Harold S. Geneen Charitable Trust Awards Program for Coronary Heart Disease Research Awardees 2021-2025

"Targeting CaMKII O-GlcNAcylation in Heart Failure with Preserved Ejection Fraction"

Bence Hegyi, M.D., Ph.D. – 2025 Awardee
University of California, Davis

Coronary heart disease (CHD) is common in patients with heart failure (HF) with preserved ejection fraction (HFpEF) and remains the leading cause of death in the United States and globally. HFpEF affects >3 million Americans, severely deteriorates the quality of life, and has high mortality rates, while effective therapies are still very limited. HFpEF is a multi-organ disease associated with metabolic and hemodynamic impairments including diabetes mellitus (DM), obesity, and hypertension. Structural and functional changes in HFpEF, DM, and CHD predispose the heart to contractile deficit, particularly to impaired muscle relaxation (diastolic dysfunction) and rhythm irregularities (arrhythmias) that can be life-threatening.
Calcium/calmodulin-dependent protein kinase II (CaMKII) regulates multiple cellular processes, including excitation-contraction coupling and gene transcription. Upregulation of CaMKII has been associated with heart failure (HF), cardiac hypertrophy, and arrhythmias. CaMKII knockout (KO) and pharmacological CaMKII inhibition were cardioprotective following CHD and myocardial infarction, in HF with reduced ejection fraction, and in diabetic hyperglycemia. CaMKII has recently been implicated in the pathophysiology of HFpEF; however, the precise role of CaMKII in HFpEF remains unclear. Cardiac stress mediates key posttranslational modifications of CaMKII leading to sustained pathological activation. We recently demonstrated that diabetic hyperglycemia induces posttranslational modification of CaMKII by O-linked β-N-acetylglucosamine (O-GlcNAc) on Serine 280, inducing autonomous kinase activity and promoting diabetic cardiomyopathy and arrhythmias. This mechanism may also play a key role in diabetic HFpEF and represent a novel therapeutic target.

The aim of this proposal is to investigate the contribution of CaMKII to the HFpEF phenotype. The main hypothesis is that CaMKII is enhanced in HFpEF, inducing cardiac dysfunction, and that selective therapeutics targeting CaMKII O-GlcNAcylation in cardiac myocytes have important functional benefits. We will use integrative multi-scale and multi-parametric approaches, including molecular (transcriptomics, proteomics, FRET), cellular (electrophysiology, Ca imaging, cell contraction recording), and in vivo (echocardiography, ECG) functional analysis to test two translational aims.

Specific Aim 1. Define the precise molecular mechanism of pathological CaMKII activation and its role in HFpEF. We will test and compare CaMKII activation in two translational murine models of HFpEF, each exhibiting distinct disease pathophysiology: 1, high-fat diet combined with nitric oxide synthase inhibition, and 2, a new model that we developed and established using leptin receptor-deficient type 2 diabetic db/db mice with chronic aldosterone infusion.
Specific Aim 2. Test the molecular, cellular, and whole-heart functional benefits of targeting CaMKII O-GlcNAcylation in HFpEF using pharmacological agents (preclinical selective inhibitors for CaMKII and O-GlcNAc-transferase, and clinical SGLT2 inhibitor, empagliflozin, that attenuates hyperglycemia) and genetic manipulations (CaMKII knockout and site-specific knock-in mice we generated recently using CRISPR-Cas9).

This study will improve the fundamental mechanistic understanding of HFpEF pathophysiology and uncover the role of CaMKII activation via O-GlcNAcylation, a novel molecular signaling mechanism in diabetic HFpEF. This study will demonstrate the feasibility and translational potential of targeting CaMKII O-GlcNAcylation that could lead to more effective tailored therapies for patients with diabetic HFpEF.

Harold S. Geneen Charitable Trust Awards Program for Coronary Heart Disease Research Awardees 2021-2025

"Defining the Role of Circulating Bile Acids in Coronary Heart Disease"

Cholsoon Jang, Ph.D. – 2025 Awardee
University of California, Irvine

Coronary heart disease (CHD), triggered by elevated blood cholesterols and vascular inflammation, is the leading cause of death globally. While studies using rodents provided numerous insights into CHD mechanisms, human-like coronary atherosclerosis is not observed in the most common CHD models, Apoprotein E (ApoE) or low-density lipoprotein receptor (LDLR) knockout mice. Therefore, investigators developed LDLR knockout minipigs, which develop coronary atherosclerosis and resemble humans regarding diet, metabolic rate, day-night cycle, organ systems, biochemistry, and pathophysiology. Using unbiased mass spectrometry-based metabolomics in LDLR knockout minipigs, we found strikingly elevated blood bile acids (BAs). Consistently, elevated BA levels are observed in CHD patients and correlated with coronary atherosclerosis, suggesting the causal relationship between BAs and CHD. Strikingly, our metabolomics analysis of systemic arterial blood versus 10 organ-specific venous blood in LDLR knockout pigs revealed that many organs including kidneys, heart, intestine and muscle release BAs. RNA-sequencing also unveiled abnormal overexpression of BA-synthesizing enzymes in these organs. This was surprising because the current BA paradigm indicates the liver as the sole BA-producing organ. Given that BAs have detergent-like toxicity and are normally absent in systemic blood, elevated systemic blood BAs can exert toxicity on vascular endothelial cells, the cell type constantly exposed to them. We thus hypothesize that elevated blood BA induces vascular inflammation, a well-known CHD risk factor, thereby contributing to coronary atherosclerosis. If so, reducing BAs would be a novel therapeutic strategy for mitigating CHD.

In Aim 1, we will determine the causal relationship between BAs and coronary atherosclerosis. Compared to human or pig BAs, mouse BAs are much less toxic due to higher solubility in blood. Thus, we will determine whether human BAs trigger vascular inflammation and coronary atherosclerosis in mice. First, we will infuse human BAs into jugular-vein catheterized ApoE or LDLR knockout mice for several days and weeks to mimic patients with chronically elevated blood BAs. Then, we will measure vascular inflammation and coronary atherosclerosis using authentic staining methods. Our pilot infusion study in wild-type catheterized mice that we surgically generated demonstrated feasibility. These studies will determine the causal role of elevated blood BAs in coronary atherosclerosis.

In Aim 2, we will explore whether BA normalization can mitigate CHD via suppressing vascular inflammation. We found that fibroblast growth factor 19 (FGF19), a hormone that normally suppresses BA synthesis, is dramatically depleted in LDLR knockout pigs and human CHD patients. Moreover, FGF19 and BA levels are inversely correlated, suggesting that decreased FGF19 is the underlying cause of the BA elevations in LDLR knockout pigs and CHD patients. If so, restoring FGF19 levels can reverse BA abnormalities and reduce BA's toxic effects and vascular inflammation. Thus, we will provide LDLR knockout pigs with recombinant FGF19 proteins and measure organs' BA release, blood BA levels, vascular inflammation, and coronary atherosclerosis.

These studies, which combine multiple advanced technologies in highly relevant CHD pig and mouse models, will elucidate the undefined mechanism of circulating BAs and FGF19 in CHD pathogenesis.

Harold S. Geneen Charitable Trust Awards Program for Coronary Heart Disease Research Awardees 2021-2025

"CD40: Gatekeeper of B1 Cell Homeostasis in Atherosclerosis"

Esther Lutgens, M.D., Ph.D. – 2025 Awardee
Mayo Clinic

Targeting immune responses in atherosclerotic cardiovascular disease (CVD), especially in coronary heart disease, has been proven successful in clinical trials. To pursue immunotherapeutic solutions in CVD, insights into cell-specific immune responses in atherosclerosis are urgently needed. The co-stimulatory CD40L-CD40 dyad is a master regulator of immune responses and atherosclerosis. CD40L-CD40 interactions were originally described to allow cognate interactions between T cells and antigen presenting cells, but it has become clear that CD40L and CD40 are expressed on a plethora of cell-types and subsets.
We have identified an important, cell divergent role for the co-stimulatory CD40L-CD40 dyad in modulating atherosclerosis. Whereas deficiency of T-cell CD40L decreases atherosclerosis and induces plaque stability, deficiency of platelet CD40L only affects atherothrombosis. Deficiency of macrophage CD40 reduces atherosclerosis by enhancing efferocytosis and reducing necrotic core content, while deficiency of dendritic cell CD40 impairs Th1 responses and induces an increase in plaque collagen content. Understanding the cell-type specific actions and signaling pathways of CD40L and CD40 is paramount because CD40(L) expressing cell types and cell-type specific signaling intermediates can be targeted specifically to combat atherosclerosis and limit unwanted side effects. This strategy leaves CD40L-CD40 interactions required for proper immunity intact.
CD40 is highly expressed on B cells, that, depending on their subset, can either drive or inhibit atherosclerosis. Our preliminary data show that depletion of CD40 on B cells increases atherosclerosis, which we found to be caused by a reduction in B1 cells, the innate type B cell that produces atheroprotective anti-oxidation specific epitope (OSE) IgMs. CD40 deficient B1 cells have an impaired cellular metabolism, thereby hampering adequate anti-OSE IgM responses. Transfer of CD40-competent B1 cells in B-cell CD40-deficient atherosclerotic mice restores anti-OSE IgM levels and prevents the increase in atherosclerosis.

This application's central hypothesis is that B1 cell CD40 protects against atherosclerosis, by maintaining B1 cell metabolic homeostasis during hyperlipidemia, thereby warranting adequate atheroprotective anti-OSE IgM responses. Understanding the role of CD40 in maintaining B1 cell homeostasis in atherosclerosis will reveal the mechanisms how CD40 ensures the production of athero-protective anti-OSE IgM antibodies. To test this hypothesis, we will pursue the following Specific Aims:

Aim 1. Define the role of B1 cell CD40 in different stages of atherosclerosis. We will study the effects of deficiency of B1 cell CD40 in early, intermediate, and advanced stages of atherosclerosis in our CD19-CD40flflApoE-/- mice. We will analyze plaque burden, plaque phenotype, antibody titers and the composition and activation status of the immune system in the aorta, lymphoid organs and blood using flow/mass cytometry and scRNAseq/CITEseq approaches.
Aim 2. Delineate the mechanisms and functions of CD40-associated B1 cell signaling in atherosclerosis. Our preliminary results indicate that CD40 maintains cellular and metabolic homeostasis of B1 cells. We will delineate how B1 cell CD40-signaling is initiated and via which pathways B1 cell CD40-mediated anti-OSE-IgM production is warranted.

This project will provide insights in how CD40 mediates anti-OSE-IgM antibodies by B1 cells, thereby providing future therapeutic targets aimed at promoting anti-OSE-IgM production to combat atherosclerotic CVD, including coronary heart disease.

Harold S. Geneen Charitable Trust Awards Program for Coronary Heart Disease Research Awardees 2021-2025

"Targeting Acetaldehyde Dehydrogenase 2 in Smoking-induced Coronary Artery Disease"

Hongchao Guo, Ph.D. – 2024 Awardee
University of Utah

Coronary artery disease (CAD) causes approximately one of six (~9 million) annual deaths in the United States. Moreover, due to an aging population and still limited innovative prevention and treatment strategies, the global prevalence of CAD is rising. Because CAD is a complex pathophysiological process that involves both genetic and environmental interactions, precision medicine represents a promising approach to prevention and treatment for CAD patients with genetic variants. This knowledge gap is to understand genetic variants' physiological impact and causal relevance.

The common aldehyde dehydrogenase 2 (ALDH2) alcohol flushing variant known as ALDH2*2 affects ~8% of the world's population. Even in heterozygous carriers, this missense variant leads to a severe loss of ALDH2 enzymatic activity and has been linked to an increased risk of CAD. Endothelial cell (EC) dysfunction plays a determining role in all stages of CAD pathogenesis, from initiation to atherothrombotic complication. However, the contribution of ALDH2*2 to EC dysfunction and its relation to CAD are not fully understood. In addition, ALDH2 is vital in detoxifying toxic aldehydes derived from cigarette smoking. Given that smoking accounts for 33% of all deaths from CAD in the United States, it is important to elucidate the contribution of ALDH2*2 in smoking-induced CAD.

With complementary advances in patient-derived induced pluripotent stem cells (iPSCs), my existing studies revealed that ALDH2*2 induces EC dysfunction (Guo et al., Science Translational Medicine 2023). Our preliminary results further found that EC dysfunction is further worsened by smoke exposure. Transcriptional profiling revealed the expression of a transcriptional factor KLF5 is significantly increased in ALDH2*2 iPSC-ECs when treated with cigarette smoke extract. KLF5 has been shown to mediate endothelial angiogenic dysfunction in diabetic endothelial cells. For this proposal, We hypothesize that KLF5 mediates smoke and ALDH2*2-induced EC dysfunction. The aims of this proposal are to define the mechanism of KLF5 in ALDH2*2 and smoking-induced EC dysfunction, and to examine the beneficial effect of KLF5 inhibitor on smoking-induced vascular dysfunction in ALDH2 deficiency mice. Completion of this study will inform whether tobacco consumption should be considered with caution in ALDH2*2 carriers and describe the therapeutic role of KLF5 inhibitor in potentially mitigating risk for CAD in this population.

Harold S. Geneen Charitable Trust Awards Program for Coronary Heart Disease Research Awardees 2021-2025

"Defining the Role of ER Stress Proteins in Calcium-Dependent Arrhythmias"

Shanna Hamilton, Ph.D. – 2024 Awardee
University of Arizona College of Medicine - Tucson

Coronary heart disease (CHD) causes 370,000 US deaths annually and is the most common cardiovascular disease (CVD). Many patients with myocardial infarct (MI) associated with CHD die suddenly from arrhythmia caused by irregular, ectopic activity that can accumulate and lead to lethal ventricular arrhythmias. Abnormal calcium (Ca^{2+}) handling gives rise to arrhythmogenesis and impaired contraction in many CVD settings, including MI, heart failure (HF), and catecholaminergic polymorphic ventricular tachycardia (CPVT). While implantable cardioverter-defibrillators can prevent sudden cardiac death in patients with arrhythmias, they do not offer absolute protection, and repeated shocks are associated with increased mortality. It is imperative to find new treatments targeting irregular cardiac Ca^{2+} homeostasis to improve patient outcomes across various CVDs.
The sarcoplasmic/endoplasmic reticulum (SR/ER) stress response is triggered in cardiomyocytes when intra-organelle homeostasis, including Ca^{2+} handling, is disturbed. Growing evidence suggests ER stress is implicated in CVDs associated with Ca^{2+}-dependent arrhythmias. Although strategies to reduce ER stress have shown cardioprotective effects, these have not translated to the clinic. Resolving molecular mechanisms linking ER stress proteins with disturbed Ca^{2+} homeostasis will aid in therapeutic design to reduce arrhythmias in diseases associated with CHD and inherited arrhythmia syndromes.

I recently demonstrated in acquired CVD, ER stress drives the upregulation of ERO1alpha, an enzyme that facilities protein folding. Concomitantly, ERO1alpha increases reactive oxygen species (ROS) that can perturb Ca^{2+} transporter activity by redox posttranslational modifications. Peroxiredoxin-4 (PRDX4) enzyme is thought to serve as an antioxidant to degrade ERO1alpha-induced ROS, and recent reports have suggested PRDX4 knockdown increases oxidative stress and contribute to HF. However, the contribution of ER stress and the ERO1alpha-PRDX4 axis to Ca^{2+}-dependent arrhythmias is not yet defined.

As the major SR Ca^{2+} release channel, hyperactivity of the ryanodine receptor (RyR2) is central to Ca^{2+}-dependent arrhythmogenesis in multiple CVDs. Hereditary gain-of-function RyR2 mutations are linked to the malignant syndrome CPVT. We and others have shown RyR2 gain-of-function not only results in perturbed Ca^{2+} release but can lead to secondary changes in cell physiology and signaling that markedly contribute to the arrhythmic phenotype. This remodeling includes increasing ROS from the mitochondria. As RyR2 is sensitive to oxidative stress, this can further increase channel activity in a vicious feedback cycle. Whether RyR2 gain-of-function drives ER stress remodeling and a similar feedback cycle remain unexplored.
The overarching goal of this proposal is to define the contribution of ER stress evoked by Ca^{2+} mishandling to cardiac arrhythmogenesis.

Specific Aim 1: We will define the contribution of ERO1alpha and PRDX4 to RyR2 hyperactivity in arrhythmia induced by RyR2 gain-of-function using a newly created, highly arrhythmogenic rat model of CPVT.
Specific Aim 2: We will genetically target these proteins using adeno-associated viral vectors as a novel therapy to reduce RyR2 activity in both CPVT and acquired disease associated with CHD.
This study will demonstrate the feasibility of targeting ER stress as an alternative approach to attenuating RyR2 dysfunction, with translational potential to many CVDs linked to CHD and Ca^{2+}-dependent arrhythmias.

Harold S. Geneen Charitable Trust Awards Program for Coronary Heart Disease Research Awardees 2021-2025

"Promoting resolution of atherosclerosis via caloric restriction-induced macrophages"

Ada Weinstock, Ph.D. – 2024 Awardee
University of Chicago

Obesity causes chronic systemic inflammation that is the main driver of its myriad comorbidities, such as cardiovascular diseases. Weight loss leads to resolution of obesity-related inflammation and reduces cardiovascular complications, with reasons largely unknown. Supporting this, we recently showed that moderate caloric restriction (CR)-induced weight loss in obese mice causes atherosclerosis resolution.

Macrophages play a critical role in atherosclerosis resolution, most importantly by clearing dying cells in a specialized phagocytic process termed 'efferocytosis', thus diminishing necrotic cores (the most unstable and vulnerable plaque areas, rich in dead cells and degraded matrix). Mechanistically, we recently demonstrated that CR promotes the accumulation of a new macrophage subtype in atherosclerotic plaques and adipose tissue, distinguished by high expression of the IgG receptor Fcgr4 (protein name CD16a). Importantly, these macrophages were key to atherosclerosis resolution, possibly due to their superior efferocytotic capability, which reduced plaque necrotic cores and inflammation. This suggests that CR-macrophages induction is an attractive possible therapy for atherosclerosis in obesity. The central hypothesis in this proposal, strongly supported by extensive preliminary data, is that CR-macrophages are integral for weight loss-induced atherosclerosis resolution. We proposed two specific aims to elucidate underlying molecular mechanisms contributing to CR-macrophage-mediated atherosclerosis resolution and explore their therapeutic potential.

In Aim 1 we will test the hypothesis that CR-macrophages are sufficient to resolve atherosclerosis in obese mice. For this, we have developed a new in vitro model to recapitulate the CR-macrophage phenotype from bone marrow-derived macrophages. In this aim we will leverage this system to better understand CR-macrophage function and explore them as therapy. At the completion of this aim, we will elucidate novel immunological functions of CR-macrophages and identify molecular pathways by which they facilitate atherosclerosis resolution.

In Aim 2 we will test the hypothesis that elevated CD16a levels in macrophages promote atherosclerosis resolution. Using nanomedicine (created in collaboration with Dr. Yun Fang at UChicago) to increase CD16a levels in vitro and in vivo, macrophage functions will be investigated. For this, we have engineered novel nanoparticles that are effectively taken up by macrophages and successfully deliver functional Fcgr3a mRNAs, the human homologue of Fcgr4. These nanoparticles will be used to promote the expression of CD16a in atherosclerosis bearing mice, to investigate whether overexpression will alleviate established disease. At the completion of this aim, we will identify the specific contribution of CD16a in macrophages to atherosclerosis resolution.

Impact: Our proposed studies will unearth novel immunological mechanisms underlying atherosclerosis resolution induced by CR. These findings can be leveraged to develop interventions to induce CR-Mø appearance, potentially reversing atherosclerosis, establishing a new paradigm in disease management.

Harold S. Geneen Charitable Trust Awards Program for Coronary Heart Disease Research Awardees 2021-2025

"Improved Cardiac Stem Cell Therapy for Coronary Heart Disease"

Phung Thai, Ph.D. – 2023 Awardee
University of California, Davis

Coronary heart disease (CHD), the most common type of cardiac disease, remains the leading cause of morbidity and mortality. After myocardial infarction (MI) from coronary artery disease, significant loss of cardiomyocytes can result in heart failure with lethal consequences. Heart failure is associated with a very high mortality rate with a five-year mortality of 50%. Stem cell-based therapy represents a promising therapeutic avenue for the treatment of end-stage heart failure. However, the primary challenge of cardiac stem-cell based therapy is the survival and retention of transplanted stem cells due to the hostile milieu of the host environment. Due to the robust inflammatory response mediators, approximately 90% of transplanted cells are lost within just the first few days, severely limiting cardiac regenerative potential. The amplification of inflammatory responses is mediated by NLRP3 (NACHT, leucine-rich containing family, pyrin domain-containing-3) inflammasomes, leading to inflammation-induced cell death, or pyrpotosis. The objective of this study is to determine the critical role of NLRP3 inflammasome activation in human- induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) transplantation. A proof-of-concept study will be performed using CRISPR/Cas9 gene edited-hiPSC-CMs, to enhance stem cell survival and retention, leading to the enhancement of cardiac function post transplantation in a rabbit MI model.

The central hypothesis: to be tested is that CRISPR/Cas9-mediated gene silencing in hiPSC-CMs would increase hiPSC-CM engraftment and survival, resulting in improvements in cardiac function in a rabbit MI model. The hypothesis is firmly grounded on our recent published study demonstrating the critical roles of inflammation in cardiac stem cell loss after transplantation using small molecule inhibitors. Two specific aims are proposed to test the hypothesis.

Specific Aim 1: Determine the mechanistic role of the NLRP3 inflammasome in hiPSC-CMs.
Specific Aim 2: Determine the therapeutic potential of gene-edited hiPSC-CMs in a rabbit MI model.

Translational and clinical impact: By utilizing CRISPR/Cas9 gene editing technology in hiPSC-CMs, this project will be the first to determine the critical roles of inflammasomes in cardiac cell-based therapy. Additionally, it will determine the therapeutic potential of CRISPR/Cas9 gene silencing in hiPSC-CM transplantation in a clinically relevant rabbit MI model. Successful completion will pave the way for future studies readily translatable to the clinic for precision medicine, since CRISPR/Cas9 gene-edited hiPSC-CMs are highly scalable, using patient-specific stem cells that can be delivered via intracardiac route with catheters, to generate new functional myocardium and neoangiogenesis. The proposed study has broad therapeutic ramifications. Embedded in these findings are essential paradigm shifts for the improvement of cardiac stem cell-based therapy that may be exploited for the treatment of end-stage HF.

Harold S. Geneen Charitable Trust Awards Program for Coronary Heart Disease Research Awardees 2021-2025

"Mitochondrial Protein Quality Control and Calcium Signaling in Myocardial Ischemia-Reperfusion Injury"

Dhanendra Tomar, Ph.D. – 2023 Awardee
Wake Forest University Health Sciences

The root cause of heart failure and cardiac diseases is abnormal metabolism and death in cardiomyocytes. Cardiomyocytes rely on mitochondria for energy needs. Abnormal mitochondria induce death and are linked with many heart diseases. In a failing heart, mitochondrial abnormalities are usually characterized by anomalous calcium flux. Dysregulated mitochondrial calcium signaling is known to elicit multiple cardiac stress conditions, including bioenergetic crisis, reactive oxygen species generation, mitochondrial permeability transition pore opening, leakage of apoptogens, and loss of mitochondrial membrane potential, all of which can lead to metabolic failure and the loss of myocytes. Therefore, defining the molecular mechanisms underlying the mitochondrial stress response may offer novel treatment strategies for cardiovascular diseases (CVD), heart failure (HF), and numerous other metabolic failure-driven disorders. The primary route for calcium entry into the mitochondrial matrix is through the mitochondrial calcium uniporter channel (mtCU). The mtCU activity is tightly controlled by the gatekeeping regulator mitochondrial calcium uptake protein 1 (MICU1), which controls the mtCU's open probability. The MICU1 protein has a very short half-life compared to other mtCU components, suggesting that a very specific protein quality control system is present for this protein. Besides that, loss of MICU1 is associated with numerous chronic disease states and contributes to mitochondrial calcium overload that is associated with cellular bioenergetic failure and onset of cell death. The proposed research aims to delineate the regulatory mechanisms for MICU1 protein turnover and how it determines the cardiomyocyte's fate in response to cardiac ischemia-reperfusion injury.

Harold S. Geneen Charitable Trust Awards Program for Coronary Heart Disease Research Awardees 2021-2025

"Deciphering Function of GATA4 uORF in Myocardial Infarction"

Peng Yao, Ph.D. – 2023 Awardee
University of Rochester

Short peptide-encoding sequences in the 5' untranslated region (5'UTR) of messenger ribonucleic acids (mRNA), called upstream open reading frames (uORFs), are widespread in ~50% of human mRNAs. Translating these uORFs reduces the protein output of an mRNA main open reading frame (mORF). Our bioinformatic analysis of human and mouse ribosome profiling databases uncovered a group of cardiac mRNAs containing translated uORFs, such as transcription factor (TF), including GATA4. Biochemical analyses suggest that stabilizing a double-stranded RNA (dsRNA) structure downstream of the start codon of uORFs enhances their translation, thereby inhibiting mORF translation. This mechanism is mitigated by DEAD-box RNA helicase DDX3X that unwinds dsRNA and inactivates uORF and promotes mORF translation. uORF-inactivating start codon knock-in (KI) mice show mild spontaneous cardiomyocyte (CM) hypertrophy and will be used to characterize cardiac phenotype under myocardial infarction (MI). Based on this new "see-saw" mechanism of DDX3X-regulated, dsRNA-dependent, uORF-mediated translational inhibition of mORF, we have developed antisense oligonucleotides (ASOs) that can reduce uORF translation by disrupting dsRNA structures. The uORF-inhibitory ASO unwinds the dsRNA structure and inhibits uORF translation, thereby enhancing GATA4 mORF protein expression. Human AC16 CMs treated with this ASO exhibit increased GATA4 protein translation and promoted CM hypertrophy. As GATA4 is known to antagonize CM apoptosis, promote angiogenesis, and inhibit cardiac fibrosis, we propose to treat an MI model with uORF-inhibitory ASO to enhance GATA4 protein translation, promote CM survival, and improve cardiac function. Our central hypothesis is that genetic or ASO targeting inactivates GATA4 uORF, promotes GATA4 mORF translation, and improves ischemia heart disease.

We have two specific aims:
Aim 1. Characterize cardiac phenotypes of GATA4 ΔuORF knock-in mice under MI.
Aim 2. Determine cardioprotective effects of a uORF-inactivating ASO under MI.

My lab is among few groups studying translational control mechanisms in cardiac biology and disease. We aim to understand the biological role of uORF translation and their regulation and identify ways to manipulate their activity to determine the biological impact and therapeutical potential. These studies will provide novel insights into translational control mechanisms in CMs, underscore the impact of uORF in cardiac biology, and develop novel RNA-based therapeutics to treat MI. This novel type of translation-manipulating ASOs can be designed to target additional mRNAs for treating other human diseases by either enhancing protective protein production or reducing pathogenic protein expression. This project's broad, long-term objective is to serve as a proof-of-concept model to establish a platform technology for future therapeutic development.

Harold S. Geneen Charitable Trust Awards Program for Coronary Heart Disease Research Awardees 2021-2025

"Mechanisms of Coronary Microvascular Disease in Atrial Fibrillation"

Cevher Ozcan, M.D. – 2022 Awardee
University of Chicago

Coronary microvascular disease (CMD) is a new frontier in cardiovascular disease and contributes substantially to ischemic heart disease. It is associated with an increased risk of morbidity and mortality. Recently we found that CMD is highly prevalent in patients with atrial fibrillation (AF). Also, our examination of human heart autopsy samples showed CMD in patients with COVID-19, which was more common with concomitant COVID-19 and AF. Yet, the molecular mechanism of the cause-and-effect relationship between CMD and AF is unknown. AF is the most common sustained arrhythmia with a high risk of all-cause mortality. Both AF and CMD have major implications for cardiovascular health, and therefore understanding of the molecular mechanisms of CMD in AF is critical for developing an effective preventive strategy and the risk stratification.

The proposed project aims to determine 1) pathogenesis of the cause-and-effect relationship between CMD and AF, 2) role of mitochondrial complexes-mediated oxidative and metabolic stress in molecular mechanisms of CMD in AF, and 3) novel therapies and markers to prevent CMD in AF by targeting mitochondrial reactive oxygen species (mROS) and complex II (succinate dehydrogenase; SDH). SDH is essential in metabolism and mROS generation. We propose that excessive mROS generation cause CMD in AF by triggering structural-functional microvascular remodeling (SFMR). Our central hypothesis is that SDH-mediated mROS and associated energy-metabolic dysregulation is a molecular mechanism of CMD in AF through SFMR. Therefore, the SDH/mROS axis can serve as a novel therapeutic target for the prevention of CMD in AF, and thereby provide relief to COVID-19 patients. We will determine the role of mROS/SDH axis is genesis of SFMR. Dysregulated proteins in plasma and myocardial tissue during the initiation and progression of SFMR/CMD in AF will be discovered as potential biomarkers. Moreover, we will test the efficacy of mROS/SDH modulators in prevention of SFMR/CMD in AF.

Our preliminary studies showed significant SFMR with abnormal coronary flow reserve, perivascular inflammation and fibrosis, reduced microvascular density, and heterogeneous vascular distribution in myocardium with AF. These changes were associated with mitochondrial dysfunction, metabolic dysregulation, energy deficit, increased mROS and inflammation linked to SDH/ROS homeostasis. Proteomics showed dysregulated proteins in association with inflammation, metabolic/oxidative stress and matrix remodeling.

Our specific aims are to determine 1) whether AF causes CMD through SFMR, 2) the mitochondrial mechanisms of CMD in AF, and 3) the therapeutic efficacy of SDH/mROS modulators in prevention CMD in AF. We will probe mitochondrial complexes with particular focus on SDH and mROS in molecular mechanisms of CMD in AF. Functional relationship and downstream pathways of SDH/mROS will examined.

Our hypothesis will be tested in mouse and swine models of AF. We will use an integrative multi-parametric approach including proteomics, molecular analysis, histopathology and coronary/electrophysiology studies. Novel biomarkers and an in-depth understanding of in CMD disease processes will be discovered. Furthermore, mROS/SDH modulators will tested in prevention of CMD as an innovative treatment strategy. This proposal has significant clinical implication for risk stratification and management of patients with CMD in AF and/or COVID-19 with cardiac involvement.

Harold S. Geneen Charitable Trust Awards Program for Coronary Heart Disease Research Awardees 2021-2025

"Targeting Inflammation and Exercise in Arrhythmogenic Cardiomyopathy: A Novel Therapeutic Strategy for Heart failure"

Joseph Palatinus, M.D., Ph.D. – 2022 Awardee
University of Utah

Heart Failure is a leading cause of hospitalization and death in the United States and other developed countries with over 6 million cases reported in 2019. Despite state-of-the-art care for heart failure 50% of patients die within 5 years of diagnosis highlighting the urgent need for new therapeutic approaches. There is growing evidence that inflammation plays a key role in the pathogenesis of heart failure.

Arrhythmogenic cardiomyopathy (ACM) is a genetic cardiac disease that results in heart failure, arrhythmias, and sudden cardiac death and is the most common cause of sudden death in young athletes. Patients with this disease are born with normal hearts but at adolescence develop reduced heart function and fibrofatty replacement of heart muscle with fat and scar. The disease is exacerbated with exercise and patients are advised to avoid intense exercise. There are no treatments for this disease other than heart transplant.

We have discovered that an exercise responsive proinflammatory transcription factor, nuclear factor Kappa (NFkB) is upregulated in this disease model. Furthermore, our preliminary data indicate that osteopontin, (a potent inflammatory cytokine) levels are increased in this disease and correlate significantly with the degree of cardiac impairment observed in a mouse model of ACM.
Why exercise increases sudden death in ACM and how to therapeutically target inflammation in heart failure are areas of great interest in the field and answering these questions will yield novel therapeutic avenues.

We have extensive experience in our lab using adeno-associated viral vectors for gene therapy in the heart and will leverage this experience to address this gap in understanding. We hypothesize that exercise induced NFkB activation in cardiomyocytes and subsequent increased inflammation directly contributes to the reduced systolic function and dysrhythmias observed in ACM. The aims of this grant are to 1) Determine how NFkb regulates inflammation in the DSG2 mutant model of ACM in the setting of both forced and voluntary exercise. 2) Determine if Adeno-Associated virus (AAV) mediated overexpression of the NFkB inhibitor A20 reduces inflammation and prevents arrhythmias and sudden cardiac death in the DSG2 model of ACM. Successful completion of this project will define a new paradigm by which cardiomyocyte-origin inflammatory mediators are central to the pathogenesis of ACM. The results will introduce a novel anti-inflammatory-based therapy for ACM and heart failure in general.

Harold S. Geneen Charitable Trust Awards Program for Coronary Heart Disease Research Awardees 2021-2025

"Harnessing the angiogenic potential of the fetal epicardium to treat ischemic heart disease"

Eric Small, Ph.D. – 2022 Awardee
University of Rochester

Coronary artery disease (CAD) disrupts the vascular network that supplies the heart with oxygen and nutrients, and is a major risk factor for myocardial infarction (MI), an obstruction in a coronary artery that damages cardiac muscle. The heart has a surprising capacity to heal and support life even after catastrophic injury such as an MI. However, collateral vessels that bypass a blockage in a major artery are not sufficient to support meaningful repair. Therefore, MI generally leads to scar formation and the loss of cardiac function, often progressing to heart failure (HF), the most prevalent cause of death in the U.S.

Remarkably, the newborn heart heals completely and without a scar, in a process called regeneration. Heart regeneration requires the rapid growth of coronary blood vessels via angiogenesis to support the newly forming heart muscle. Based on our preliminary data and published reports, we hypothesize that developmental and regenerative coronary angiogenesis is guided by the epicardium, a single cell-layer of multi-potent cardiac progenitor cells on the surface of the heart. Using single cell RNA-sequencing of non-myocytes at key developmental timepoints, we recently identified a population of epicardium-derived cells we call "vascular guidepost cells". Vascular guidepost cells express an important cocktail of angiogenic chemokines that are important for coronary artery growth and patterning; however, these angiogenic factors are absent in the adult heart, and not induced after an MI. Therefore, the adult heart is not a conducive environment for angiogenesis or collateral artery formation. The overall goal of this proposal is to develop a gene therapy strategy to facilitate coronary angiogenesis and improve functional recovery after an MI. We will use viral vectors and cutting-edge mRNA-based therapeutics to deliver candidate angiogenic growth factors to the heart in an experimental MI model in mice. We will identify individual factors, or combinations of factors, that stimulate new vessel growth, reduce scarring, and improve cardiac function. We will also use single cell RNA-sequencing to establish how epicardium-derived factors stimulate blood vessel growth in order to improve future heart regeneration strategies.

In summary, this study will harness fetal epicardium-derived angiogenic factors to stimulate coronary artery formation after MI. Importantly, the development of mRNA and viral-based gene therapy approaches that improve coronary artery growth may be broadly applicable to regenerative medicine strategies and the treatment of numerous diseases associated with vascular obstructions, such as stroke and peripheral artery disease.

Harold S. Geneen Charitable Trust Awards Program for Coronary Heart Disease Research Awardees 2021-2025

"Targeting SARS-CoV-2 Viral RNA with CRISPR-Cas13 in ACE2-expressing Cell Types using CoV Spike-pseudotyped Lentiviral Vectors"

Douglas Anderson, Ph.D. – 2021 Awardee
University of Rochester

Infectious viruses are among the greatest threats to human health. While vaccines remain an important and standard approach, numerous factors limit their efficacy, including: 1) immune non-responders, 2) time required to design and test vaccine candidates and 3) ever mutating viral strains. To date, only a handful of vaccines have been successfully developed, relative to the vast number of infectious viruses, highlighting a dire need for alternative, platform-based therapeutics to combat infectious disease.

In response to the current escalating pandemic caused by COVID-19, we re-programmed our CRISPR-Cas13 RNA cleavage technologies to target conserved and essential SARS-CoV-2 viral RNA sequences. In proof-of-principle cell-based experiments, we have identified functional guide-RNAs which result in 90% knockdown of a SARS-CoV-2 reporter gene. These preliminary data suggest that directly targeting viral RNA may offer a robust strategy to clear virus from infected cells, to limit disease severity and spread. However, major challenges remain to enable CRISPR-Cas13 to be utilized a therapeutic, including 1) ensuring that viral mutations are not able to 'escape' RNA targeted therapeutic approaches and 2) the safe and effective delivery of CRISPR-Cas13 components to virus-infected cell types. In this regard, SARS-CoV-2 presents a unique challenge, as 1) numerous viral mutations have been identified across the globe and 2) the SARS-CoV-2 Spike protein allows entry into diverse cell types present in respiratory, renal, and cardiovascular tissues. To address these challenges, we have developed strategies to 1) utilize a CRISPR guide-RNA array to target multiple viral RNA sequences at once and 2) deliver CRISPR-Cas13 components in safe, integration-deficient lentiviral vectors which can be pseudotyped with SARS-CoV-2 Spike envelope proteins to target virus infected cells.

This proposal outlines the essential next steps towards developing and demonstrating the capability of CRISPR-Cas13 as a therapeutic approach for combating SARS-CoV-2 to limit disease severity and spread of COVID-19. Further, the programmable RNA targeting capability of CRISPR-Cas13 and the novel pseudotyped viral delivery tools developed in this proposal may have broad utility for targeting and treating other infectious RNA viruses or human genetic RNA diseases.

Harold S. Geneen Charitable Trust Awards Program for Coronary Heart Disease Research Awardees 2021-2025

"The Impact of Macrophage IL-1β Expression on SARS-CoV-2 Virulence in the Setting of Experimental CAD"

Alan Morrison, M.D., Ph.D. – 2021 Awardee
Brown University

COVID-19 is an emerging respiratory and systemic illness caused by the virus, SARS-CoV-2. SARS-CoV-2 is a novel coronavirus first identified as being transmitted from animals to humans in Huanan seafood market in the city of Wuhan, China. Reports indicate that coronary artery disease (CAD) from atherosclerosis and atherosclerotic risk factors like advanced age, diabetes mellitus (DM), and hypertension place patients at higher risk for more severe disease and increased mortality. CAD and associated risk factors are associated with increased systemic inflammation. Our proposal seeks to define inflammatory mechanisms that increase virulence in COVID-19 for patients with CAD. We have developed the world's first experimental mouse model of macrophage conditional deletion of the potent inflammatory cytokine, interleukin-1beta (IL-1beta). We have studied the impact of inflammatory macrophage IL-1beta on worsening outcomes in atherosclerotic vascular disease and during injury-mediated angiogenesis. There is emerging data in the literature to suggest macrophages may play an important role in COVID-19 pathology. Preliminary data demonstrate that mice with CAD risk factors of aging and DM demonstrate elevated serum IL-1beta levels. Moreover, macrophages from aged or DM mice expressed increased expression of ACE2, the primary receptor for the SARS-CoV-2 virus. Finally, macrophage conditional IL-1beta-deletion led to reduced macrophage ACE2 expression. We hypothesize that enhanced systemic IL-1β expression by macrophages in the setting of atherosclerosis or atherosclerotic predisposing conditions increases virulence of infection with SARS-CoV-2.

We will carry out 2 major aims: 1) to define mechanism(s) of IL-1beta-induced ACE2 expression in experimental CAD models, and 2) to develop and validate macrophage IL-1beta expression to be a critical virulence factor in the setting of atherosclerosis in a novel mouse model of COVID-19. Because of limitation in the current mouse strains and in order to carry out these SARS-CoV-2 studies in a preclinical model, an entirely new mouse strain, using a humanized ACE2 under regulation of the native mouse ACE2 promoter will be generated. By defining the molecular mechanisms of IL-1beta-induced ACE2 expression, we will identify new molecular targets for small molecule inhibition with the long-term goal of developing novel treatment strategies that suppress ACE2 in macrophages to mitigate viral severity in patients afflicted with COVID-19.

Harold S. Geneen Charitable Trust Awards Program for Coronary Heart Disease Research Awardees 2021-2025

"The Dynamic Angiogenic Network Underpinning Coronary Artery Disease"

Casey Romanoski, Ph.D. – 2021 Awardee
University of Arizona College of Medicine - Tucson

Coronary Artery Disease (CAD) is the primary cause of death in the Western World. Risk for CAD is determined by about 50% inherited genetics and 50% environmental and lifestyle factors. While the genes underlying genetic risk have been well-studied for lipid-related genes, little is understood about how genetic pre-disposition manifests through innate properties of the blood vessel wall. This is despite evidence that blood vessels contribute to the genetic risk and that therapies that correct aberrant function would likely act in synergy with current care for patients with CAD.

For the first time, this work will elucidate the effects of CAD genetic risk as it is manifested through the angiogenic human Endothelial Cell (EC). We focus on angiogenesis because of its documented impairment in cardiovascular diseases, including ischemic heart disease, ageing, vascular dysfunction, cardiac profusion, and in co-morbidities such as obesity and diabetes. Despite its relevance, no reports to our knowledge have systematically integrated the genome-wide human angiogenic regulatory program with genetic CAD risk.

Our central hypothesis is that the dynamic angiogenic EC program is a part driver of natural CAD risk. By elucidating this program, we will disentangle causal CAD alleles, genes, and mechanisms. We have assembled a highly skilled and collaborative team to identify novel CAD biology through these related, yet independent aims:

AIM 1: Identify and validate dynamic transcripts and defining regulatory elements with corresponding TFs that control activities of EC sub-populations during angiogenesis using quantitative single cell sequencing and morphological image analysis.

AIM 2: Identify and validate common genetic variants that increase CAD risk by way of vascular function through allele-specific analysis of molecular data with perturbations during angiogenesis. This work is innovative because it combines the dynamic, single cell regulatory program of angiogenesis with the latest in CAD human genetics to understand mechanisms underpinning common risk to heart disease. The potential translation of our findings would be synergistic with lipid therapies to improve disease prevention and treatment.