

## CHD Research Awards

### Key Facts and Recipients – January 2016

#### Quick Facts

- Seven projects have been funded as part of the first round of research awards
- The grants total just over \$803,000
- Recipients are from California, Georgia, Massachusetts, Maryland, Wisconsin and two are from Ohio
- Two of the recipients received pre-doctoral grants
- Four of the recipients are female and three are male

### Regulation of atrioventricular canal restriction by Tmem2

*Dr. Lucile Ryckebusch, UC San Diego*

AHA Affiliate - Western States Affiliate (California)

#### Description

The cardiac atrioventricular (AV) valve is a highly specialized anatomical structure that prevents regurgitation of blood flow between the cardiac chambers. As such, correct valve development is required for appropriate hemodynamics of circulation. The AV valve develops in a discrete region of the heart, the AV canal (AVC). The long-term goal of my research is to understand the molecular mechanisms that regulate the specification and differentiation of the AVC and make it distinct from the adjacent cardiac chambers. Currently, my work focuses on the role of the transmembrane protein Tmem2, an important player in restricting the boundaries of the AVC. With support from an AHA postdoctoral fellowship, I have shown that Tmem2 confines AVC differentiation by limiting Wnt signaling. Here, I propose to build upon this work by delving deeper into the mechanism through which Tmem2 influences Wnt signaling and by establishing which domains of Tmem2 are required for its function. In this one-year proposal, I aim to (1) test whether Tmem2 restricts Wnt signaling by modulating the extracellular matrix, and (2) determine which domains of Tmem2 are required for restriction of AVC differentiation. Together, these studies will provide new insights regarding poorly understood mechanisms underlying AV valve development and will help us to understand how these processes could be disrupted by congenital heart defects.

### Calcium handling remodeling during development and disease

*Dr. Mary Wagner, Emory University*

AHA Affiliate - Greater Southeast Affiliate (Georgia)

#### Description

Surgical repair for congenital heart defects (CHD) continue to improve and while surgical repair of some forms of CHD result in normal or near-normal physiology, there are many children who are left with palliated CHD or abnormal hemodynamic loads on their hearts. Many of the treatments for these children were designed for the adult cardiac patient and do not take into account the unique physiology of the early postnatal heart. Furthermore, contractility has been shown to increase with developmental age and may be due, in part, to alterations in calcium handling. Thus, the overall goal of this proposal is to identify alterations of calcium handling in the developing heart and to determine if increased pressure loading of the immature heart alters the development of the calcium handling system. We utilize the developing rabbit heart for these studies but also use our unique collaboration with the cardiovascular surgeons at Children's Healthcare of Atlanta to obtain ventricular tissue from children undergoing surgical repair for congenital heart defects where part of the repair includes the therapeutic removal of tissue. In our first

aim we will systematically examine cytosolic calcium dynamics of the developing rabbit heart and correlate these changes with cellular structural changes, namely the development of cell membrane invaginations called T-tubules. We hypothesize that lack of T-tubules in the newborn rabbit alters the spatial distribution and pharmacologic modulation of the calcium transient, and it is the cyclic changes in calcium that are the signal for cellular contraction. We hypothesize that there are developmental changes in the effects of kinases and beta-adrenergic stimulation on calcium movement in the rabbit heart that correspond to T-tubule alterations. In the second aim, we will examine calcium movement in the developing human heart. We will compare cells from patients < 1 week old to those 2-12 months old to determine if the developmental changes we see in the rabbit hold true for human heart development. Our preliminary data suggest that older infants have adverse remodeling that is not seen in the developing rabbit heart. In our third aim, we will determine if pressure overload in an immature rabbit, created by pulmonary artery banding, will induce adverse remodeling of the calcium handling system and thus explain the differences we find in human compared to rabbit development. A better understanding of how the immature heart responds to hemodynamic stress may lead to improved therapies for these young patients.

## **Hydrogen Gas as a Neuroprotective Agent during Circulatory Arrest for Congenital Heart Surgery**

*Dr. John Kheir, Boston Children's Hospital*

AHA Affiliate - Founders Affiliate (Massachusetts)

### **Description**

The repair of congenital heart defects requires the use of cardiopulmonary bypass to permit visualization and handling of the heart and vessels. In infants and children, the repair of some defects requires the temporary partial or complete cessation of blood flow in the entire body, a phenomenon known as deep hypothermic circulatory arrest (DHCA). This is made possible only by the protective effects of deep hypothermia (~18C). Although deep hypothermia is essential to the safety of circulatory arrest, it is not completely protective. Several studies have shown that up to 73% of children undergoing complex heart surgery with this technique have evidence of new brain injury following heart surgery. As children grow, this causes delays in learning, attention, fine motor skills and other cognitive defects, all consistent problems among congenital heart patients.

This grant will explore a new technique to protect the brain during heart surgery. Hydrogen (H<sub>2</sub>) gas is a naturally occurring gas which has unique protective properties. Because oxygen and hydrogen have similar properties, H<sub>2</sub> diffuses easily to all the same places. Inside the mitochondrion, H<sub>2</sub> has been shown to bind up and neutralize a highly toxic mediator of brain injury known as the hydroxyl radical. Following an injury, hydroxyl radicals directly damage DNA and cause localized or even global cell death, leading to the neurologic impairments described above. Several studies have demonstrated that animals exposed to H<sub>2</sub> gas following an insult exhibit significantly improved survival and less brain injury than without it. Our group will utilize a tried and true animal model of cardiopulmonary bypass to test whether the use of H<sub>2</sub> gas prior to and following on heart surgery positively impacts survival and neurologic injury, comparing it with controls. Treated animals will undergo daily detailed neurologic exams, a brain MRI, and measurement of several important markers of end organ injury. If successful, the investigators plan to conduct a clinical trial in the near future.

## **Cell-autonomous control of heart progenitors in niche**

*Dr. Chulan Kwon, Johns Hopkins*

AHA Affiliate - Mid-Atlantic Affiliate (Maryland)

### **Description**

Congenital heart defects remain the most frequent type of birth defect and the leading cause of birth defect-related deaths in humans. Cardiac progenitor cells (CPCs) serve as building blocks to make the heart during embryogenesis and abnormal CPC development is closely associated with the etiology of congenital heart defects. Thus, it is crucial to understand the biology of CPCs. However, CPCs are highly heterogeneous and it is unknown if stem cell-like CPCs exist. Consequently, understanding the mechanisms of CPC maintenance remains a fundamental challenge. We discovered a renewing population of CPCs during development, and this proposal aims to elucidate the mechanisms of CPC renewal regulated by the cell-autonomous factors. This knowledge will provide first insights into the mechanistic understanding of the self-renewal of CPCs, which will open up new avenues of research in congenital heart defects.

## **Requirements for Cyp26 enzymes in second heart field addition and ventricular maintenance**

*Ariel Rydeen, Children's Hospital, Cincinnati*

AHA Affiliate - Great Rivers Affiliate (Ohio)

### **Description**

Congenital heart defects (CHDs) are the most common type of birth defect with one third of CHDs being outflow tract (OFT) defects. Understanding the mechanisms underlying proper heart and OFT development is necessary for improving and designing novel therapies aimed at the prevention and treatment of CHDs. Normal heart development requires proper regulation of retinoic acid (RA) signaling. Vitaminosis A or excess RA in mothers during development can lead to RA embryopathies, which often include OFT defects. Cyp26 enzymes limit embryonic RA signaling through metabolizing RA into easily degraded derivatives, suggesting their loss could lead to congenital defects similar to excess RA. Moreover, alterations in Cyp26 enzyme function have been implicated in several human diseases, including DiGeorge syndrome, Klippel-Feil anomaly and Antley-Bixler syndrome, which also incur OFT defects. Although previous studies show Cyp26 deficient embryos have cardiovascular defects, the precise nature of the heart defects have not been addressed. Our preliminary data using zebrafish indicates that depletion of Cyp26 enzymes first leads to a disruption of second heart field (SHF) addition to the OFT and then a previously unrecognized progressive loss of differentiated ventricular cardiomyocytes (VCs), which is due to cells migrating away. Additionally, we observe an ectopic population of cardiac-like cells in the pharyngeal region of Cyp26 deficient embryos, suggesting increased RA signaling leads to ectopic differentiation and migration of cardiac fated cells. In Specific Aim 1, we will use Kaede-based lineage tracing to determine the origin of the ectopic cardiac-like cell population in Cyp26 deficient embryos. In order to identify the mechanisms underlying the progressive loss of VCs, we performed RNAseq and identified MMP9 as a candidate effector of excess RA signaling in Cyp26 deficient embryos. Importantly, treatment with pan-MMP inhibitor can restore SHF addition. In Specific Aim 2, we will use loss-of-function experiments to specifically address if increased MMP9 activity in Cyp26 deficient embryos promotes SHF addition to the OFT. Ultimately, the information obtained from our studies will help to broaden our understanding of the signaling networks and mechanisms driving heart development and cardiomyocyte maintenance, which will allow for generation of new strategies aimed at preventing OFT defects and improving the quality of life for individuals living with CHDs.

## **Noninvasive oxygen saturation estimation with quantitative MRI T2 mapping**

*Juliet Varghese, The Ohio State University*

AHA Affiliate - Great Rivers Affiliate (Ohio)

### **Description**

Congenital heart defects are characterized by reduced blood oxygen saturation, due to the mixing of arterial and venous blood. Increased survival into adulthood necessitates the need for non-invasive diagnostic tests that can reduce the need for invasive procedures in the management of congenital heart disease patients. As magnetic resonance transverse relaxation time T2 is affected by hemoglobin O2 saturation, we aim to develop a non-invasive, rapid, novel MRI method that is designed to minimize the effects and artifacts caused by flowing blood, and provide a reliable measurement of blood T2. This T2 measurement will then enable an accurate estimation of oxygen saturation in the chambers of the heart and deep vessels, even in regions having limited accessibility with other techniques. The method could be easily incorporated into MRI protocols for evaluation of patients with congenital heart defects, and may also have applications in peripheral arterial and other cardiovascular diseases.

## **Mechanistic analysis of mechanical alterations to improve therapeutic interventions for aortic coarctation**

*Dr. John LaDisa, Marquette University*

AHA Affiliate - Midwest Affiliate (Wisconsin)

### **Description**

Coarctation of the aorta (CoA) is a constriction of the thoracic aorta and is one of the most common congenital cardiovascular (CV) defects. Treatment by surgical correction has saved the lives of thousands of children, but they still have a reduced lifespan from CV morbidity, mostly due to refractory hypertension. Identifying associated mechanisms is difficult in humans due to confounding variables (e.g. severity of CoA at correction, time to follow-up, concurrent anomalies). Thus, in spite of surgical repair being available for >70 years, the causes of morbidity after correction for CoA remain elusive. This proposal to the AHA/Children's Heart Foundation (CHF) partnership addresses long-term morbidity after correction for CoA using an innovative animal model devoid of these confounding variables. Our model mimics the pathology of CoA in humans, and permits correction at different times using dissolvable suture. This model is used with computational fluid dynamics to quantify detailed mechanical alterations from the coarctation that we hypothesize are responsible for vascular remodeling, endothelial dysfunction, and eventually lead to long-term morbidity after correction. Mechanical alterations are compared to structural, functional, and mechanistic vascular changes using histology, myography, and protein analysis, respectively. Our preliminary data revealed adverse vascular changes resulting solely from CoA, and indicates these changes may be permanent. Microarray analysis revealed differentially expressed genes after correction of CoA in our model, including decreased expression of SERCA (sarco/endoplasmic reticulum calcium ATPase). This proposal builds on the preliminary data with 2 aims. Aim 1 will determine the minimum mechanical stimuli for persistent vascular remodeling with endothelial and smooth muscle cell dysfunction by varying the severity and duration of CoA within clinical ranges. Aim 2 will correlate down-regulation of the SERCA message with protein expression, and the persistence of pathological changes after correction for CoA. Results from these aims have the potential for clinical translation by proposing a fresh criteria for when correction of CoA should be performed, and identifying potential targets for monitoring persistent vascular alterations. Our goal of translating results to enhance health, reduce morbidity, and allow individuals treated for CoA to live longer healthier lives is aligned with the goals of the AHA/CHF partnership.