2015 Research Annual Report

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SUMMARIES OF 2015 NATIONAL INSTITUTES OF HEALTH GRANTS AWARDED TO HFHS

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Summaries of 2015 National Institutes of Health Grants Awarded to HFHS

Part I – Department of Internal Medicine

- Allergy and Immunology
- Cardiology/Cardiovascular Research
- Endocrinology and Metabolism
- Hypertension and Vascular Research
- Sleep Medicine

Allergy and Immunology

Principal Investigator: Edward Zoratti, M.D.
Pets and the Infant Microbiome: Effect on Immune Maturation & Atopic Asthma—Project 2 (NIH P01A1089473-01A1)

The Program Project Grant (PPG) application seeks an increased understanding of the relationships between dog or cat exposure during infancy and a lower risk of allergic asthma. We believe that this protective association is related to different patterns of microbial stimulation during immune development. Four synergistic Projects will examine our hypothesis that the presence of pets in a home results in a more diverse bacterial community composition (BCC) of the dust in the home which in turn influences the development of the gut BCC of a newborn infant living in the home. A more diverse gut BCC shifts the maturation of the infant's immune system such that later immune responses are less likely to produce IgE antibody responses and allergic asthma. Project 2 will recruit a new birth cohort of children either living with or without a dog, measure infant stool BCC, and follow the cohort with detailed studies of immune function until 18 months of age to determine the impact of dog exposure on immune maturation. The Projects are supported by five Cores which each provide essential services to all four Projects.

Cardiology/Cardiovascular Research

Principal Investigator: David Lanfear, M.D.
Impact of Race and Genetic Factors on Beta-Blocker Effectiveness in Heart Failure (NIH 1R01HL103871-03)

Heart failure (HF) is an enormous public health problem with over 500,000 cases annually, and African American individuals share a disproportionate amount of this burden including a higher prevalence and mortality when compared with white individuals. Beta adrenergic antagonists (beta-blockers, BB) are the foundation of modern HF care, but their effectiveness in African Americans is not clear. Pivotal clinical trials of BB in HF were woefully underpowered to assess African American patients, and many experts have suggested a differential BB benefit in African American patients when compared with white patients.
This issue requires additional data and clarity because improved understanding and elimination of such disparities is a national research priority (Healthy People 2010). Multiple factors may contribute to a racial disparity in BB effect such as genetic factors, medication adherence, and comorbid illnesses. All of these factors must be characterized in detail in order to evaluate which factor(s) contribute to this. Existing pharmacogenetic studies have suggested that specific variants may explain racial differences in BB effectiveness, but these studies have not quantified drug exposure or adherence and have not included a sufficient number of African Americans. In order to answer these questions, we propose a racially diverse, prospective, pharmacogenomic registry of 1000 HF patients. Our center has important advantages to achieve this including the fact that roughly half of our HF patients are African American, and we have experience and infrastructure in quantifying adherence and drug exposure using pharmacy claims data. Using this cohort we will assess the influence of race and genetic factors on BB effectiveness, measured by clinical events (time to hospitalization or death) and health status. Ultimately these data will clarify the benefit of BB in African Americans, and contribute to improved targeting of BB therapy to those with highest likelihood of favorable response while avoiding those likely to respond unfavorably.

Public Health Relevance: Heart failure is an enormous public health problem and African American individuals share a disproportionate amount of this burden including a higher prevalence and mortality when compared with white individuals. Beta blockers are the foundation of modern heart failure care, but their effectiveness in African American individuals is not clear. This project seeks to clarify whether beta blockers are equally effective in African American patients when compared with white patients and identify the underlying factors that impact this difference, particularly genetic factors.

Endocrinology and Metabolism

Principal Investigator: D. Sudhaker Rao, M.D.
Pathogenesis of Atypical Femur Fractures on Long Term Bisphosphonate Therapy (NIH 1R01AR062103-01A1)

Bisphosphonates (BP) have been used successfully for over a decade to prevent and treat osteoporosis and reduce osteoporotic fractures. However, since 2005 there have been many reports of atypical femoral fractures (AFF) in patients on prolonged BP therapy. Recently, it has been found that a prodromal bone deterioration (PBD) usually appears before the development to AFF. However, many PBDs may be asymptomatic, not necessarily progress to AFF, and heal spontaneously after discontinuation of BP therapy. Therefore, the prevalence of PBD may be much higher than AFF. To date, there is no evidence to support this hypothesis. It has been reported that many patients with PBD and AFF have severely suppressed bone turnover (SSBT). However, since not all patients with PBD/AFF also have SSBT, factors other than SSBT might contribute to the development of PBD/AFF. Our preliminary study suggests that PBD/AFF may be associated with osteocyte death, bone hypermineralization, and microdamage accumulation which compromise bone mechanical properties. These facts collectively led us to formulate the hypothesis that BP treated patients
who develop SSBT and consequent increase in bone age, in conjunction with previous
osteocyte deficiency, are predisposed to micropetrosis, accumulation of fatigue microdamage,
PBD, and eventually to stress fracture manifested as AFF. To pursue this hypothesis we
propose the following specific aims: Aim 1 is to determine the prevalence of PBD and AFF
in 1,000 patients with postmenopausal osteoporosis, either treated with BP for more than 2
years (500 subjects), or never BP treated (500 subjects). X-rays of the femurs will be
performed to systematically screen the patients for PBD/AFF. PBD can be defined as an X-
ray finding of focal cortical thickening associated with a fracture line at the lateral femoral
cortex.

Suspected PBD patients, whose x-ray does not show clear fracture line at focally thickened
cortex, will be evaluated further using x-ray tomosynthesis, isotope bone scan or MRI. Aim 2
will determine the contribution of osteocyte deficit to PBD/AFF and SSBT in iliac bone
biopsies obtained from long term BP treated patients. Degree of bone mineralization and
bone nano-mechanical properties will also be assessed on these biopsies.

Hypertension and Vascular Research

Principal Investigator: Oscar A. Carretero, M.D. Project 1 and Project 3
Principal Investigator: Pamela Harding, Ph.D. Project 2
Autocoids in Hypertension: Pathogenesis and End Organ Damage
(NIH 2P01HL028982-31A1)

This PPG was started in September, 1982. The central theme is "the study of the role of
vasoactive systems (autocrine, juxtacrine, paracrine and endocrine) in the regulation of renal
function and blood pressure (BP) and mediation of end organ damage (EOD)". The general
hypothesis to be tested is that there is a balance between systems that promote water and
sodium retention, hypertension and EOD, including Angiotensin II (Ang II), prostanoids,
reactive oxygen species and inflammation, and systems that antagonize these effects like
Ac-SDKP, activation of the Ang II type 2 receptor (AT2), kinins, NO, PGE2/EP4, and the
newly discovered cross-talk between the connecting tubule and the afferent arteriole
(CTGF) which may participate in both natriuresis and renal damage. Alterations of this
balance in favor of the former are responsible for retention of water and sodium and
development of hypertension and EOD, while alterations of this balance in favor of the
latter have therapeutic effects. We will use molecular, physiological, and pharmacological
approaches' to study vasoactive systems at the subcellular, cellular, and isolated organ levels
in hypertension in rats and various transgenic and gene knockout mice. We will mainly use
Dahl salt-sensitive rats (Dahl SS) and Ang II-induced hypertensive rats as models. In Project
I, using Dahl SS rats, we will study whether N-acetyl-seryl-aspartyl-lysyl-proline protects
against EOD by decreasing adaptive immunity. In Project II we will study whether
expression of cyclooxygenase-2 and generation of PGE2 via the EP4 receptor protects
against EOD in Ang II-induced hypertension. In Project III, using Dahl SS rats, we will
study whether CTGF causes glomerular damage via afferent arteriole dilatation and increases
in capillary glomerular pressure. In Project IV, using Dahl SS rats, we will study whether a
decrease in the renal thick ascending limb AT2-signaling participates in the pathogenesis of
hypertension. The Four Cores - Administrative (A), Analytical and Morphological (B),
Mutant Mouse (C), and Biostatistics (D) - will support the scientific efforts of the
investigators. This PPG provides integration of our efforts, collaboration, sharing of ideas
Principal Investigator: Oscar A. Carretero, M.D.

Project 1:

In hypertension, end organ damage (EOD) is due in part to the mechanical forces exerted by high blood pressure (BP); however, other mechanisms such as inflammation, oxidative stress, the RAS, and genetic predisposition, all play key roles in its pathogenesis. In hypertension, Acetyl-Ser-Asp-Lys-Pro (Ac-SDKP), a naturally occurring peptide hydrolyzed mainly by ACE, reduces cardiovascular and renal inflammation and fibrosis without lowering BP. We have evidence that Ac-SDKP mediates some of the anti-fibrotic and anti-inflammatory effects of ACE inh and also prevents experimental autoimmune myocarditis in rats. Thus we propose to test the general hypothesis that in hypertension Ac-SDKP shifts the balance between pro-inflammatory/pro-oxidative and anti-inflammatory/anti-oxidative systems in favor of the latter by decreasing innate and adaptive immunity and thus slowing the development of EOD. Furthermore, the effects of Ac-SDKP on BP and EOD are related to the degree of participation of innate and adaptive immunity in the pathogenesis of hypertension and EOD. This hypothesis will be studied in 3 aims. **Aim I:** In hypertensive Dahl salt-sensitive rats (Dahl SS) and in mice with systemic lupus erythematosus and hypertension, a model of autoimmune disease, Ac-SDKP acts as an immune modulator, reducing innate and adaptive immunity and thus EOD. Some of the effects of Ac-SDKP depend on the degree of participation of innate and adaptive immunity in the pathogenesis of hypertension and EOD. **Aim II:** The effects of ACE inh on the pro-inflammatory transcription factor NF-KB, TH cells and Treg cells are mediated by an increase in Ac-SDKP. **Aim III:** The effects of Ac-SDKP are multiphasic; central to these effects are decreases in: 1) the pro-inflammatory transcription factor NF-KB, 2) differentiation and maturation of dendritic cells (DCs), 3) DC transformation of T cells in effector T cells, and 4) TH cell proliferation, activation, migration, and differentiation into pro-inflammatory phenotypes. The effects of Ac-SDKP on TH are partly due to an increase in Treg cells. Project I is related to III and IV which also study Dahl SS; 2) II and III, which also study the pathogenesis of EOD; and 3) II and IV which also study Ang II. Project 1 will use all 4 Cores.

Project 3:

In hypertension, high glomerular capillary pressure (Pgc) leads to glomerulosclerosis. In African-Americans with salt-sensitive (SS) hypertension, high salt intake causes an increase in estimated Pgc, which could explain their high rate of hypertensive renal disease. Dahl SS rats on high salt intake have hypertension, high Pgc and significant glomerular injury compared to SHR with similar blood pressure. Connecting tubule glomerular feedback (CTGF) is a cross-talk that dilates the afferent arteriole (Af-Art) when Na is increased in the connecting tubule (CNT). General hypothesis: In SS hypertension, during high salt intake there is an imbalance between factors that cause Af-Art constriction (myogenic response and TGF) versus dilatation (CTGF) in favor of the latter, leading to an increase in Pgc and glomerular damage. **Aim I**, Hypothesis, In normotensive animals, chronic high salt intake causes TGF
resetting due to heightened CTGF via increased release of EETs and PGE2 by the CNT. Mice with a gain-of-function mutation of ENaC have increased CTGF and reduced TGF, while mice with deletion of ENaC in the CNT have decreased or no CTGF and enhanced TGF. **Aim II,** hypothesis: In hypertensive Dahl SS rats CTGF is increased, causing TGF resetting leading to increases in PGC and glomerular damage. Conversely, in SHR CTGF is decreased, causing an enhancement of myogenic response and TGF which in turn decreases PGC and protects the glomerulus from damage. In SHR, high salt will increase CTGF, causing attenuation of the myogenic response, TGF resetting, increased PGC, and glomerular damage. In Ang II-induced hypertension in mice with increased ENaC activity, glomerular damage will be greater due to an increase in CTGF, while in mice with selectively decreased ENaC in the CNT glomerular damage will be lower, due to a decrease in CTGF. **Aim III,** hypothesis: In hypertensive Dahl SS rats, CTGF is augmented due to increases in ENaC, COX-2 and PGE2. In contrast, in SHR CTGF is attenuated due to increased soluble epoxide hydrolase and decreased EET release. Project III is closely related to 1): I and IV which also study Dahl SS; 1) I and II which also study the pathogenesis of EOD; and II which also studies arachidonic acid metabolites. Project III will use all 4 Cores.

**Principal Investigator: Pamela Harding, Ph.D.**

**Project 2:**

Uncontrolled hypertension (HTN) is a major cause of end organ damage (EOD) and a risk factor for cardiovascular morbidity and mortality. Although prostaglandin E2 (PGE2) was historically thought to be a mediator of inflammation, more recent evidence suggests that it may be pro or anti-inflammatory, depending on the involvement of specific PGE2 EP receptor sub-types that signal through divergent signaling pathways. We previously reported that aged male mice lacking the EP4 receptor on cardiomyocytes develop heart failure characterized by reduced ejection fraction, left ventricle dilation and fibrosis, coupled with elevated expression of chemokines (fractalkine and MCP-5) in the left ventricle. This proposal examines whether the protective and anti-inflammatory effects of PGE2 via EP4 are mediated by reduced fractalkine and MCP-5. It tests the general hypothesis that **EP4, activated by PGE2, reduces the EOD that occurs in Angiotensin II (Ang II)-dependent hypertension and myocardial infarction (MI) by inhibiting the production and/or release of the inflammatory chemokines fractalkine and MCP-5.** **Aim I** will study whether PGE2 via its EP4 receptor reduces production and/or secretion of fractalkine and MCP-5 via its EP4 receptor and cAMP in cardiac myocytes and fibroblasts and opposes the deleterious effects of Ang II. **Aim II** will study whether EP4-dependent reductions in fractalkine and/or MCP-5 improve cardiac function both in vivo and in vitro. **Aim III** will study whether PGE2 via its EP4 receptor and inhibition of fractalkine and/or MCP-5 synthesis and/or release prevents EOD by reducing infiltration of inflammatory cells into the myocardium in models of Ang II-dependent HTN and myocardial infarction (MI). The proposal will utilize a novel mouse model coupled with state-of-the-art molecular techniques to address these aims. These studies are of utmost importance in determining the role of PGE2 and EP4 in cardiac hypertrophy and EOD. Project II is closely related to: 1) Projects I and III which also study the pathogenesis of EOD; 2) Project IV which also studies AT1 receptors and superoxide; and 3) Project III which also studies arachidonic acid metabolites. Project II will use all 4 Cores.
Principal Investigator: Mariela Mendez, Ph.D.
Hydrogen Peroxide Stimulates Renin Release: Role in Hypertension and Diabetes 
(NIH R03DK105300-01)

Hypertension and diabetes are the principal cause for chronic kidney disease (CKD). In both diseases, a high percentage of patients show activation of the renin angiotensin system (RAS). Renin is the rate-limiting enzyme in the activation of the RAS. Thus, understanding the mechanism and proteins involved in the release of renin may offer alternative targets for hypertension and CKD. Renin is stored in dense-core granules in juxtaglomerular (JG) cells, located at the pole of the renal afferent arteriole, in the kidney cortex. In hypertension and diabetes, reactive oxygen species, including hydrogen peroxide (H2O2), are enhanced in the kidney cortex. We found that hydrogen peroxide stimulate renin release from JG cells. However, the enzymes responsible for production of hydrogen peroxide in JG cells have not been identified. Hydrogen peroxide is mainly a product of superoxide dismutation or enzymatic formation by NADPH oxidases (NOX1-5). The NOX4 isoform preferentially produces hydrogen peroxide and its expression in the renal cortex is enhanced in diabetes and hypertension. Our preliminary data shows that NOX4 is expressed in renin granules in JG cells, suggestive of intragranular production of H2O2. However it is not known whether NOX4 produces the pool of hydrogen peroxide that stimulates renin release from JG cells; and whether NOX4/H2O2-induced renin release contributes to increase blood pressure and kidney damage in diabetic nephropathy. In addition the mechanism by which H2O2 stimulate renin release is unknown. In other cells H2O2 induces signaling by oxidation of protein thiols. In this proposal we will test the hypothesis that the NADPH oxidase isoform NOX4 produces hydrogen peroxide in juxtaglomerular cells and stimulate renin release, thereby increasing blood pressure and contributing to glomerular damage. We will also explore the protein targets by which H2O2 stimulates renin release. In Aim 1 we will use primary cultures of juxtaglomerular cells, isolated afferent arterioles and Akita mice to test the role of NOX4-derived hydrogen peroxide in renin release in vitro and in vivo. In Aim 2 we will use a proteomics approach and subcellular fractionation of renin granules, to identify proteins that are oxidized by hydrogen peroxide in juxtaglomerular cells. This approach will allow us to collect critical preliminary data for an RO1 submission and focus on new protein targets of hydrogen peroxide that mediate renin release.

Sleep Medicine

Principal Investigator: Drake, Christopher, Ph.D.
Behavioral Treatment of Menopausal Insomnia; Sleep, Depression, Daytime Outcomes 
(NIH R01NR013959-01A1)

Insomnia is recognized as one of the most prevalent and costly sleep disorders and is associated with considerable morbidity including significantly reduced quality of life, impaired work performance, and increased risk for major depressive disorder. Insomnia is a key symptom of the menopausal transition with 40-50% of postmenopausal women (> 17 million) having insomnia. Insomnia associated with menopause has a pattern of sleep disturbance predominantly characterized by sleep maintenance difficulties including frequent awakenings and arousals, reduced sleep efficiency, and overall fragmented sleep. It has recently been demonstrated that this pattern of sleep disturbance, difficulty maintaining
sleep, increases throughout the progression of menopause. We have recently found sleep maintenance problems in menopause are associated with reduced work performance, increased healthcare utilization, and impaired quality of life. Historically, menopausal symptoms including sleep disturbance, were treated using hormone replacement therapy (HRT). However, evidence linking HRT to increased risks of heart disease and cancer have led to a 40% reduction in the use of sex steroid hormones by postmenopausal women and highlight the need for alternative approaches to treatment. Importantly, the American Association of Clinical Endocrinologists guidelines for management of menopause do not address treatment of menopausal-related insomnia due to the absence of research findings in this area. Cognitive-behavioral therapy for insomnia (CBT-I) yields equivalent short-term efficacy and superior long-term durability to pharmacological treatment of insomnia. However, the efficacy of cognitive behavioral therapy for insomnia comorbid with menopause, one of the primary focuses of the present proposal, has not been tested. Traditional CBT-I has disadvantages however, including the need for a trained therapist and significant time commitment on the part of the patient. Therefore, widespread availability of multicomponent CBT-I is limited by the relatively low number of CBT sleep specialists, complexity of therapy, and patient burden. Thus, another aim of this project is to test the acute and long-term efficacy of a single component behavioral therapy for menopausal-related insomnia. Given the significant daytime impairment present in insomnia comorbid with menopause including depression, quality of life, and fatigue, a final aim of this proposal is to determine the efficacy of CBT-I on these measures in women with menopausal-related insomnia.

The project will test the efficacy of cognitive-behavioral therapy for insomnia (CBT-I), as a safe and effective evidence-based alternative to medication for sleep disturbance associated with menopause. Because CBT-I is costly and time intensive, we will also test the efficacy of abbreviated single component sleep restriction therapy (SRT) in the treatment of menopausal-insomnia. Importantly, we will also test the efficacy of each of these treatments on improvements in depression, fatigue, and quality of life.

Principal Investigator: Timothy Roehrs, Ph.D.

Risks for Transition from Therapeutic Hypnotic Use to Abuse
(NIH R01DA038177-01A1)

The acknowledged drugs of choice for the pharmacological treatment of insomnia are the benzodiazepine receptor ligand hypnotics (BzRL). Our nighttime studies show that with therapeutic doses used either short-term or chronically, the abuse liability of BzRLs in insomnia is not seen universally and is relatively low. The data from our last grant, a first-ever study, showed the abuse liability of chronic zolpidem use in insomniacs was low. Yet case reports and retrospective studies continue to report BzRL dependence and for the majority of these cases the abuse developed through initial therapeutic use. In our study some subjects showed an increase in dose across time. Understanding the transition from therapeutic use to abuse and identifying risk factors, such as specific patient and drug characteristics, is both mechanistically and clinically important. Our preliminary data have shown that a subset of insomniacs, those insomniacs that have signs of hyperarousal as reflected by elevated Multiple Sleep Latency Test (MSLT) scores, increased their nightly zolpidem dose across time. BzRLs have differential receptor binding affinities and associated anxiolytic or
antidepressant properties. Zolpidem has selective alpha 1 BzRL affinity and little mood activity and thus may show less risk for transition from therapeutic use to abuse than another currently frequently prescribed BzRL with less alpha subtype selectivity such as eszopiclone. We propose to study the abuse liability of a selective (zolpidem) vs nonselective (eszopiclone) hypnotic during chronic use (six months) in an at-risk subpopulation (insomniacs with hyperarousal shown by elevated MSLTs). The proposal is highly innovative as it reflects a paradigm shift in understanding the abuse liability of hypnotics. In the end, this proposal will generate a unique set of data addressing a number of previously clinically important unanswered questions regarding hypnotic abuse by insomniacs (i.e., its likelihood as a function of arousal state and specific hypnotic pharmacology, of dose escalation over time and change in mood/drug effect ratings over time). It will provide clinicians with behavioral indicators of abuse risk.

Part II – All Other Clinical Departments

- Dermatology
- Imaging Research Program
- Neurology
- Radiation Oncology
- Urology

Dermatology

Research Summaries

Principal Investigator: Qing-Sheng Mi, M.D., Ph.D.

microRNAs and NKT Cell Development and Function (NIH R56AI119041-01)

Natural killer T (NKT) cells are an evolutionarily conserved subset of T cells that are developmentally and functionally distinct from conventional T cells. The ability to quickly secrete large quantities of a variety of cytokines upon activation enables NKT cells to be potent regulators of diverse immune responses. The deficiencies in NKT cell number and function have been linked to the development of many diseases. However, a significant gap remains in our understanding of how the development and function of NKT cells are precisely regulated. MicroRNAs (miRNAs), a recently discovered class of evolutionarily conserved small non-coding RNAs, negatively regulate the expression of protein-coding genes and thereby control essential biological functions and contribute to the development of many diseases. We were the first to report that the deletion of Dicer (a key enzyme for miRNA biogenesis) during hematopoiesis results in a significantly reduced NKT cell number and impaired NKT cell maturation and function, without alternating conventional T cell development in the thymus, suggesting that miRNAs are required for NKT cells. Our long-term goal is to understand how miRNAs regulate NKT cell development and function. While more than 1000 experimentally reported miRNAs, very few specific miRNAs are linked to NKT cells so far. Our objective here is to define specific miRNAs and their targets that regulate NKT cell development and function. Using miRNA arrays, we recently identified dynamic expression of miRNAs, including miR-155, and miR-17-92 cluster,
during NKT cell development and activation. These findings plus our recent other report lead to our central hypothesis that these dynamically expressed miRNAs serve as critical regulators controlling NKT cell development and function through fine-tuning of specific target genes. Here we will further test this hypothesis. We will investigate how dynamic and miR-155 and miR-17-92 expression regulates NKT cell development and function using specific miRNA mutant mice with the gain or loss of miRNA gene. The results from proposed studies may not only illuminate the new immunological and molecular mechanisms underlying NKT cell development, but may also facilitate the development of new and more efficient intervention strategies for autoimmune diseases, infection, and cancer based on the NKT cell therapy.

**Imaging Research Program**

**Research Summaries**

**Principal Investigator: Soltanian-Zadeh, Hamid, Ph.D.**

**Decision Support System for Temporal Lobe Epilepsy (NIH R01EB013227-01A1)**

With the ever-increasing role of medical images in diagnosis, treatment planning, and evaluation of treatment effects, extraction of quantitative information from these images and efficient use of the results have become a necessity. In recent years, we have developed novel three-dimensional (3D) knowledge-based methods to segment brain structures from magnetic resonance images (MRI) automatically. These methods need to be optimized, fine-tuned, and compared to other methods for the segmentation of specific brain structures that may be involved in medical temporal lobe epilepsy (mTLE). Feature extraction methods also need to be developed and optimized to characterize (i.e., determine local and global multi-parametric intensity distribution, texture, shape, surface area, surface curvatures, and volume of) the brain structures. Multi-modality analysis using multi-parametric MRI and SPEC needs to be developed for improved sensitivity and specificity. We have also developed our preliminary version of a content-based human brain image database system to hold the image analysis results with other clinical information (e.g., textual data) in a manner that can be searched, retrieved, and queried conveniently from any computer station. This system needs integrated methods for data preparation, missing value treatment, interactive rule-extraction, visualization, and user-inference to serve as a decision support system in clinical practice. A user-friendly, web-based interface will be critical for the ultimate use of the system by researchers and clinicians. Last but not least, the database needs to be populated with data from a large number of patients so that it can be confidently used for hypothesis testing and clinical applications. The goal of this project is to develop novel approaches for the above needs. Image analysis and feature extraction methods will segment and characterize hippocampus, amygdala, entorhinal cortex, thalamus, putamen, and other brain structures from MRI. The methods will be tested, evaluated, and validated using clinical data of epilepsy patients. Clinical diagnosis based on EEG studies and surgery outcome will be used as “god standards” for evaluation and validation of the image analysis methods. The proposed decision support system will be populated with multi-modality data of 350 epilepsy patients to evaluate correlation between a variety of risk factors, imaging features, clinical diagnosis (lateralization), and post-operative outcomes, and to assist physicians with improved clinical diagnosis, reduced intracranial EEG studies (reduced risk and suffering of
patients as well as their healthcare cost), optimal treatment options, and prediction of outcome in prospective studies. The proposed research will be a breakthrough in the application of computerized methods for medical image quantification and object characterization, and will advance image analysis science in the direction of integrating knowledge-based image segmentation and characterization methods with pattern recognition and data mining technology in decision support systems. The proposed approaches are applicable to the identification, segmentation, and characterization of other biological structures. They are also applicable to virtually any image analysis task for which object segmentation, quantification, and characterization are used. This project will develop a decision support system for assisting physicians to improve diagnosis and prognosis of epilepsy patients while reducing the healthcare cost. It will process multi-modality medical images and extract quantitative information from them. The image analysis results will be used along with the results of other clinical tests as well as the patients’ history and characteristics to reduce the need for intracranial electrographic studies, predict post-operative outcomes, and suggest optimal treatment options for the new patients.

Neurology

Principal Investigator: Jieli Chen, Ph.D.
Neurorestorative Therapy of Stroke with HUCBC in Type Two Diabetic Mice (NIH 1R01NS083078-01A1)

Diabetes mellitus (DM) leads to a 3-4 fold higher risk of experiencing ischemic stroke. Hyperglycemia and diabetes instigate a cascade of events leading to vascular endothelial cell dysfunction, increased vascular permeability and poor recovery after ischemic stroke. Diabetic animals exhibit more severely injured white matter (WM) than non-DM animals after stroke. There is also a differential response to treatment of stroke between DM and non-DM subjects. Effective therapy of stroke in the non-DM population may not necessarily transfer to the DM population, prompting the need to develop therapeutic approaches specifically designed to reduce neurological deficits after stroke in the DM population. Our preliminary data show that T2DM significantly decreases microRNA - 126 (miR-126) and Angiopoietin-1 (Ang1) expression in the circulation and in the ischemic brain of mice. Human umbilical cord blood cell (HUCBC) treatment of stroke in T2DM mice starting at 3 days after stroke significantly improves recovery of neurological function as well as increases miR-126 and Ang1 expression in the ischemic brain. Therefore, based on our robust preliminary data, we propose to use HUCBCs for the treatment of stroke in the T2DM mice and to investigate the role of intercellular communication via miR-126 encapsulated within Exosomes/Microvesicles (EMVs) in mediating the therapeutic benefit on HUCBCs for ischemic stroke. This application includes three Aims. Aim 1 will test if miR-126 mediates HUCBC treatment induced neurorestorative effects after stroke in T2DM mice. We hypothesize that miR-126 mediates HUCBC treatment–induced vascular integrity, axonal outgrowth, and WM remodeling, and improves functional outcome after stroke in T2DM mice. Aim 2 will test whether miR-126 generated by HUCBCs is transferred to brain endothelial cells (BECs) and parenchymal cells via EMVs. We hypothesize that HUCBCs secrete EMVs containing miR-126 which are taken up by BECs and parenchymal cells. Aim 3 will investigate whether miR-126 regulation of Ang1 promotes the HUCBC-induced neurorestorative effects after stroke in T2DM mice. We hypothesize that: 1) HUCBC
treatment of stroke in T2DM mice increases Ang-1 signaling activity in the ischemic brain; 2) miR-126 regulates Ang1 expression and thereby regulates vascular remodeling, axonal outgrowth and oligodendrocyte survival and differentiation; 3) Restoration of Ang1 with an Ang1 mimic peptide will rescue the neurorestorative effects of knockdown of miR-126 in HUCBC after stroke in T2DM mice. In this application, we are the first to propose that, generation of miR-126 encapsulated in EMVs by HUCBCs contributes to its robust therapeutic restorative effects and that miR-126 and its regulation of Ang-1 mediate HUCBC-induced neurovascular and WM remodeling, and thereby improve stroke functional recovery in T2DM mice. This proposal is highly clinically relevant and if successful, will significantly impact the treatment of diabetic and possibly all stroke patients.

Principal Investigator: Michael Chopp, Ph.D.

MSCs Induce Brain Plasticity via tPA (NIH R01AG037506-03)

Cell-based therapies have shown enormous promise in reducing neurological deficits associated with stroke. One of the most effective of these therapies is bone marrow stromal cells (MSCs), that has been demonstrated to be highly neurorestorative. In this application, we will investigate the mechanisms by which MSCs produce this neurorestorative effect. Our preliminary data strongly indicate that MSC treatment of stroke promotes neurite remodeling of brain. We propose that when administered after stroke, MSCs activate tissue plasminogen activator (tPA) within parenchymal cells, and tPA mediates neurite remodeling leading to improvement in neurological function. Therefore, the following three hypotheses are tested

Hypothesis 1 a) MSCs increase tPA activity in parenchymal cells; b) Increased tPA activity increases neurite remodeling; c) Increased neurite remodeling contributes to improvement of functional outcome after stroke. Hypothesis 2 a) MSCs up-regulate tPA activity in astrocytes, neurons and endothelial cells via the Shh signaling pathway; b) MSCs down-regulate TGF-β1/PAI-1 via the Shh signaling pathway and thereby increase tPA activity. Hypothesis 3 tPA activity increased by MSCs promotes neurite remodeling via plasmin-dependent proteolytic cleavage of pro-neurotrophins pro-nerve growth factor (pro-NGF) to NGF, pro-brain derived neurotrophic factor (pro-BDNF) to BDNF. These hypotheses dissect the interactions of exogenous MSCs and endogenous parenchymal cells and their affect on tPA activity, neurite remodeling and neurological function after stroke. Our studies employ genetically modified tPA-/-, Plg-/--mice as well as an array of novel and well-established experimental techniques in our laboratory. To our knowledge, our work is the first to investigate tPA activity as a key unifying factor to amplify beneficial actions of exogenous cells in the CNS. This project is a coherent and highly interwoven effort to elucidate the molecular and cellular pathways by which injured brain can be remodeled by cell-based therapies. Our ultimate goal is to delineate the mechanistic underpinnings of cell-based therapy in the restorative treatment of stroke. The therapeutic implications of our studies for all neurological disease and injury are evident. Our study will provide essential insight into how the injured brain is remodeled and neurological function improved using a cell-based therapy. Restorative therapy using exogenously administered cells is not limited by a narrow therapeutic window and can be administered to all stroke patients. Our goal to identify how these administered cells interact with the endogenous brain cells will likely bring to fruition restorative cell-based therapy for the treatment of stroke and neural injury.

Principal Investigator: Michael Chopp, Ph.D.
**miR-17-92 Exosome Treatment of Stroke (NIH R01NS088656-01A1)**

Exosomes, small lipid microvesicles (30-150 nm), are active biological containers, which transport regulatory genes and proteins between cells and form a major biological communication conduit, facilitating a plethora of biological responses. The regulatory molecules contained in the exosomes include microRNAs (miRNAs), short (22-25 nt) non-coding RNAs which regulate gene translation and play primary roles in mediating a vast range of biological functions. In this proposal, based on strong preliminary data, we propose to manufacture a distinct exosome population which contains increased levels of the miR-17-92 cluster as a proof-of-principle and a mechanistic demonstration of a new method of treating stroke and possibly other neurological diseases and injury. We test the premise, that by modulating their miRNA content, exosomes can be designed to enhance plasticity of axons and thereby further promote neurological recovery post stroke. Success of this novel approach may lead to a new designer-based paradigm for the treatment of stroke and neurological disease. The following Specific Aims and associated Hypotheses are proposed:

**Specific Aim 1:** To employ exosomes derived from multipotent mesenchymal stromal cells (MSCs) to treat stroke in order to enhance neurovascular remodeling and thereby, functional recovery post stroke. Hypothesis: Exosomes, derived from MSCs when administered to rats after stroke promote neurovascular remodeling which improves functional outcome.

**Specific Aim 2:** To alter specific miRNAs contained within exosomes generated by MSCs as a means to enhance axonal plasticity and neurological recovery post stroke. Hypothesis: Administration of exosomes with increased miR-17-92 cluster to rats post stroke promotes axonal remodeling and enhances functional outcome. There are multiple layers of innovation in our application: we generate biological exosome carriers tailored for specific miRNAs; we use these exosomes to treat stroke, without the administration of exogenous cells; we employ electrophysiological methods, laser capture, fiber track tracing, a battery of neurological tests, and an array of novel approaches, e.g. microfluidic chambers, and ex vivo slice cultures, to mechanistically determine the molecular pathways of the target exosomes which mediate axonal outgrowth. Development of this designer exosome-based therapy, also serves as a prototype for capitalizing on the characteristics of exosomes to transport specific miRNAs and for the manufacture of designer exosomes. Developing a therapy for stroke that is exosome-based, opens up a wide variety of means to deliver targeted regulatory genes to enhance multifaceted aspects of central nervous system (CNS) plasticity and to amplify neurological recovery for neural injury and neurodegenerative diseases.

**Principal Investigator:** Xu Cui, Ph.D.

**ABCA1 Regulates White Matter Remodeling and Oligodendrogenesis after Stroke (NIH R01NS092917-01)**

Stroke is a major cause of white matter (WM) damage which induces long-term disability. There is limited WM remodeling in the adult brain. Many neuroprotective treatments of stroke have failed in clinical trials because they cannot protect WM. Therefore, there is a compelling need to investigate the mechanism underlying WM remodeling and oligodendrogenesis of the adult brain and to develop effective long-term stroke therapy. Cellular cholesterol modulates axonal and dendritic outgrowth and is required for myelination. The level of HDL-cholesterol is related to the progression and recovery of
stroke patients. ATP-binding cassette transporter A1 (ABCA1) is a major cholesterol transporter and plays critical roles in regulation of HDL-cholesterol and ApoE synthesis and metabolism in the central nervous system. Brain specific-ABCA1 deficient (ABCA1-B/-B) mice have very low brain HDL-cholesterol/ApoE level, and exhibit neuronal ultrastructure changes and functional deficits. Both HDL-cholesterol and ApoE increase neurite outgrowth in culture conditions. Our preliminary study shows that ABCA1-B/-B mice exhibited increased WM damage and reduced oligodendrogenesis and exacerbated neurological functional deficits after stroke. Primary cultured neurons derived from ABCA1-B/-B mice show decreased neurite outgrowth, which can be attenuated by HDL treatment. ABCA1-B/-B astrocyte-conditioned media also decreased wild type neurite outgrowth after hypoxic ischemia. Therefore, we propose the following three specific aims: 

**Aim1** To investigate whether brain-deficient in ABCA1 exhibits decreases in WM-remodeling and axonal growth after stroke. ABCA1-B/-B and floxed-control mice will be subjected to stroke, WM-changes and oligodendrogenesis will be measured. 

**Aim2** To investigate molecular mechanism underlying ABCA1 in regulation of WM-remodeling and oligodendrogenesis after stroke, we will examine whether ABCA1 regulates brain HDL and ApoE level, and whether brain HDL and ApoE levels mediate ABCA1-induced WM-remodeling and oligodendrogenesis after stroke. 

**Aim3** To investigate cellular mechanisms of ABCA1 in regulation of WM-remodeling and oligodendrogenesis, we will examine neurons and oligodendrocytes and the cross talk of astrocytes with neurons and oligodendrocytes on ABCA1-induced WM-remodeling and oligodendrogenesis in vitro and in vivo. We expect that ABCA1 deficient brain will exhibit significant decreases in HDL and ApoE level, and decreases WM-remodeling and oligodendrogenesis as well as reduced functional outcome after stroke. The level of HDL/ApoE in brain or cerebrospinal fluid will, at least partially, mediate ABCA1-induced WM-remodeling and oligodendrogenesis in the ischemic brain after stroke. To our knowledge, no one has investigated the functional effect of ABCA1 on oligodendrogenesis and WM-remodeling post-stroke recovery, especially by using ABCA1-B/-B mice. The new insights gleaned from this study will contribute to our understanding of the beneficial role of ABCA1/HDL-C/ApoE in brain plasticity which will impact development of rational restorative approaches to improve neurological outcome for stroke patients.

**Principal Investigator: Xianshuang Liu, M.D.**

**Translational Study of miR-146a Gene Therapy for Diabetic Peripheral Neuropathy (NIH R01DK102861-01A1)**

Peripheral neuropathy is the major complications of diabetes. There is a compelling need to develop effective therapeutic approaches specifically designed to improve neurological function in the damaged peripheral nervous system after diabetes. MicroRNA-146a (miR-146a) has been implicated in the regulation of multiple immune diseases. However, the role of miR-146a in diabetic peripheral neuropathy (DPN) has not been investigated. In a novel set of experiments, our preliminary data show that intravenous administration of miR-146a remarkably improved sciatic nerve vascular function, axonal myelination and peripheral nerve function in diabetic mice, indicating that miR-146a may have a beneficial effect on the clinical treatment of DPN. In this application, we therefore seek to investigate the mechanisms underlying the therapeutic effects of miR-146a on DPN. We propose that miR-146a by improving vascular function and suppressing pro-inflammation factors ameliorates
DPN. The associated hypotheses are: 1. Treatment with chemically engineered miR-146a improves neurological outcomes in DPN in dose and therapeutic window dependent manners. 2. Elevation of miR-146a levelssuppresses its target genes, IRAK1/TRAF6 and their down-stream pro-inflammatory factors in vascular endothelial cells and monocytes of type II diabetic mice, thereby, leading to the improvement of neurovascular function and consequently ameliorating peripheral neuropathy. To investigate the effect of miR-146a on neurological outcomes, type II diabetic mice which develop severe peripheral neuropathy will be treated with miR-146a at various time points and doses after onset of DPN. To investigate the underlying molecular mechanisms, the effects of miR-146a overexpression and knockdown on target genes and inflammatory genes that mediate miR-146a-enhanced neurovascular function will be determined. These studies are innovative and will provide novel insights into mechanisms underlying the neurological dysfunction of DPN and likely lead to the development of a new miRNA-based gene therapy.

Principal Investigator: Lei Wang, M.D.
Phosphodiesterase-5 is a Therapeutic Target for Peripheral Neuropathy in Diabetic Mice (NIH R01NS075084-02)

Peripheral neuropathy is a common and major complication of diabetes, the underlying mechanisms of which are not fully understood. Using a mouse model of type II diabetes, the present study investigated the role of phosphodiesterase-5 (PDE5) in peripheral neuropathy. BKS.Cg-m/+ Leprdb/J (db/db) mice were treated with sildenafil, a specific inhibitor of PDE5, at doses of 2 and 10 mg/kg or saline. Levels of PDE5 and morphometric parameters in sciatic nerve tissue as well as the motor and sensory function were measured in these mice. In diabetic mice, PDE5 expression in sciatic nerve tissue was significantly upregulated, whereas the myelin sheath thickness, myelin basic protein (MBP), and subcutaneous nerve fibers were significantly reduced. Treatment with sildenafil significantly improved neurological function, assayed by motor and sensory conducting velocities and thermal and mechanical noxious stimuli, concomitantly with increases in myelin sheath thickness, MBP levels, and subcutaneous nerve fibers. In vitro, hyperglycemia upregulated PDE5 in Schwann cells and reduced Schwann cell proliferation, migration, and expression of brain-derived neurotrophic factor (BDNF). Blockage of PDE5 with sildenafil increased cyclic guanosine monophosphate (cGMP) and completely abolished the effect of hyperglycemia on Schwann cells. Sildenafil upregulated cGMP-dependent protein kinase G I (PKG1), whereas inhibition of PKG1 with a PKG inhibitor, KT5823, suppressed the inhibitory effect of sildenafil on Schwann cells. These data indicate that hyperglycemia substantially upregulates PDE5 expression and that the cGMP/PKG signaling pathway activated by sildenafil mediates the beneficial effects of sildenafil on diabetic peripheral neuropathy.

Principal Investigator: Lei Wang, M.D.
Thymosin Beta4 Promotes the Recovery of Peripheral Neuropathy in Diabetic Mice (NIH R01DK097519-01)

Peripheral neuropathy is one of the major complications of diabetes. There is a compelling need to develop effective therapeutic approaches specifically designed to improve neurological function in the damaged peripheral nervous system after diabetes. Thymosin Beta4 (Tβ4), a major intracellular G-actin sequestering peptide, has multiple biological
functions, including promotion of remodeling of injured and damaged tissues, and increasing angiogenesis after myocardial infarction. However, the role of Tβ4 in diabetic peripheral neuropathy has not been investigated. In a novel set of experiments, our data show that Tβ4 remarkably improved sciatic nerve vascular function and peripheral nerve function in diabetic mice, indicating that Tβ4 may have a beneficial effect on the clinical treatment of diabetic peripheral neuropathy. In this application, we therefore seek to investigate the mechanisms underlying the therapeutic effects of Tβ4 on the treatment of diabetic peripheral neuropathy. We propose that Tβ4 by improving vascular function ameliorates diabetic peripheral neuropathy. Our hypotheses are: 1. Treatment with Tβ4 improves neurological function of peripheral neuropathy in diabetic mice. 2. The Ang/Tie2 signaling pathway mediates the therapeutic effect of Tβ4 on neurovascular function in diabetic peripheral neuropathy. 3. The PI3K/Akt signaling pathway underlies the effect of Tβ4 on Ang1/Ang2 expression. To investigate the effect of Tβ4 on neurological outcome, type II diabetic mice which develop severe peripheral neuropathy will be treated with Tβ4 at various time points after onset of diabetes. To investigate the molecular mechanisms that mediate Tβ4–enhanced neurovascular function in diabetic mice, the effect of Tβ4 on expression of Ang/Tie2, and activation of PI3K/Akt signaling pathway will be examined. Using pharmacological inhibitors and siRNA gene knockdown techniques, we will investigate the cause-effect of the Ang/Tie2 and PI3K/Akt signaling pathways on regulating Tβ4-enhanced neurovascular function and axonal outgrowth. These studies are innovative and will provide new insight into mechanisms underlying the neurological dysfunction of diabetic peripheral neuropathy and lead to the development of a new treatment using Tβ4. Peripheral neuropathy often stemming from diabetes is a major disability affecting millions of Americans. In this proposal, employing preclinical studies in the diabetic animal, I seek to develop a novel treatment for peripheral neuropath with Tβ4. Tβ4 is currently in a phase II clinic trial for the treatment of patients with acute myocardial infarction. In this proposal, I also elucidate the molecular mechanism by which Tβ4 is therapeutically effective. This research will provide the essential pre-clinical data for translation to a phase 1 clinical trial.

Principal Investigator: Hongqi Xin, Ph.D.

Exosome Transfer of miR-133b Mediates MSC Induced Neurological Recovery after Stroke (NIH R01NS081189-01A1)

Multipotent mesenchymal stromal cells (MSCs) have potential therapeutic benefit in many diseases including neurological diseases and injury. MSC-based therapies enhance recovery from stroke. We have previously demonstrated that exogenously administered MSCs interact with neural cells, increase the production of neurites, reduce expression of axonal inhibitory molecules and stimulate the production of growth and plasticity positive factors in neural cells which promote neurorestoration and recovery of neurological function. However, it is unknown how MSCs interact with neural cells, alter their protein expression, and thereby promote functional recovery. In the present proposal, we provide fundamental and novel mechanistic insight into how cell-based therapies promote recovery. MicroRNAs (miRNAs) act as master switches regulating the translation of many genes, and exosomes are membrane vesicles, 40-100nm in diameter, that are secreted by a wide range of cell types. We propose that MSCs increase specific miRNA levels in neural cells via exosomes, which subsequently stimulate neurite outgrowth and functional recovery. Based on our preliminary data, we will primarily focus on miR-133b, as an important target miRNA. Two specific aims are proposed. Aim 1: To investigate whether exosomes primarily mediate cell-cell
communication by direct transfer of miR-133b to neural cells and/or indirectly by stimulating
miR-133b expression in neural cells, which subsequently promote neurite outgrowth and
functional recovery after stroke. Aim 2: To investigate the mechanisms by which miR-133b
promotes neurite remodeling after treatment of stroke with MSCs. This study opens up
important and novel ways to elucidate how exogenously administered cells communicate with
and alter neural cells to activate restorative events. Confirming our hypothesis represents a
major leap forward in our understanding of cell-cell communication and will lead to novel
ways to augment brain recovery.

Principal Investigator: Zheng Gang Zhang, M.D., Ph.D.
MicroRNAs and Neurogenesis after Stroke (NIH 1R01NS075156-03)

MicroRNAs (miRNAs) regulate biological function of neural progenitor cells and
oligodendrocyte progenitor cells (OPCs). Our preliminary data show that stroke substantially
changed miRNA expression profiles in adult neural progenitor cells and oligodendrocytes. In
this application, we propose to test the hypothesis that miRNAs in neural and OPCs play a
pivotal role in mediating adult neurogenesis and oligodendrogenesis in the ischemic brain. In
Specific Aim 1, we will investigate the effect of inactive miRNA processes in neural
progenitor cells and OPCs on stroke-induced neurogenesis and oligodendrogenesis by
conditional and inducible Dicer ablation in Ascl1 lineage cells (Ascl1-
CreTM/Dicerflox/flox). In Specific Aim 2, we will investigate whether the sonic hedgehog
(Shh) signaling pathway interacts with the miR-17-92 cluster to increase neurogenesis and
oligodendrogenesis. In Specific Aim 3, we will investigate the effect of the miR17-92 cluster
on biological function of neural and oligodendrocyte progenitor cells in the ischemic brain by
deletion or overexpression of the miR17-92 cluster in neural progenitor cells and OPCs after
stroke. These studies will provide novel insights into miRNAs in regulating stroke-induced
neurogenesis and oligodendrogenesis, which could potentially lead to new therapies to
amplify neurogenesis and oligodendrogenesis in injured brain. Neurogenesis and
oligodendrogenesis are associated with functional recovery after stroke. Molecular
mechanisms underlying generation of new neurons and oligodendrocytes in ischemic brain
have not been fully understood. Our preliminary data suggest that MicroRNAs (miRNAs),
short noncoding RNA molecules, could be essential components in mediating stroke-induced
neurogenesis and oligodendrogenesis. In this application, we propose three experiments to
investigate the role of miRNAs in regulating adult neurogenesis and oligodendrogenesis in the
ischemic brain. We will first delete Dicer to inactive miRNA processes in neural
progenitor cells and oligodendrocyte progenitor cells (OPCs) after stroke. We will then
examine a linkage between the sonic hedgehog (Shh) signaling pathway and miR17-92
expression in mediating neurogenesis and oligodendrogenesis. Finally, we will ablate or
overexpress the miR17-92 cluster in neural progenitor cells and OPCs. These studies will
provide novel insights into miRNAs in regulating stroke-induced neurogenesis and
oligodendrogenesis, which could potentially lead to new therapies to amplify neurogenesis
and oligodendrogenesis in injured brain.

Principal Investigator: Zheng Gang Zhang, M.D., Ph.D.
Ac-SDKP for Treatment of Acute Stroke (NIH R01NS079612-01A1)

Stroke is a leading cause of death and disability worldwide and approximately 72% of
people who suffer a stroke are over the age of 65. Tissue plasminogen activator (tPA) is the only drug approved by the Food and Drug Administration (FDA) for treatment of acute stroke (within 4.5h). The most feared complication after tPA treatment of stroke is an increased risk of cerebral hemorrhage. Our preliminary data indicate that N-acetylsereryl-aspartyl-lysyl-proline (Ac-SDKP), a peptide normally presented in human plasma, in combination with tPA reduced infarct volume by more than 50% and improved neurological outcome, but did not increase the incidence of hemorrhagic transformation in young adult rats. In this application, we propose to develop a combination therapy of Ac-SDKP and tPA for treatment of acute stroke in aged rats and to investigate molecular mechanisms underlying the combination therapy on the neurovascular unit. In Specific Aim 1, using MRI and 3D laser confocal microscopy, we will investigate the effect of Ac-SDKP alone and Ac-SDKP in combination with tPA on recanalization of the occluded MCA, cerebral microvascular perfusion and vascular integrity, brain hemorrhage, and ischemic neuronal damage in aged rats subjected to embolic middle cerebral artery occlusion (MCAO). In Specific Aim 2, we will examine whether Ac-SDKP suppresses the ischemia- and tPA-activated nuclear transcription factor-κB (NF-κB) pathway in cerebral vessels, which leads to enhancement of cerebral microvascular patency and integrity by reduction of thrombosis. In Specific Aim 3, we will examine whether Ac-SDKP blocks the ischemia- and tPA-activated transforming growth factor β (TGFβ) signaling pathway in cerebral vessels and astrocytes, which leads to reduction of thrombosis by down regulation of plasminogen activator inhibitor 1 (PAI-1). These studies could potentially provide a new therapy to minimize the adverse effect of tPA on ischemic neurovascular damage, leading to improved neurological outcomes after acute stroke.

**Radiation Oncology**

**Principal Investigator: Svend Freytag, Ph.D.**

**Gene Therapy and Radiation Therapy for Prostate Cancer (NIHR01CA160289-02)**

Our translational research program has been developing a multi-modal, gene therapy-based approach for the treatment of cancer. We have evaluated the toxicity and preliminary efficacy of our investigational approach in five clinical trials of non-metastatic prostate cancer. Our early stage results indicate that our approach is safe and has the potential to improve local tumor control. Although local tumor control is important, new therapies for high-risk prostate cancer must also target metastatic disease if they are to have an impact on survival. Hence, we have added a fourth modality to our investigational approach by generating a third-generation adenovirus armed with interleukin 12 (IL-12) that has the potential to eradicate both local and metastatic disease. This new adenovirus has generated encouraging preliminary results in preclinical studies, and we plan to move it into the clinic targeting high-risk prostate cancer. In specific aim 1, we will test the hypothesis that IL-12 will improve the efficacy of replication-competent adenovirus-mediated suicide gene therapy and radiation in an immune-competent, orthotopic model of prostate cancer. C57BL/6 male mice bearing intraprostatic TRAMP-C2 tumors will receive an intratumoral injection of Ad5- yCD/mutTKSR39rep-mIL12 followed by 2 weeks of 5-fluorocytosine (5-FC) + ganciclovir (GCV) prodrug therapy and pelvic radiation. Primary endpoints are local and metastatic tumor control. Secondary endpoints include T cell activation, NK and CTL activity, serum and tumor cytokine levels, and development of anti-tumor immunity. In
specific aim 2, we will test the hypothesis that cyclophosphamide (CP) can be combined safely with replication-competent adenovirus-mediated suicide and IL-12 gene therapy and that the combined therapies exhibit synergy in vivo. Efficacy will be examined in the immune-competent, orthotopic TRAMP-C2 tumor model without and with CP. Efficacy endpoints are identical to those in specific aim 1. Toxicity will be examined in C57BL/6 male mice and Syrian hamsters, the latter of which are permissive for human adenovirus replication. In specific aim 3, we will test the hypothesis that replication-competent adenovirus-mediated suicide and IL-12 gene therapy can be combined safely with intensity modulated radiation therapy (IMRT) and androgen suppression therapy (AST) in men with newly-diagnosed, high-risk prostate cancer. Fifteen men (5 cohorts, 3 patients each) with high-risk prostate cancer (Stage e T3 or Gleason e 8 or PSA >20 ng/mL) will receive a single injection of Ad5- yCD/mutTKSR39rep-hIL12 at five dose levels (1 x 1010 vp to 1 x 1012 vp in half-log increments). The adenovirus will be injected intraprostatically under transrectal ultrasound-guidance. Two days later, men will receive 2 weeks of 5-FC + valganciclovir (vGCV) prodrug therapy concomitant with 80 Gy IMRT and e 2 years of AST. The primary endpoint is toxicity through day 90. Secondary endpoints are: 1) prostate biopsy at 2 years, 2) freedom from biochemical/clinical failure (FFF), 3) disease-specific survival, 4) overall survival, and 5) serum cytokine levels. We believe this research will have high impact because it may lead to better treatments for aggressive forms of prostate cancer. Public Health Relevance: The broad, long-term goal of the proposed research is to develop better cancer treatments. This research will improve further an investigational therapy that has already demonstrated promising activity in early stage trials of prostate cancer. We believe this research will have high impact because it will lead to better treatments for aggressive forms of prostate cancer.

Urology

Principal Investigator: Nallasivam Palanisamy
Functional Characterization of Pseudogenes as New Biomarker in Prostate Cancer (NIH 7R21CA176330-02)

Prostate cancer remains the most commonly diagnosed cancer and second leading cancer-related death in men in the United States with 238,590 new diagnosis and ~29,720 deaths in 2013 (ACS). Accurate diagnosis and early intervention will reduce the number of deaths due to metastatic prostate cancer. Prostate cancer diagnosis is based initially on the controversial serum prostate specific antigen (PSA) level. To overcome the problems associated with testing PSA level, identification of accurate and reliable cancer-specific molecular markers will reduce unnecessary biopsies for those with benign conditions and direct patients with aggressive disease for appropriate treatment. The genetic basis of prostate cancer can be classified into groups based on specific driving molecular aberrations. About 50-60% of prostate cancer patients are known to have E26 transformation specific (ETS) gene rearrangements and overexpression of SPINK1 was identified in 5-10% of ETS-negative prostate cancer. Recently, we reported the identification of “druggable” gene fusions involving RAF kinase genes (SLC45A3-BRAF and ESRP1-RAF1) in 1-2% of ETS negative prostate cancer. The genetic basis of the remaining 30-40% of ETS-negative prostate cancer remains unknown. In this proposal we utilized next generation sequencing technology to study the transcriptional landscape of
prostate cancer on a genome-wide scale for pseudogene expression. In our pilot study using 89 prostate cancer samples we identified 8 candidate pseudogenes and a novel fusion gene involving a pseudogene expressed only in prostate cancer. Based on the encouraging preliminary results on the functional role of the candidate pseudogenes, we will prioritize our studies with a focus on the functional characterization of CXADRP2 and KLK4-KLK1.

**In specific aim 1:** We will expand the validation of candidate pseudogenes in a large cohort of prostate cancer to understand their unique role in prostate cancer. In our preliminary validation studies, we confirmed the expression of CXADRP2 in ETS fusion-negative prostate cancer and KLK4-KLK1 in ETS fusion positive prostate cancer. Further using RNA *in situ* hybridization assay we showed significant increase in the expression of KLK4-KLK1 in high Gleason score (GS-7 or above) prostate cancer indicating its potential role in aggressive prostate cancer. We also presented preliminary data on the expression of these two pseudogenes in post-DRE urine samples, indicating the potential to screen by non-invasive methods. With the strong correlation with ETS-positive and ETS-negative prostate cancer for CXADRP2 and KLK4-KLK1; respectively, we will correlate the expression with known prostate specific non-coding RNA PCA3 and PCAT-1 to understand the mutually exclusive function of pseudogenes in subsets of prostate cancer. Given the unique 54 amino acid peptide from KLK1, we will develop KLK1 specific antibody to screen by western blot analysis and immunohistochemistry. **In specific aim 2:** We showed strong functional data using *in vitro* and *in vivo* studies. We also show that either of the gene has any effect on the other, suggesting an independent mechanism in prostate cancer. We will perform gene expression microarray analysis in cells with overexpression to understand the molecular mechanisms involved and potential signaling pathways affected by the pseudogenes. Further, based on the encouraging results from the *in vivo* CAM assay, we will validate the oncogenic properties in murine models. We anticipate that validation of these new molecular markers will add into the armamentarium of prostate cancer diagnostics to identify patients that will develop metastatic disease who require early and aggressive treatment strategies.

**Part III – Population and Health Sciences**

- Center for Health Policy and Health Services Research
- Department of Public Health Sciences

**Center for Health Policy and Health Services Research**

**Principal Investigator:** Brian Ahmedani, Ph.D.
**Treatment Utilization Before Suicide (TUBS) (NIH R01MH103539-01A1)**

Adult suicide rates in the United States rose by almost 30 percent between 1999 and 2010. These rates have not markedly improved in decades. To date, previous suicide attempts and psychiatric diagnoses are largely the only known clinical risk factors for suicide death. Recent research shows that most individuals who die by suicide make a health care visit in the weeks and months prior to their death. Most of these visits occur in primary care or outpatient medical specialty settings. However, over half of these visits do not include a psychiatric diagnosis.
Thus, there is limited evidence available from health care users in the US general population to inform targeted suicide screening and risk identification efforts in general medical settings. New research is needed to investigate the general medical clinical factors associated with increased suicide risk among individuals without a known risk factor. This research project uses data on more than 4000 individuals who died by suicide and made health care visits to one of eight health care systems across the United States in the year prior to their death. These health systems are members of the Mental Health Research Network and have affiliated health plans. They are able to capture nearly all health care for their members via the Virtual Data Warehouse (VDW). The VDW consists of electronic medical record and insurance claims data organized using standardized data structures and definitions across sites. These data are matched with official regional mortality data. This project includes the following specific aims:

1) identify clinical factors from general medical visits prior to suicide across sites, 2) compare clinical factors to a matched sample of health care users across sites, and 3) investigate indications of psychiatric and other concerns in general medical chart notes prior to suicide. This is the first study with a large enough sample in the US general population to be able to study general medical treatment utilization prior to suicide death. This project will allow the identification of previously unknown factors that increase risk of suicide death, including general medical diagnoses, medications, health care procedures, and types of visits. These results will inform decisions about how to focus suicide prevention efforts in general medical settings.

Principal Investigator: Keoki Williams, M.D.

Combined Transcriptomics and Genomics to Find Asthma Genes in Admixed Populations (NIH R01HL118267-01A1)

African American individuals are more likely to develop asthma and are nearly three times as likely to experience serious asthma complications when compared with European American individuals. Genome wide association studies have identified a number of genetic risk markers for asthma, but many of the associations observed in European and European American patients have not replicated in African American individuals. This may be the result of allele frequencies, linkage disequilibria, or disease-related genes which differ by ancestry. Detailed characterization of the transcriptome can aid in the identification of asthma-related genes by circumventing some of the aforementioned problems associated with genotype association alone. Therefore, this proposal seeks to combine transcriptomics and genomics to identify asthma-related genes and the expression quantitative trait loci (eQTL) which appear to regulate these genes. We propose using RNA sequencing (RNA-seq) to characterize the transcriptome of African American individuals with and without asthma. RNA-seq is superior to traditional microarrays at quantifying transcript abundance, but this method has not been widely used in U.S. minority populations to date. The Study of Asthma Phenotypes and Pharmacogenomic Interactions by Race-ethnicity (SAPPHIRE) cohort is an ideal group in which combine these analytic approaches. In addition to being one of largest and best characterized asthma cohorts in the U.S., genome wide genotype data and banked whole blood RNA already exist for a large number of SAPPHIRE participants. In Specific Aim 1, we will use RNA-seq to identify expression differences in previously identified asthma-related genes among African American individuals by asthma status. Pre-existing genotype data will then be used to identify eQTL for these differentially expressed,
asthma-associated genes. In Specific Aim 2, we will use admixture mapping to identify chromosomal regions where ancestry is associated with asthma. The genes in these regions will be interrogated for differential expression by asthma status. The resulting potentially novel, ancestry-specific asthma genes will also be assessed for eQTL. As a subset of African American SAPPHIRE participants have RNA collected at both their initial evaluation and the time of an asthma exacerbation, in Specific Aim 3 we will assess whether the genes identified in the preceding aims are also associated with asthma exacerbations. Lastly, Specific Aim 4 will attempt to replicate our findings in a separate group of African American participants with and without asthma. In summary, asthma is a complex disease with potentially distinct genetic predictors by ancestry. Persisting inequities in asthma complications by race-ethnicity underscore the need for improved disease biomarkers and therapeutic targets. As a step in this direction, we proffer an integrative approach with greater statistical power to identify asthma-related genes and their regulatory elements.

Department of Public Health Sciences

Principal Investigator: Gwen Alexander, Ph.D.
Encouraging Young Adults to Make Effective Nutrition Choices: MENU Gen Y Study (NIH R01HD067314)

The MENU Gen Y Study will focus on developing an age-targeted web-based intervention designed to increase daily intake of fruit and vegetables for young adults (age 21 – 30 “Gen Y”) using features that appeal to and factors relevant to this targeted age group.

Principal Investigator: Andrea Cassidy-Bushrow, Ph.D.
Delivery Mode, Environment and the Gut Microbiome: Influence on Childhood Body Size (NIH R01HD082147-01A1)

Caesarean section (CS) delivery, which accounts for ~32% of all US births, has been associated with offspring obesity. Little is known about the mechanisms linking CS with obesity risk. The gut microbiome, which varies by mode of delivery, is also associated with childhood obesity. In our established racially and socioeconomically diverse birth cohort (WHEALS; Wayne County Health, Environment, Allergy and Asthma Longitudinal Study), the early-life gut microbiome is associated with body mass index (BMI) category at age 2 years; CS is associated with both a distinct early-life gut microbiome and with increased BMI at age 2 years; and the presence of pets in the home, which increases microbial diversity, reduces the association between CS and BMI. Our data provide evidence for a mediating role of the gut microbiome in the CS-obesity relationship. However, to provide stronger evidence requires additional study. This project builds on extant data in WHEALS and on-going data collection in a subset of these children to examine the role of the gut microbiome in the CS-obesity association. Children will be invited for a research clinic visit for comprehensive body size assessment and blood draw at age 10-12 years. Gut microbiome composition and predicted function will be measured in banked early-life (1 and 6 months of infancy) stool samples and in samples from these children at age 10-12 years using the 16S rRNA and ITS2 biomarker genes and the Illumina MiSeq platform. A
metabolomics analysis will be conducted in a subset of these stool samples. Adiposity will be measured as BMI at ages 2 and 10-12 years, BMI trajectory from birth to age 10-12 years, and anthropometric, bioimpedance and inflammatory measures at ages 10-12 years. Combined, we anticipate 630 unique children will have 10-year adiposity measures and at least one early-life microbiome measure (~405 with 1 month and ~381 with 6 month stool samples, which includes ~300 children with paired 1 and 6 month samples). Of these children, 400 will also have gut microbiome measured at age 10-12 years. Our specific aims are to: (1) examine if mode of delivery is associated with childhood adiposity; (2) examine if the gut microbiome is associated with childhood adiposity; and (3) examine whether the gut microbiome mediates relationships between mode of delivery and measures of adiposity. Such a complementary “omics” approach has never been applied to the study of childhood obesity and is likely to provide critical insights into disease development in early-life as well as potential targets amenable for intervention.

Overall Principal Investigator: Christine Cole-Johnson, Ph.D. Project 3, Project 4 Principal Investigator: Ganesa Wegienka, Ph.D. Project 1 Pets and the Infant Microbiome: Effect on Immune Maturation & Atopic Asthma (NIH P01A1089473-01A1)

This Program Project Grant (PPG) study seeks an increased understanding of the relationships between dog or cat exposure during infancy and a lower risk of allergic asthma. We believe that this protective association is related to different patterns of microbial stimulation during immune development. Four synergistic Projects will examine our hypothesis that the presence of pets in a home results in a more diverse bacterial community composition (BCC) of the dust in the home which in turn influences the development of the gut BCC of a newborn infant living in the home. A more diverse gut BCC shifts the maturation of the infant's immune system such that later immune responses are less likely to produce IgE antibody responses and allergic asthma. Project 1 examines the relationships between the presence of a dog or cat in a home, the BCC of dust in the home, the BCC of stools of infants living in homes and allergic sensitization at 2 years of age. An innovative, culture-independent microarray (G3 PhyloChip) will be used to characterize the BCCs utilizing previously collected samples from the ongoing WHEALS birth cohort study. Changes in home BCC following introduction of a dog are also examined. Project 2 will recruit a new birth cohort of children either living with or without a dog, measure infant stool BCC, and follow the cohort with detailed studies of immune function until 18 months of age to determine the impact of dog exposure on immune maturation. Project 3 uses mouse models of allergic asthma to further examine the influence of house dust from homes with and without dogs on immune responses that result in lung inflammation. The use of mouse models allows more detailed studies of immune functions from multiple compartments of the body than are possible with human children. Project 4 again utilizes the existing WHEALS birth cohort to examine the relationships between dog or cat exposure during the first year of age, 6-month infant stool BCC, and the presence of allergic asthma at 9 years of age. The Projects are supported by five Cores which each provide essential services to all four Projects. Subcontracts to the University of Michigan, UCSF, and Georgia Regents University, as well as the summaries of the Cores are not described here.

Principal Investigator: Christine Cole-Johnson, Ph.D.
Project 3:

Allergic asthma is common in Westernized countries and its prevalence has increased greatly during the last century. Interestingly recent research has shown that exposure to pets during infancy correlates with a lower risk of developing allergy and asthma in later life. In addition, data generated by the PPG investigators have demonstrated that pets can be associated with alterations to the microbiota in house dust. It is well known that soil and dust are ingested by children, and alterations in gut microbiome have been associated with risk for allergic asthma development. In order to further our understanding of the mechanisms that lead to the alterations observed clinically, this project has established and utilized models of pulmonary disease with exposure to dust from homes with dogs versus no pets. Using two different mouse models of allergic asthma (cockroach antigen and ovalbumin), we will study pulmonary immune responses after oral exposure to house dust using samples collected from homes with dogs and without pets. We will test the overall hypothesis that the composition of the dust microbiome from homes with dogs versus no pets differentially alters pulmonary immune responses during allergen exposure by changing the GI microbiota or bacterial community composition (BCC), resulting in systemic changes in antigen presenting cell and bone marrow progenitor cell programming and allergic outcomes. To test this hypothesis, we will specifically focus on clinically relevant mechanisms involved in the ability of pulmonary-derived allergen responses to induce Th2 cytokines, mucus hypersecretion, physiologic changes (AHR), and changes in innate responses locally as well as systemically. Our studies will: 1) establish that exposure of mice to dust from homes with dogs versus no pets will result in differential changes in the GI BCC, 2) demonstrate that the dog dust-altered gut microbiome alters pulmonary allergic responses, 3) establish that the dog dust-induced changes alter dendritic cell responses in the lung, 4) identify that changes in innate responses are a systemic effect by examining bone marrow cell responses, and 5) utilize innovative mouse models to derive the cellular mechanisms of the altered responses. BCC will be measured using the G3 PhyloChip. Thus, these studies, in close collaboration with the other projects in this PPG, will clarify a number of previously unexplored questions and will guide exploration into this novel area of research.

Project 4:

The overall objective of Project 4 is to assess the relationship between exposure to pets, the infant home and gut microbiomes, and allergic asthma at 9 years of age, using an ethnically diverse, population-based general risk birth cohort (the WHEALS cohort). A premise with growing evidence is that lack of exposure to particular patterns of microbial stimuli during early infancy results in a heightened T-helper (Th) 2 response in the maturing immune system, likely due to a suboptimal regulatory capacity, which in turn is associated in childhood with increased immunoglobulin (Ig)E, allergy, and clinical allergic conditions such as asthma. Epidemiological studies have revealed that atopic conditions have increased over the latter half of the twentieth century. Humans, in earlier centuries had lifestyles associated with closer direct contact with soil, animals and other humans, suggesting exposure to environments with richer and more diverse microbial burdens. We hypothesize that evolutionary adaptation to such microbial exposures with respect to immune recognition and regulation may result in untoward consequences when humans are presented...
with the different, and probably more limited, patterns of microbial exposures found in modern Westernized societies. Our theory is that in many settings, pets, as well as farm animals in close proximity, render the home microbiome, or bacterial community composition (BCC,) to be more similar to early 20th century environments with respect to an increased bacterial richness, diversity and a more even distribution of taxa. This home microbiome impacts directly through effects on the infant gastrointestinal tract BCC the immunogenesis of the infant and subsequently the development of clinically important outcomes such as childhood atopic asthma. Using a new technology (the G3 PhyloChip), capable of cost-effectively identifying, to a great depth, bacteria in environmental and biological samples, our collaborative team has preliminary data suggesting that the presence of dogs and cats is associated with distinct home and infant gut microbiomes characterized by dramatic increases in bacterial diversity, richness and evenness. Using newly measured outcome variables measured by questionnaire and clinical examinations in the WHEALS cohort, in conjunction with PhyloChip analyses of stored infant stool and dust samples, we will test whether distinct patterns of pet exposure, home microbiome and infant gut microbiome are associated with current allergic asthma at age 9 years.

**Principal Investigator: Ganesa Wegienka, Ph.D.**

**Project 1**

The overarching hypothesis of Project 1 is that pet ownership is associated with exposure to a wider diversity of bacteria in house dust, and that these exposures profoundly influence the bacterial community composition (BCC) of the infant gastrointestinal microbiome, maturation of immune responsiveness and subsequently, the development of allergy and allergic asthma. This Project thus proposes two population-based studies and a longitudinal panel study to shed light upon mechanisms that may explain the observed protective effects of exposure to household pets during infancy against development of atopy and high total IgE in infancy. We propose to use an advanced, highly sensitive and semi-quantitative method for bacterial detection, the G3 PhyloChip. This method offers an unprecedented capacity for detailed, high-resolution profiling of complex microbial communities, detecting in parallel common and uncommon members of assemblages present in house dust and infant stool samples. For Aims 1 and 2, we propose to examine samples already collected and stored from a large, carefully characterized, racially and socio-economically diverse, cohort of children (the WHEALS cohort). In Aim 1 we propose using an innovative case-cohort design to compare samples from infants who became atopic at age 2 years versus samples from a randomly selected sub-cohort (serving as the control group). Using the sub-cohort in Aim 2, we will determine whether, and in what fashion, bacterial community composition of both house dust and infant stool are impacted by pet-keeping and if they are related to each other. The study of bacteria, or bacterial communities, identified as deriving from dog keeping will be enabled by a small prospective panel study proposed as Aim 3, to analyze the changes in microbial community composition of house dust in child-occupied but previously pet-free households into which a dog is introduced. Because the 16S-rRNA PhyloChip provides information on relative abundance of every bacterial taxon detected, we aim, through statistical analyses, to take advantage of this semi-quantitative data to identify particular bacterial species as critically important in protection against atopy development.

**Principal Investigator: Christine Cole-Johnson, Ph.D.**
Personalizing Care for Obese Patients in an Urban Health System
(Agency for Healthcare Research and Quality R24HS022417-01)

Today, patients and providers are presented with more health care options than ever before. There is considerable doubt and lack of understanding of science not only among patients, but also among physicians. Without systematic, evidence-based guidance for the appropriate and efficient use of the multitude of treatment options, as well as the consideration of patient preferences, the rapid growth and complexity of treatments will only add to the existing confusion about which option is best for each individual patient. The objective of the proposed Patient-Centered Outcome Research Center (PCORC) is to fully leverage and further develop the research infrastructure and clinical assets of Henry Ford Health System (HFHS) in order to conduct Patient-Centered Outcomes Research (PCOR). The PCOR evidence will guide care in order to achieve patient-desired outcomes in our urban and suburban patient populations located throughout Metropolitan Detroit. The Center will be organized into four different Cores (Patient Engagement Core, Study Design and Analysis/Measurement Core, Patient Data Network Core, and Implementation/Dissemination Core) with different functions. The Center currently has three PCOR projects proposed that are focused on our selected theme of caring for the obese patient. The HFHS PCORC will maximize programmatic and scientific efficiency within our health care system setting, promote shared use of resources and standardization of processes and procedures, promote training in PCOR/Comparative Effectiveness Research (CER) methods, and facilitate rapid dissemination of research findings to the medical community and translation of those results into our system's clinical practice.

The Center will build on existing research capabilities by developing expertise in novel PCOR methods through didactic course work, educational sessions at national meetings and content-specific educational seminars. These educational experiences will allow the Center staff to learn how to develop and conduct innovative PCOR studies that will provide evidence for the many pressing issues in patient care. The Center will demonstrate its proficiency through the conduct of the proposed projects.

The proposed PCORC is relevant to public health because it provide research infrastructure and methods for the design and conduct of patient-centered outcome research (PCOR) projects in patients within our health care system. The results of those projects will provide evidence-based and patient-centered guidelines available to physicians in their offices, on a real-time basis in order to ultimately provide care that places each individual patient and his/her preferences at the center of decision-making.

Principal Investigator: Albert Levin, Ph.D.
Comprehensive Analysis of Gene-Environment Interaction in Sarcoidosis
(NIH R21HL129023-01)

Sarcoidosis is a multi-organ granulomatous, inflammatory disease of uncertain etiology, with 90% of cases presenting pulmonary involvement. Despite convincing evidence that sarcoidosis likely arises when a genetically-susceptible host is exposed to a causative environmental agent, studies of environmental agents and disease risk suggest that there is more than one single environmental trigger. Further, there have been few gene-environment interaction (GxE) studies of disease etiology published to date; those few have focused on
the class II genes within the Human Leukocyte Antigen (HLA) region, the most studied and validated region associated with sarcoidosis risk. In the United States, African Americans are at highest risk for sarcoidosis; the adjusted annual incidence among African Americans is roughly three times that of European Americans (35.5/100,000 versus 10.9/100,000). Among African Americans, the disease is also more likely to be chronic and severe. We recently performed the first gene-environment-wide interaction study (GEWIS) of insecticide exposure and sarcoidosis risk in the Ancestry Mapping of Sarcoidosis Study (AMASS), a large study of sarcoidosis in African Americans. Using our recently-developed methodology, we identified a genome-wide significant GxE association in the FUT9 gene. To build on this effort, the current proposal will focus on the family-based portion of AMASS (503 families, including 818 sarcoidosis cases and 632 healthy family members), which has a rich resource of both environmental exposure and genetic (genome-wide genotyping, targeted/full exome sequencing, and imputed HLA Class I and II genes) data available. First, we propose to identify environmental exposures associated with sarcoidosis risk (Aim 1). Where existing sarcoidosis studies have been limited to investigating environmental exposures in isolation, we will move beyond this trend by using latent-class analysis (LCA) to identify patterns across multiple environmental exposures. Associated environmental exposures (individual and/or LCA-identified multi-exposure groups) will be evaluated for GxE effects (Aim 2). Current family-based GEWIS have focused on the exhaustive strategy of testing all genetic variants. To attempt to improve power, we will extend a two-step strategy to our study of related and admixed individuals and compare both exhaustive and two-step GEWIS strategies via simulation. The strategy with the highest power that also controls the type-1 error rate will be selected for GxE testing for the environmental factors from Aim 1. In summary, we propose multiple innovations to the fields of both GxE analysis and sarcoidosis research that increase the chance for better understanding of the etiology of this debilitating disease.

**Principal Investigator:** Mei Lu, Ph.D.

**Pragmatic Cluster Randomized Trial of an Asthma Intervention for Urban Teens (NIH R01HL114981-01)**

Clinical trials are critical for medical decision making, however, under the current paradigm, clinical trials are fraught with problems, including low enrollment of eligible patients, poor study compliance and high cost for the specialized infrastructure for study conduct. Most trials are of an explanatory nature (i.e., efficacy under ideal, highly controlled conditions) rather than pragmatic (evidence of effectiveness in a real clinical situation). Comparative effectiveness methods (CEM) seek to reduce the inefficiency and high cost of clinical trials while comparing effective interventions in a real world setting with decisions tailored to individual patient needs. CEM strategies, such as electronic initiatives, the use of centralized databases with remote data capture (RDC), and integration of the electronic medical record (EMR) with RDC for trial data collection, patient enrollment, and data management, are revolutionizing the conduct of clinical trials through cost reduction and improved efficiency. The objective of this proposal is 1) to implement a seamless clinical trial lifecycle using EMR initiatives and 2) to design and conduct a Phase II trial using EMR-RDC integration to evaluate an asthma management program for urban teens in a clinical setting. Urban clinics from Henry Ford Health System of Detroit and Kaiser Permanente of Atlanta will participate. Both are members of the HMO Research Network (HMORN) which has developed an innovative Virtual Data Warehouse (VDW) for common EMR data collection. The specific
Aims of this proposal are: Aim 1 Design a Phase II pragmatic cluster randomized trial of a web-based asthma management intervention in teens with asthma attending urban clinics, using adaptive approaches to plan a future Phase III trial. Aim 2: Use electronic initiatives for patient enrollment and management including EMR back-end and front-end for patient recruitment, online consent, and electronic monitoring of intervention compliance and follow-up. Aim 3: Establish and maintain a centralized Oracle Clinical infrastructure with RDC integration to EMR/VDW and electronic patient reported outcomes (ePROs). Our pragmatic approach with electronic initiatives will (a) minimize the need for new infrastructure for trial conduct; (b) maximize electronic retrieval of clinical endpoints (thereby minimizing the need for non-routine care clinic visits); (c) allow exploration of web-based patient assent/consent; (d) allow electronic monitoring of compliance to study regimen, and (e) maximize external validity. We will randomize 9 clinics with 250 teens for this Phase II trial (pilot study) to test feasibility, validity and cost of the trial. Using elements of the adaptive design for clinical trials, we will assess futility and feasibility of the proposed intervention. If no futility is observed, we will seek funding for a Phase III trial with more HMORN sites and a larger study population.

Public Health Relevance: Our multidisciplinary team, with extensive experience in clinical trial design, will conduct a pragmatic cluster randomized trial with an adaptive design. This PCRT will use state-of-art centralized infrastructure, including the integration of an EMR and EMR-RDC database, to evaluate a behavioral intervention in the outpatient clinic setting. This approach will significantly increase study recruitment and reduce study-associated costs.

Principal Investigator: Christine Neslund-Dudas, Ph.D.
Exploration of Cadmium as an Endocrine Disruptor in Prostate Cancer Disparities (NIH R21ES024379-01)

Tremendous race disparities exist in prostate cancer incidence and mortality. Reasons for these disparities remain unknown but are likely due to both environmental and biological factors. In vitro studies suggest that the heavy metal cadmium is an endocrine disruptor of the androgen receptor. The androgen receptor is essential for normal prostate growth and development but is also the primary drug target for treatment of prostate cancer as it controls a plethora of downstream targets involved in disease progression. Previous studies indicate that compared to European-Americans, African-Americans have had higher urinary and blood cadmium levels and, have higher androgen receptor protein levels and different patterns of AR-target expression in prostate tissue. Our preliminary data (N=59) suggests that African-Americans may have higher prostate tissue cadmium levels than European-Americans. Our data also suggests that cadmium is associated with androgen receptor protein expression in prostate tumor tissue but that race modifies the association. Therefore, race differences in the association between cadmium and the androgen receptor could lead to differences in expression/signaling of downstream androgen receptor targets involved in tumor aggressiveness and disease recurrence. In this R21 study, we propose to further investigate cadmium as an endocrine disruptor of the androgen receptor by capitalizing on a well-defined, ethnically diverse cohort of prostatectomy cases (N=415, 44% AA, diagnosed 1999-2004) that originally participated in the Gene-Environment Interaction in Prostate Cancer (GECAP) (R01 ES11126) study. In prostate tumor and adjacent non-tumor tissue,
we will measure cadmium as well as a panel of additional toxic and essential metals that can affect cadmium toxicity. We will also measure the whole-genome transcriptome in tumor tissue. The transcriptome includes all coding and non-coding RNAs transcriptionally expressed by genes in a population of cells. We will determine whether prostate tumor tissue cadmium level is associated with androgen receptor protein expression in the larger cohort and will determine if race does indeed modify the association between cadmium and androgen receptor expression. Further, we’ll determine whether prostate tumor tissue cadmium level is associated with prostate cancer aggressiveness or biochemical recurrence. In addition, we will examine the combined association of cadmium level and androgen receptor protein expression with regard to the prostate tumor transcriptome in African – and European- Americans. From our preliminary data and other existing reports, we hypothesize that race differences in the association of cadmium and the androgen receptor result in differences in expression of downstream androgen receptor targets that play a role in disease progression, and further, that these differences may in part explain race disparities in prostate cancer progression. If our hypothesis is confirmed, the GECAP data set contains an enormous resource of demographic, clinical and medical history, dietary, occupational and genetic information to investigate variables that may explain these findings.

**Principal Investigator: Benjamin Rybicki, Ph.D.**

**A Nested Case-Control Study of Prostate Carcinogenesis (NIH R01ES011126-11A1)**

Chronic inflammation, which is caused by infectious agents or exposure to environmental factors such as heterocyclic amines, is believed to play a role in up 20% of adult cancers. In prostate, genetic, molecular pathology, and toxicology data suggest that inflammation-related processes are involved in cancer development, but these data conflict with results of epidemiological studies that show an inverse correlation between inflammation and prostate cancer risk. This may be due to bias in the factors that lead men to undergo prostate biopsy, as well as complexity of the inflammatory phenotype itself. Our proposed study will address this paradox by dissecting inflammation at the cellular, molecular, and clinical level. The Henry Ford Health System biorepository contains benign prostate tissue specimens collected from over 9,000 men over the past 20 years, including over 1,000 men who subsequently developed prostate cancer. Using this unique cohort with its annotated clinical baseline and follow-up data, we will conduct a nested case-control study of 700 prostate cancer case-control pairs. Characterizing inflammatory markers in these pre-disease specimens will allow us to determine the nature of tumor-suppressive vs. tumor-supportive inflammatory signatures. We will also measure telomere length in the same benign prostate tissue specimens in which we characterize inflammation to assign a malignancy-potential signature to each specimen. Approximately 1 million prostate biopsies are performed annually in the US, two-thirds of which reveal benign condition. Our cohort includes a large group of patients who are at high risk of prostate cancer despite a negative biopsy. An in-depth characterization of inflammation in the benign prostate, before histologic signs of malignancy become apparent, will provide insight into the type of inflammatory milieu associated with eventual tumor development as well as cancer progression and recurrence. A better understanding of the clinical implications of chronic inflammation of the prostate so often observed in older men can have significant impact upon millions of men where currently a negative biopsy offers little reassurance in terms of prostate cancer outcomes.
Pediatric allergy and asthma are a costly public health burden, but so far substantial research efforts have yielded no prevention strategies. A likely reason is that despite longstanding recognition by the medical community that the term ‘asthma’ refers to a collection of diseases, researchers have historically treated the syndrome as a single disease entity. Epidemiologically, the collapse of different phenotypes (observed disease patterns) and endotypes (phenotypes further delineated by pathophysiological processes) into a single category corrupts associations between risk factors and diseases. Thus, progress in allergic disease research has been hampered. Prior attempts have been made to identify such phenotypes and endotypes, but a combination of incomplete data and oversimplified statistical methods have limited progress. We propose to apply sophisticated latent class analyses in a large general risk cohort combined with immunological markers to finely discriminate asthma and allergy disease phenotypes and endotypes and then use this information to conduct risk factor analyses. Using this approach in our WHEALS birth cohort, we have already characterized four classes at age 2 years: 1) Low to No Sensitization; 2) Highly Sensitized; 3) Milk and Egg Dominated Sensitization; and 4) Peanut and/or Inhalant allergen – No Milk Sensitization. Total IgE levels varied between the groups, as did the rates of eczema and doctor diagnosis of asthma (at age 4 years). The Highly Sensitized had the greatest rates, the Low to No Sensitization had the lowest rates, and the other two classes had rates intermediate between the Low and High Sensitization groups. These data suggest the use of latent classes, rather than the use of the “traditional” definition of atopy (any allergen-specific IgE (sIgE) ≥ 0.35 IU/mL), more specifically identifies those on a trajectory for allergic disease, yielding advancement in both allergic disease research and clinical care. Using the predominantly (62%) African American birth cohort WHEALS, we will: Aim 1) Determine which early life allergic disease phenotypes identified at age 2 years are associated with lung function (spirometry and methacholine challenge) at age 10 years; Aim 2) a) Identify the allergic disease endotypes for 10 year old children based on annual report of wheeze; lung function, eNO, obesity, cytokines, and white cell counts and extensive immunophenotyping [assessment of cellular markers to identify and quantify activation of regulatory T cells (Tregs), basophils and dendritic cells (DCs)] at age 10 years; and total IgE and sensitization (sIgE and skin prick tests) at ages 2 and 10 years; and, b) Estimate associations between early life risk factors (e.g., delivery type, pet exposure, etc.) and the identified Aim 2a endotypes; and, 3) Compare and contrast the risk factor associations with the endotypes in Aim 2 to the risk factor associations determined using “traditional” definitions of atopy and asthma (doctor diagnosis and medication use and/or symptoms in the last year). Analyses will be performed for all 900 WHEALS cohort children and separately for Black children and White children to assess racial differences.
Administration


Allergy and Immunology


Anesthesiology


Behavioral Health Services


Bioresources


Cardiology / Cardiovascular Research


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**Ophthalmology and Eye Care Services**


**Orthopaedics**


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