growth factor or cytokine production in osteoblasts. Such a detailed analysis of signaling complexes in which SH3BP2 participates will greatly contribute to better molecular understanding of inflammatory bone diseases. Importantly, the identification of the proteins that control TNF–alpha production would provide new targets for the development of more effective and safer drugs for the treatment of inflammatory bone diseases of children such as juvenile rheumatoid arthritis.

## January 2007 Award Recipients

### Kate G. Ackerman, M.D.

*Assistant Professor of Pediatrics, Harvard Medical School*

*Department of Medicine*

*Brigham and Women's Hospital*

*(currently at the Center for Pediatric Biomedical Research, Rochester, NY*

### **"Mechanism of *Barx1* Mediated Congenital Diaphragmatic Hernia"**

**Key words:** *Barx1*, CDH, congenital diaphragmatic hernia, diaphragm, pulmonary hypoplasia, *Wt1*, *COUP-TFII*

### **Scientific Abstract**

Congenital diaphragmatic defects are common birth defects that occur as frequently as cystic fibrosis and have high morbidity and mortality. Despite their significant impact, the molecular mechanisms underlying diaphragm development are not understood. The few genetic models of posterior diaphragmatic hernia include mice with loss of *Wt1* or *COUPTFII* expression. Mutations or deletions of *WT1* or *COUPTFII* are associated with congenital diaphragmatic hernia in humans.

Embryos from an ENU mutagenesis screen were examined for diaphragmatic defects. Of 1721 embryos from 34 families, four models of diaphragmatic defect were recovered. Of these, one had reproducible posterior diaphragmatic defects. This line, called *heartburn*, also has a shortened esophagus, abnormal stomach, hiatal hernia and cleft palate. The *heartburn* mutation was mapped and localized to a 20 MB interval on mouse chromosome 13. In this interval, a candidate gene, *Barx1*, was previously reported to be critical for normal stomach development. Sequencing of *Barx1* revealed a splice site mutation predicted to cause early truncation of Barx1 protein with loss of important homeobox domains.

The *Barx1* diaphragmatic phenotype is similar to *Wt1* and *COUPTFII* models suggesting that these genes interact in a pathway critical for normal diaphragmatic development. *Nkx3.2 Cre* activity (*Cre* activity resulting in *COUPTFII* deficient posterior hernia) is present along the posterior rim of the medial diaphragm, but not in the major diaphragmatic parenchyma. Examination of human diaphragmatic hernias shows that the diversity of phenotypes includes one with no posterior muscle rim. Our findings suggest that there is phenotypic sub-type of diaphragmatic defect that may be attributed to loss of *Barx1*, *Wt1*, or *COUP-TFII* function. This proposal will evaluate the relationship between these genes during diaphragmatic development and will elucidate

whether a posterior attachment defect (rather than a tissue loss defect) is responsible for this diaphragmatic hernia phenotype.

**Sarah Fortune, M.D.**

*Assistant Professor*

*Department of Immunology and Infectious Diseases*

*Harvard School of Public Health*

**“At the Host–Pathogen Interface: Characterization of a Candidate Mycobacterial Toxin”**

**Key Words:** Tuberculosis, virulence, toxin, alternative secretion

**Scientific Abstract**

*Mycobacterium tuberculosis* is a devastating pathogen that causes epidemic disease and latently infects much of the world’s population including the majority of children in the developing world. In the infected host, *M. tuberculosis* resides within macrophages where it creates a protected niche within these cells which should otherwise eradicate it. The molecular mechanisms by which *M. tuberculosis* accomplishes this are unclear. Other pathogenic bacteria often use specialized secretion systems that translocate virulence factors into the host cell to modulate host cell function. In *M. tuberculosis*, a novel secretory system, known as ESX1, has recently been shown to be required for the virulence of the organism. Our hypothesis is that *M. tuberculosis* uses the ESX1 system to translocate virulence proteins into the host cell. A proteomic screen for proteins secreted by the ESX1 apparatus was performed. It identified a protein that is similar to a family of bacterial toxins with phosphodiesterase activity. We hypothesize that this protein, which we have named “candidate mycobacterial toxin” or Cmt, is the first of the long-anticipated mycobacterial virulence factors that directly modify host cell targets and thus contribute to virulence. Our proposal will to test this model by: 1) determining whether the subcellular of Cmt is consistent with it acting on a host cell target and 2) characterizing the role of Cmt in the virulence of *M. tuberculosis*. Results from this work will form the basis for an R01 grant submission on the mechanism of Cmt and ESX1 mediated virulence.

**Richard Iain Gregory, Ph.D.**

*Assistant Professor of Pediatrics*

*Department of Biological Chemistry and Molecular Pharmacology, HMS*

*Stem Cell Program, Children's Hospital Boston*

**"The Role of MicroRNAs in DiGeorge Syndrome"**

**Key words:** MicroRNA, DiGeorge syndrome, MiR-185

### **Scientific Abstract**

The goal of this proposal is to explore the role of microRNAs in DiGeorge syndrome. The variable clinical features of this syndrome include congenital heart defects, characteristic appearance, immunodeficiency, and developmental and behavioral problems. Despite its high incidence (1:4000), the fact that >90% cases are caused by heterozygous deletion of a region containing many genes has made the task of identifying the particular genes that underlie the pathogenesis a challenge. However, mouse models recapitulate many features of the disease. These studies, together with mutation screening in non-deleted patients, revealed that haploinsufficiency of the T-box transcription factor, TBX1, causes many of the DiGeorge syndrome phenotypes. However, TBX1 is not responsible for other common features such as mental retardation. Therefore other genes located within the typically deleted chromosomal region (TDR) must contribute to DiGeorge syndrome.

MicroRNAs (miRNAs) are a large family of regulatory RNAs that repress target genes at the posttranscriptional level and are important for various biological pathways. Significantly, a particular miRNA, miR-185, is expressed from the DiGeorge syndrome TDR. Indeed, we propose that miR-185 is a likely candidate gene for some of the features associated with DiGeorge syndrome. It will therefore be important to examine the expression, biogenesis, and gene regulatory function of miR-185 in order to determine the role of this newly identified gene in DiGeorge syndrome.

**Aim 1: Investigate the role of miR-185 in DiGeorge syndrome.** We will characterize miR-185 expression during development. Using reconstitution and biochemical assays we will explore the mode of miR-185 biogenesis to determine how this unusually short (18-nt) miRNA is generated. Also, we will perform DNA sequencing analysis to identify mutations in miR-185 in DiGeorge syndrome non-deleted patients.

**Aim 2: Identification of miR-185 target genes.** We are developing a novel biochemical approach for the large-scale identification of mRNAs that are regulated by miR-185.

**Tracy Richmond, M.D., M.P.H.**

*Instructor*

*Department of Medicine, Division of Adolescent Medicine*

*Children's Hospital Boston*

**“School Physical Activity Programs’ Contribution to Racial/Ethnic Disparities in Adolescent Physical Activity and Obesity”**

**Key words:** racial/ethnic disparities, adolescent, physical activity, obesity, school environments

**Scientific Abstract**

The purpose of the proposed project is to determine whether differential availability of school programs, specifically Physical Education classes and/or interscholastic sports, contribute to racial/ethnic disparities in adolescent physical activity and/or obesity. By understanding the impact of these programs, we will be uniquely positioned to better understand how to develop effective school-based interventions to increase physical activity, decrease obesity, and reduce racial/ethnic and socioeconomic disparities in adolescent health.

Specifically, we propose to investigate the following hypotheses:

*1. Schools with higher proportions of racial/ethnic minority students and/or students living at or below the poverty level are less likely to have interscholastic sports available and are likely to have lower PE requirements for graduation.*

*2. African-American and Hispanic adolescents and those of lower SES have on average lower rates of PE enrollment and interscholastic sports participation than White adolescents and those of higher SES. Differential enrollment/participation can be explained by differential availability and requirements by school.*

3. Higher participation in PE and/or interscholastic sports during high school is associated with higher levels of total physical activity as an adolescent and in the future as a young adult.

4. Independent of their baseline weight, students with higher enrollment in PE and higher sports participation will have on average lower BMIs, less weight gain from adolescence into early adulthood, and lower incidence of obesity compared to those with lower PE enrollment and/or sports participation.

We will use three waves of the National Longitudinal Study of Adolescent Health (Add Health), including the recently released Add Health Education dataset. We will use multivariate linear and logistic regression to examine associations within single levels (separately at individual and school levels). We will then use multilevel modeling to simultaneously examine associations at the individual and school levels.

**Christopher Sasseti, Ph.D.**

*Assistant Professor of Pediatrics*

*Department of Molecular Genetics and Microbiology*

*University of Massachusetts Medical School*

**“Virulence Gene Regulation in Mycobacterium Tuberculosis”**

**Key words:** tuberculosis, gene expression, virulence, pathogenesis

**Scientific Abstract**

Mycobacterium tuberculosis, the causative agent of tuberculosis, must adapt to a constantly changing host environment in order to cause disease and be successfully transmitted to a new host. In many bacteria, the functions specifically required for survival in the host (“virulence factors”), are coordinately controlled by a single transcriptional regulatory network. While several virulence factors have been identified in M. tuberculosis no single regulatory system has been shown to coordinate their expression. We have identified a single protein, cpsA, that may serve this function. We have found that cpsA-deficient mutants are unable to survive in an animal model of tuberculosis. The cpsA regulon contains at least three distinct virulence factors, the loss of which, are likely to be responsible for this phenotype. This proposal has two major goals. First, we will determine how the loss of each cpsA-regulated virulence factor contributes to the profound attenuation of the cpsA mutant. Secondly, we will more precisely define the mechanism by which cpsA, a protein with no recognizable DNA-binding motifs, mediates its transcriptional effects. As cpsA orthologs are widespread in gram-positive bacteria, we expect this work to also have broad implications for a variety of infections that affect children.