Late-Breaking Basic Science Oral Abstracts I

**Inhibition Of Prolyl-tRNA Synthetase As A Novel Mediator Of Cardiac Fibrosis**

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**Introduction:** Prolyl-tRNA synthetase (PRS), a member of aminoacyl tRNAs synthetases (ARS), is an enzyme that conjugates amino acid proline to its cognate tRNA to generate prolyl-tRNA to be used in protein synthesis. Since ARS are essential for protein synthesis and viability, dysregulation of ARS has been proposed to many human diseases. Cardiac fibrosis is characterized by excess production and deposition of ECM proteins by activated fibroblast. Although proline is a major component of collagen and ECM proteins, the exact mechanism and involvement of PRS in cardiac fibrosis has not yet been elucidated.

**Hypothesis:** We hypothesized that suppression of PRS would down-regulate collagen synthesis, which could be beneficial in cardiac fibrosis. **Results:** To validate our hypothesis, we investigated the expression levels of pro-fibrotic markers by overexpression or knockdown of PRS in vitro. Interestingly, we showed that down-regulation of pro-fibrotic markers by DWN12088 is independent of TGFβ signaling pathway, although TGFβ is required for induction of pro-fibrotic markers. In vivo study, we performed transverse aortic constriction in C57BL/6 mice to evaluate the effect of DWN12088 in cardiac fibrosis. TAC results in pressure overload-induced left ventricular hypertrophy and fibrosis, and it is one of the most widely used models to study cardiovascular diseases. 2-week oral treatment of DWN12088 markedly reduced cardiac fibrosis with ED50 of 0.2 mg/kg, based on histological examinations. In addition, we demonstrated that 2-week oral treatment of DWN12088 showed reduction in infiltration of inflammatory cells, left ventricle thickness and accumulation of collagen I. These results suggest that inhibition of PRS attenuates pressure-overloaded cardiac fibrosis and a selective inhibitor of PRS, DWN12088 could serve as a potent anti-fibrotic agent without affecting critical cellular signaling cascades.

**Key Words:** Enzyme inhibitors; Fibrosis; Heart failure

**Cytosolic RBFox1 In Cardiac Fibrosis Regulation**

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RBFox1 is known to be an RNA splicing regulator with enriched expression in cardiac muscle. Loss of RBFox1 expression is a molecular hallmark associated with pathological hypertrophy and heart failure. However, much of our current knowledge about RBFox1 focuses on nuclear RBFox1 with a major impact on global alternative splicing changes in the diseased heart. Yet, RBFox1 gene also generates a cytosolic isoform through alternative splicing (RBFox1c), but the specific function of RBFox1c in heart has not been characterized.

RBFox1c expression is significantly repressed in the mouse failing heart and hypertrophic cardiomyopathies. We performed RNA-seq combined with GO and IPA analysis to determine the impact of RBFox1c expression in culture. Among the genes suppressed specifically by RBFox1c but not the nuclear RBFox1 are groups of pro-inflammatory genes. Both Motif enrichment analysis and de novo motif discovery identified significant enrichment of RBFox1c binding motif in the 3'UTRs of the RBFox1c-regulated genes. Using CLIP analysis followed by RT-PCR, we observed RBFox1c, but not nuclear RBFox1 specifically interacted with targeted inflammatory gene 3'UTR. In the cardiac specific RBFox1c knockout mice, enhanced cardiac fibrosis was observed following TAC, associated with elevated expression of RBFox1c-dependent inflammatory genes. In contrast, cardiac specific expression of RBFox1c significantly reduced cardiac fibrosis and inflammatory gene expression following TAC, associated with improved ejection fraction and reduced hypertrophic marker gene expression. Further, we tested the effect of RBFox1c expression on cardiac fibrosis response using MVNm conditioned media. We showed the conditioned media from the hypertrophic cardiomyocytes potently induced fibroblast proliferation. However, RBFox1c expression can suppress phenylephrine and isoproterenol induced fibroblasts proliferation.

RBFox1c regulates cardiac transcriptome reprogramming at two post-transcriptional steps. The RBFox1c nucleic isoform regulates global RNA splicing reprogramming in heart, while the RBFox1c regulates inflammatory gene expression and fibrotic remodeling potentially through posttranscriptional interaction with their 3'UTR and targeted RNA degradation.

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**Key Words:** Heart failure; Genomics; Hypertrophy

Optogenetic-induced Mitochondrial Membrane Potential Depolarization and Targeting Cell Death

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**Introduction:** There is growing evidence that mitochondrial dysfunction is closely associated with the development of cardiovascular diseases, although the precise mechanism is not well defined. Normal functioning of mitochondria relies on maintaining the inner membrane potential (ΔΨm) to drive oxidative phosphorylation and redox balance. Thus, developing a tool to induce controlled mitochondrial depolarization and examine the effects on downstream intracellular processes will lead to critical information that helps to reveal the mechanisms underlying mitochondrial-induced cellular dysfunction. **Methods and Results:** In this study, we developed a new generation optogenetic-based technique for targeting mitochondrial depolarization with light. Particularly, a heterologous light-gated channelrhodopsin protein, named ChR2, was targeted to and expressed on the inner mitochondrial membrane (IMM). We showed that ChR2 formed functional cationic channels on IMM with properties similar to that on the plasma membrane, allowing light-induced targeting ΔΨm depolarization. We also showed that sustained moderate light illumination caused significant cell death in mitochondrial ChR2-expressing cells but not in the mock transfected or mitochondrial YFP-expressing cells. Finally, we provided evidence that the mitochondrial optogenetic-induced cell death is via apoptosis and is independent of the opening of the permeability transition pore. **Conclusion:** This new generation optogenetic tool can be used to study the mechanisms how a change of mitochondrial membrane permeability influences cell and organ functions.

**Key Words:** Cell physiology; Mitochondria; Apoptosis; Cellular Engineering; Cardioprotection

A Peptide Of The Amino-terminus Of Grk2 Induces Hypertrophy And Yet Elicits Cardioprotection After Pressure Overload.

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Heart failure (HF) is a leading cause of death worldwide and a growing burden on public health, and the underlying mechanisms of cardiac remodeling and compensation to HF remain a focus of research efforts towards therapeutic development. Signaling via G protein-coupled receptors (GPCRs) is critical for normal heart function and is tightly controlled by GPCR kinases (GRKs) with GRK2 (originally cGRK1), being intimately involved in HF progression. In addition to its well-characterized role in regulating GPCRs, ongoing research has demonstrated great diversity in the functional roles of GRK2. We have recently investigated GRK2 amino terminal binding interactions through the generation of transgenic (Tg) mice with cardiac-targeted expression of the amino-terminal peptide J.J Zhang: None. M.X. Liu: None. L. Zhou: None.

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**Key Words:** Heart Failure; Cardioprotection; Cardiomyopathy; Small Molecules; Small Molecule
G Protein-coupled Receptor Kinase 2 Negatively Regulates Fatty Acid Utilization and Mitochondrial Bioenergetics
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During cardiac injury or stress, G-protein-coupled receptor (GPCR) kinase 2 (GRK2) expression levels and activity are increased, leading to a desensitization of myocardial β-adrenergic receptors (βARs) and contributing to the loss of contractile reserve. Up-regulated GRK2 has been shown to be pathogenic in the post-injured heart and is involved in the promotion of heart failure (HF). There is evidence that GRK2 has other, non-βAR dependent pathological functions within cardiomyocytes. For example, GRK2 localizes to the mitochondria following oxidative stress, where it acts as a pro-death kinase and decreases fatty acid utilization. As metabolic substrate utilization and bioenergetics are key parameters in the maintenance of cardiomyocyte contractility, our objective is to explore the role of GRK2 on metabolism and bioenergetics in the adult heart. We hypothesize that desensitization of βARs via an increase in GRK2 will result in decreased fatty acid-fueled respiration and will compromise cardiomyocyte function. Conversely, ablation of GRK2 will result in increased respiration and function, under these conditions. Our results show that basal respiration, maximal respiration, and reserve respiratory capacity (RRC) are highest in the presence of palmitate versus glucose (1.6, 3, and 7.2-fold, respectively), accompanied by increased (1.3-fold) ATP levels. Moreover, basal and maximal respiration was decreased (1.6 and 1.2-fold, respectively) cardiomyocytes isolated from cardiac-specific GRK2 transgenic mice. This correlates with a decrease in ATP levels and in vivo cardiac fatty acid uptake (1-fold and 1.6-fold, respectively). Conversely, cardiomyocytes isolated from (ARKct, a peptide inhibitor of GRK2, transgenic mice or GRK2 knockout mice) increased respiratory capacity (1.5 and 1.45- respectively) and RRC (2.7-2.2-fold, respectively) with fatty acids. This correlates with an increase in ATP levels (1.2-1.9-fold). Thus, we propose that increased GRK2, as seen during heart failure, compromises fatty acid-driven mitochondrial respiration, while GRK2 inhibition under these conditions enhances RRC, which is known to improve cellular survival during stress.

Author Disclosures: J.M. Pfleger: None. W.J. Koch: None.

Key Words: Adrenergic; Energetics; Metabolism; Mitochondria; Mitochondrial energetics; heart failure; arrhythmias

Heart Failure Induced Upregulations of MicroRNAs in the Human Sinoatrial Node Associated with Pacemaker Dysfunction
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Background: Heart failure (HF), a leading cause of morbidity and mortality, involves significant dysfunction of the sinoatrial node (SAN). MicroRNAs (miRs) are abundant, non-coding RNAs that ultimately regulate protein expression at the post-transcriptional level and have been implicated in HF. However, nothing is known about the distribution and expression of miRs in the human SAN as well as their roles in regulating pacemaker channels and SAN dysfunction (SND) in HF. Methods: The human SAN was isolated and cryopreserved from failing hearts with implantable pacemakers (n=5) or non-diseased donor hearts (n=3) that were cardiologically arrested in the surgery room. Utilizing immunohistochemistry, the intact 3D intramural SAN structure was precisely identified as the fibrotic region around the SAN artery containing Connexin43-negative pacemaker cardiomyocytes (Figure). Small biopsies from the central SAN compartment were used to characterize the expression pattern of 14 miRs and their associated pacemaker channels. Ingenuity software (Qiagen) was used to predict the relationship between miRs and their targeted mRNA of SAN ion channels: Results: Out of 14 studied miRs, 5 miRs (miR-133-3p and miR-1) were significantly upregulated in SAN, but not in atria during HF. We found that miR-370-3p was predominantly expressed in the human SAN, but not atria. Whereas, both miR-133-3a and miR-1 were highly expressed in atria vs SAN. All three miRs were predicted to regulate pacemaker CNT1 and/or HCN4 channel expression. RT-PCR showed that HCN1 and HCN4 mRNA were downregulated in the HF SAN. Conclusions: This is the first study to explore the miR profiles in healthy and diseased human SAN with SND. We demonstrate that miR-370-3p and miR-1 disrupt the regulation of pacemaker CNT1 channel expression, selectively upregulated in human HF SAN. We propose that understanding the function of miRs in human SAN might lead to novel SND treatments.


Key Words: Apoptosis; Exercise tests and training; Cardiomyopathy; Redox; Ventricular arrhythmia

Late-Breaking Basic Science Oral Abstracts II
20407
Heart Failure Induced Upregulations of MicroRNAs in the Human Sinoatrial Node Associated with Pacemaker Dysfunction

24047
G Protein-coupled Receptor Kinase 2 Negatively Regulates Fatty Acid Utilization and Mitochondrial Bioenergetics

24032
Exercise Instigates Apoptosis-inducing Factor Nuclear Translocation and Myocyte Death in Arrhythmogenic Cardiomyopathy
Stephen P Cheklo, Gizem Keceli1, Peter Andersen1, Nuria Amat-Codina1, Jacopo Agrimi1, Djahida Bedja1, Marcus Stahlberg, Marc Halushka1, Cynthia A James1, Hugh Calkins1, Daniel P Judge1, Nazareno Paolocci1, Johns Hopkins School of Medicine, Baltimore, MD, Karolinska Institutet, Stockholm, Sweden

Exercise increases disease penetrance in arrhythmogenic cardiomyopathy (ACM). Yet, how exercise contributes to disease pathogenesis is unclear. Mitochondria potentiate reactive oxygen species (ROS) generation during exercise that are scavenged by antioxidants, such as thioredoxin-2 (Trx2). Here we tested if deficits in Trx2-based ROS buffering act as sub-targetable mechanism for preventing one of the most cited, yet poorly understood, pathological phenotypes (apoptosis) in ACM.


Key Words: Apoptosis; Exercise tests and training; Cardiomyopathy; Redox; Ventricular arrhythmia


Key Words: Sinoatrial node; Heart failure; MicroRNA
Regional Assessment of Pyruvate Metabolism in the Remodeled Heart Using Dynamic Nuclear Polarization Carbon13 Magnetic Resonance


Introduction: After infarction compensated remodeling of the left ventricular (LV) may be followed by adverse remodeling leading to heart failure. The mechanism of adverse remodeling maybe linked to the elevated wall stress in the dysfunctional myocardium adjacent to the infarct (border zone B2). We hypothesize that increased B2 stress results in altered metabolism which could drive the transition from compensated to adverse remodeling. To evaluate B2 and remote metabolism we compared the regional uptake and intracellular conversion of 1-13C-pyruvate using hyperpolarized (HP) 13C MR. Methods: An established pre-clinical postero-lateral infarct model of LV remodeling was used to investigate region metabolism. To accurately measure regional metabolism, we developed implantable carbon-tuned surface coils placed on the epicardium over the B2 and remote regions (Fig. Top). A coronary catheter was placed for direct injection of the HP substrate to maximize delivery and eliminate cavity blood pool signal. MRI was performed at 6-weeks post infarct with a spectra acquired every 1.5s for each region simultaneously during HP infusion under physiologic and DBB stress conditions. The resulting spectra from each coil were analyzed to measure lactate, alanine, bicarb, and total flux. Results: Under physiologic (Pre-DBB) conditions the percent difference between remote and B2 lactate, alanine, and total flux was only slightly elevated in the remote region whereas bicarb flux was greater in B2 compared to remote (Fig. Bottom). DOB stress produced an increase in remote metabolite flux compared to B2 with lactate, alanine and total flux reaching significance and bicarb flux shifting from greater in B2 Pre-DOB to greater in remote. Conclusion: These findings demonstrate an impaired metabolic response to pharmacologic stress in B2 myocardium which may provide a mechanism for the established association of metabolic stress and adverse cardiac remodeling following infarct.


Key Words: Ventricular remodeling; Cardiac metabolism; Cardiac MRI; Imaging agents; Ischemic heart disease

Late-Breaking Basic Science Posters

Therapeutic Effects of Human Pluripotent Stem Cell-derived Lymphatic Endothelial Cells Encapsulated With Nanomatrix Gel on Experimental Lymphedema

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Author Disclosures: S.I. Grijalva: None. J.H. Sung: None. B.W. Furman: None. P. Han: None. J. Li: None. H.C. Cho: None.

Key Words: Sinusoidal node; Pacing

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Background: Current systems generating lymphatic endothelial cell (LEC) from human induced pluripotent stem cells (hPSCs) have limited value due to low purity, the use of defined conditions for differentiation, and poor cell survival in vivo. Here, we developed a fully defined system to differentiate hPSCs into LECs and evaluated their therapeutic and engraftment potential when encapsulated in a nanomatrix gel (PA-RGDS). Methods and Results: hPSCs were cultured with SGSK3i inhibitor on collagen-coated plates for 2–3 days to induce differentiation into the mesodermal lineage. The mesodermally differentiated cells were then cultured with VEGF, VEGFA, EGF, and bFGF for another 6 days and double-sorted by PDPN and FLT4. These hPSC-PDPN+FLT4+ cells (hPSC-derived lymphatic endothelial cells, hPSC-LECs) showed highly purified and fully functional LEC characteristics in vitro. These hPSC-LECs express LEC markers such as PDPN, LYVE1, PROX1, and FLT4 at the mRNA level and the protein level, and formed tube-like structures in Matrigel. We next determined the lymphatic vascular reparative effects of engineered hPSC-LECs. After inducing lymphedema in the tail of mouse, hPSC-LECs, hPSC-LECs encapsulated with PA-RGDS, and hPSC-LECs encapsulated with PA-RGDS, human dermal lymphatic endothelial cells (hDLECs), hPSC-LECs, or PBS were injected into the tail of mouse. Tail thickness significantly decreased in the groups injected with hPSC-LECs with or without PA-RGDS compared to the other groups at day 28. At day 45, mice injected with PA-RGDS encapsulated hPSC-LECs showed significant decrease in the tail diameter compared to all other groups including those injected with PBS. Histological examination demonstrated that the skin thickness was significantly reduced and the density of lymphatic vessels was markedly increased when the hPSC-LECs were encapsulated with PA-RGDS compared to others. Conclusion: This study demonstrated for the first time that hPSCs can be differentiated into LECs in a clinically compatible manner with a high yield. Furthermore, nanomatrix encapsulated hPSC-LECs can substantially improve lymphema in mouse tail through enhancement of cell survival and lymphatic neovascularization. This engineered hPSC-LEC therapy represents a novel option for treating lymphedema.

Author Disclosures: S. Lee: None. Y. Sohn: None. D. Sohn: None. Y. Yoon: None. Key Words: Stem cell therapy; Lymphatic disease; Valvular myocardial ischemia, promoting cellular functions such as proliferation, glucose metabolism and angiogenesis. Therefore, we investigated the role of Zmynd8 in the pathobiology of cardiac ischemia in a model of HF-1 activation. Hypothesis: Zmynd8 modulates the HIF-1 response in ischemic heart disease by inhibiting enhancer activity of HIF-1 target genes. Methods & Results: We expressed an inducible cardiac-specific, oxygen-stable form of Zmynd8 (ZmΔ8) in a mixed strain of mice that genetically express HIF-1α (Zα). Compared to HIF-induced wild-type (WT) mice, these mice did not exhibit the expected HIF-1 phenotype of increased heart weight to body weight (6.4±0.3 vs. 4.8±0.2 vs. 5.1±0.3 mg/g; WT vs. Zα vs. uninduced; p<0.05) with reduced ventricular function and associated chamber dilation. Semi-quantitative qPCR analysis of H1- and H2C cardiac cell lines treated with CMV-ZmΔ8 expression plasmids for Zmynd8 and oxygen-stable HIF-1α resulted in striking reduction of multiple HIF-1 target genes such as PDK1 (45% reduction) compared to the H1-α plasmid alone. RNA mediated knockdown of Zmynd8 alleviated this negative regulation (65% increase). Bioinformatic analysis of human Zmynd8 and H1- and H2C CHIP-seq data indicates that Zmynd8 binds to the enhancer of 78% of HIF-1 regulated genes. This further supports our observation that Zmynd8 modulates HIF-1 activity in the heart. Conclusion: We have discovered a new regulator of HIF-1 action that modifies the hypoxic response, likely through chromatin remodelling. We suggest that this new form of regulation could modify the pathophysiology of ischemia and potentially provide new targets for therapy.

Author Disclosures: K. J. Schunker: None. C. B. Walton: None. R. V. Shohet: None. Key Words: Hypoxia; Epigenetics

24060 Alpha Calcitonin Gene-Related Peptide (CGRP) Protects Against Pressure-induced Heart Failure

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Introduction: Calcitonin gene-related peptide (CGRP), a 37 amino acid neuropeptide, is a potent vasodilator, and plays a critical role in the different functions of the sensory nervous system. A protective role for CGRP in cardiovascular diseases (hypertension, cardiac ischemia, and failure) has been well established by our laboratory and others. In the present study we determined whether long-term exogenous administration of α-CGRP protects against pressure-induced heart failure. Method: Three groups of nine-week-old C57/B6 mice were studied: one group received a sham procedure (n=4) and two groups underwent transverse aortic constriction (TAC). Two days after TAC, one group had CGRP-filled osmotic pumps (4 mg/kg bwt/day) implanted subcutaneously (n=6) while the second group was TAC only (n=7). At day 28, HFD was initiated in both groups. Male and female C57Bl/6J mice were studied. Results: In both male and female mice, HFD significantly increases body weight (31.4±3.1 vs 43.2±4.3g and 22.1±3.9g respectively), fat mass (+200% in males and +300% in females) and blood glucose (+7.9±mg/dl and +54±mg/dl, respectively) all (p<0.01). HFD tends to decrease flow reperfusion after global no-flow ischemia and to increase infarct size (p=0.08 in male and p=0.1 in male, n=5) in both sexes. In males and females, HFD increases pS6ERK75-U1 kinase and decreases Parkin levels under basal conditions (all p<0.006). Moreover, HFD tends to decrease pThr172-AMPK only in females. After I/R, Parkin levels remain lower in HFD groups without sex-difference (p=0.004) but a drastic increase of LC3-II occurs only in females under HFD (p=0.047). Conclusion: All together, these results suggest an impairment of autophagy and mitophagy pathways under HFD. A more drastic change occurs in female mice and may imply a more important response of female mice to HFD, although further studies are warranted. Sex differences in the response to metabolic syndrome and ischemic heart disease are warranted.

Author Disclosures: A. Thomas: None; S. Marek: None; K.C. Tucker: None; A.M. Andres: None; R.A. Gottlieb: None.

Key Words: Ischemia reperfusion; Obesity; Sex differences; Autophagy; Mitochondria

24077 Differential, Sex Related, Response To High-fat Diet In Mice Impairs Cardiac Autophagy And Mitophagy

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Introduction: Premenopausal women, as well as female animals in studies, have a reduced risk of cardiovascular diseases and a reduced myocardial susceptibility to ischemia/reperfusion (IR) injury. However, with constantly increasing prevalence of obesity, the impact of sex on the induced autophagy in females remains unclear. Mechanisms of autophagy and mitophagy, differentially regulated between males and females, have been shown to be cardioprotective and impacted under nutritional overload. Hypothesis: Autophagy and mitophagy pathways can be involved in both female cardiac protection and deleterious effect of obesity. Methods: Male and female C57Bl/6J mice (n = 20 per group) of 8 weeks old were fed a low-fat (LF), 10% fat or high-fat (HFD, 60% fat) diet for 12 weeks. At 12 weeks, hearts from mice were studied under basal conditions and after global no-flow ischemia and reperfusion in a Langendorff perfusion system. Results: In both male and females, HFD significantly increases body weight (31.4±3.1 vs 43.2±4.3g and 22.1±3.9g respectively), fat mass (+200% in males and +300% in females) and blood glucose (+7.9±mg/dl and +54±mg/dl, respectively) all (p<0.01). HFD tends to decrease flow reperfusion after global no-flow ischemia and to increase infarct size (p=0.08 in male and p=0.1 in male, n=5) in both sexes. In males and females, HFD increases pS6ERK75-U1 kinase and decreases Parkin levels under basal conditions (all p<0.006). Moreover, HFD tends to decrease pThr172-AMPK only in females. After I/R, Parkin levels remain lower in HFD groups without sex-difference (p=0.004) but a drastic increase of LC3-II occurs only in females under HFD (p=0.047). Conclusion: All together, these results suggest an impairment of autophagy and mitophagy pathways under HFD. A more drastic change occurs in female mice and may imply a more important response of female mice to HFD, although further studies are needed. However, this result suggests that cardiovascular disease may be more deleterious in women despite their lower cardiovascular risk. Additional studies examining sex differences in the response to metabolic syndrome and ischemic heart disease are warranted.

Author Disclosures: A. Thomas: None; S. Marek: None; K.C. Tucker: None; A.M. Andres: None; R.A. Gottlieb: None.

Key Words: Ischemia reperfusion; Obesity; Sex differences; Autophagy; Mitochondria

24037 ZMYN8 Regulates the Hypoxic Response in Cardiomyocytes

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Introduction: The Zmynd8 gene contains a variety of recognized motifs including a bro- modomain and PHD finger, which are implicated in cooperative binding to acetylated nucleosomes. It was recently identified as a key regulator of cancer progression and transcriptional repression by directing modification of histone methylation in the enhancer regions of genes. Mutations and dysregulation of Zmynd8 have been found in multiple cancer types, however its role in the cardiac myocardium is unexplored. Hypoxia-inducible factor 1α (HIF-1α) upregulation and stabilization is a common feature of both cancer and...
TRPV4 antagonist GS2193874, immediately after MI surgery and followed for 5 weeks. 2D-echocardiography revealed that the cardiac function (ejection fraction and fractional shortening) is preserved post-MI in both TRPV4KO and GS2193874-treated WT mice compared to either WT or vehicle treated mice. Further, we found reduced cardiac fibrosis at infarcted and remote zones in TRPV4KO and GS2193874-treated WT mice compared to their MI counter parts. Furthermore, TRPV4KO hearts exhibited decreased fibrosis and apoptosis (TUNEL assay) and increased capillary density (ICD3 staining) post-MI compared to WT hearts. Our results thus suggest that targeting TRPV4 protects heart from myocardial infarction-induced damage by preserving cardiac structure and function via reduced myocyte apoptosis, diminished fibrosis and increased revascularization, and identifies TRPV4 as a novel therapeutic target for heart failure.

This research has received full or partial funding support from the American Heart Association.


Key Words: Ion channels; Fibrosis; Myocardial infarction; Cardioprotection

23094

Titin Truncating Variants Predict Life-threatening Arrhythmias in Patients With Dilated Cardiomyopathy

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Introduction: There is an urgent need for better arrhythmic risk stratification in non-ischemic aedilated cardiomyopathy (DCM), where the benefit of ICD placement is unclear. Titin truncating variants (TTNV) are the commonest genetic cause of DCM and are associated with early onset non-sustained ventricular arrhythmia and atrial fibrillation (AF) in these patients. Hypothesis: We hypothesize that TTNV status can predict potentially life threatening ventricular tachycardia (VT) or fibrillation (VF) and development of new persistent AF in DCM patients with CRT-D or ICD devices. Methods: We studied 117 DCM patients with an ICD or CRT-D and documented device-recorded arrhythmia over a median period of 4.2 years. Patients were stratified by TTNV genotype (28 positive for a TTNV, 89 negative). The primary outcome was time to first device-treated VT >200bpm or VF. Secondary outcome measures included time to first development of persistent AF: Results: TTNV predicted the risk of receiving inappropriate ICD therapy for VT or VF (hazard ratio (HR) = 4.9, 95% confidence interval [CI]=2.5–8.7, P<0.001). The TTNV prediction was independent of all covariates, including replacement fibrosis measured by late-gadolinium enhancement (LOE), adjusted HR = 8.2, 95% CI 1.9–39.6, P=0.005). Individuals with both a TTNV and fibrosis had a markedly greater risk for appropriate device therapy than those with neither (HR = 16.1, 95% CI 3.5–79.3, P=0.001). TTNV were also a risk factor for developing new persistent AF (HR = 4.4, 95% CI 1.4-15.3, P=0.006). Conclusion: TTNV status is an important risk factor for clinically significant arrhythmia in patients with DCM or CRT-D or ICD devices. TTNV status alone or more importantly in combination with fibrosis may provide an effective readout for arrhythmia risk and need for ICD therapy in DCM patients.

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Key Words: Genetics; Ventricular arrhythmia; Cardiomyopathy; Heart failure; adult; Ventricular tachycardia

23928

Micorna-125b-5p Protects The Heart From Acute Myocardial Infarction By Repressing Pro-apoptotic Bak1 And Klf13 In Cardiomyocytes

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Cardiac injury is accompanied by dynamic changes in the expression of microRNAs (miRs), small non-coding RNAs that post-transcriptionally regulate target genes. MiR-125b-5p is downregulated in patients with end-stage dilated and ischemic cardiomyopathy and has been proposed as a biomarker of heart failure. We previously showed using the βArrestin-βArrestin1/3 receptor conformation that βArrestin-mediated cardioprotective signaling through βArrestin1 stimulates processing of miR-125b-5p in the mouse heart (Figure A-C). We hypothesize that βArrestin-receptor-βArrestin1-responsive miR-125b-5p confers cardioprotection against acute myocardial infarction. Using cultured cardiomyocyte (CM) and in vivo approaches, we show that miR-125b-5p is an ischemic stress-responsive gene in both infarcted and remote zones of CM. Our results suggest that miR-125b-5p may prevent apoptosis and increase myocardial infarct survival during acute myocardial infarction.

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Key Words: MicroRNA; Cardioprotection; Receptor-mediated signaling; Apoptosis

24055

Naturally Occurring Hypertension is Related to Cardiac Diastolic Dysfunction in Rhesus Monkeys

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Introduction: The lack of effective treatment for Diastolic Dysfunction (DD) is partially due to the differences between widely used genetic rabbit models and humans in the physiology and function of the heart. Previous studies, we have demonstrated that rhesus Monkeys with naturally occurring adult onset Type 2 Diabetes (T2DM) frequently have DD that is similar in characteristics to DD in diabetic patients. To further characterize DD in rhesus monkeys, we studied the relationship between hypertension and DD, and evaluated the association of rhesus monkeys with DD to Entresto (sacubitril/valsartan). Methods: Blood pressure, fasting plasma glucose and cardiac function were measured in 322 adult rhesus monkeys (Macaca mulatta, 7–22 yrs) under light anesthesia with ketamine. Monkeys with LV hypertrophy, t<0.05 cm and E/e'<10 were defined as DD. Ten monkeys with DD were enrolled in the validation study and divided into the Entresto group (n=5) and the vehicle group (n=5). Cardiac function and blood pressure were measured before and at the end of 13 weeks of treatment. Results: Among the 322 adult rhesus monkeys studied, 53 monkeys (16.46%) had SBP<140 mm Hg or DBP<90 mm Hg. Among the 174 monkeys with fasting glucose ≥80 mg/dL, 67 monkeys had isolated DD, and 8 had DD+ SD (diastolic dysfunction). The incidence of isolated DD was 31% in monkeys with SBP<140 mm Hg and 74% in monkeys with SBP>140 mm Hg. Following Entresto administration (1.66 to 13.33 mg/kg) for 13 weeks, DD and BP evaluation showed an increase of (5.2±0.31 to 6.4±1.57 cmH2O), a decrease of E/e' (12.7±2.23 to 10.5±3.29) and a decrease of SBP (126±15 to 113±16 mm Hg). These parameters remained stable and unchanged in the vehicle group. Conclusions: The incidence of naturally occurring hypertension in adult rhesus monkeys was similar to that observed in humans. Entresto reduced blood pressure, but led to no significant improvement of DD in monkeys. The extent of change in rhesus monkeys was similar to that observed in clinical trials. In rhesus monkeys, as in patients, hypertension is significantly related to cardiac diastolic dysfunction. These monkeys, therefore, provide important new opportunities to understand the pathogenesis of DD, as well as to predict the human response to new therapeutic agents.


Key Words: Diastolic function; Hypertension; Drugs; Diabetes (Type II)

24056

Longitudinal Evaluation of the Associations between Severe Hypertriglyceridemia and Cardiovascular Features

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Introduction: For at least 35 years the associations between insulin resistance, hypertriglyceridemia, hypercholesterolemia and hypertension have been described. Nevertheless, the existence of an independent association between hypertriglyceridemia (HTG) and cardiovascular diseases (CVD) remains controversial. This may be due to the insufficiency of longitudinal study data that include severe elevations of TG. The purpose of this study was to characterize the associations between systolic blood pressure and various cardiovascular features under different degrees of naturally-occurring HTG severity and to determine the

Key Words: Cardiovascular disease; Cell signaling; Cardiovascular health; Molecular biology; Gene mutations

24068

Developing a Cardiomyocyte Pipeline for Gene Edited hiPSCs

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The Allen Institute for Cell Science (AICS) is creating an open source platform of fluorescently tagged human induced pluripotent stem cell (hiPSC) lines to model cell organization and dynamics of stem cells and differentiated hiPSC-cardiomyocytes. Understanding the sub-cellular organization and the structure/function relationships of organelles within cardiomyocytes will contribute to the development of better disease models, therapies, and regenerative medicine approaches for cardiac disease. Using the WTC human iPSC line and the CRISPR/Cas9 system, we have fluorescently tagged ~20 target genes representing key cellular organelles including a few cardiac-specific genes. Edited iPSC lines are differentiated into cardiomyocytes using established protocols with either small molecules or a combination of cytokines and small molecules, which produce highly-purity monolayers of beating cardiomyocytes within 1–2 weeks. Differentiation into cardiomyocytes serves as an important quality control criterion for our gene editing efforts, but also comprises an important aspect of our predictive cell modeling efforts. We plan to study the changes in localization and organization of these targeted organelles as the stem cells differentiate into cardiomyocytes using live fluorescent imaging. Here we present our cardiac differentiation methods for multiple edited hiPSC lines and the quantitative and qualitative assays used to determine the efficacy of differentiation, including myofibril formation, cardiac protein expression, and transcriptome profiling by bulk and single cell RNAseq. Additionally, we confirm the localization of cardiac proteins such as troponin T and alpha-actinin in the myofibrillar differentiated cells using image-based assays. In experiments initiated to date we have successfully differentiated multiple gene edited iPSC lines representing major cardiac cell markers of which are specific to cardiomyocytes (αMHC and ACTC). Some of the gene edited iPSC lines are fluorescently-tagged for structures including focal adhesions, actin and microtubule cytoskeleton, mitochondria, nuclear envelope, desmosomes, and endoplasmic reticulum, which are all publically available to the community.


Key Words: Stem cells; Cellular Engineering; Cardiac development; Stem cell biology

24063

Heart Fields Are Induced by Coordinated Activity of Wnt and Bmp Signaling and Identified by CD184 and EphA2 in PSC-Derived Organoids

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Over the past few decades, major advances have been made in identifying the origins of cardiac cells from developing embryos. In particular, the discovery of the first heart field (HF) and the second heart field (SHF), led us to understand how diverse lineages and different anatomical structures of the heart arise during cardiogenesis. However, it remains unknown how the two heart fields are specified and segregated, a fundamental step toward understanding heart formation and developing pluripotent stem cell (PSC)-based therapeutic strategies. Here, we generated 3D organoids from human PSCs and cultured them under specific conditions to generate heart field/chamber-specific heart disease in cell culture.


Key Words: Stem cell biology; Cardiac development; Stem cells; Progenitor cell

23931

Selective Inhibition Of Hdac3 Prevents Diabetic Cardiomyopathy In Ove26 Mice Via Mir-200a-mediated Mrz Activation

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Although histone deacetylases (HDACs) were an early target for the initiation and development of diabetic cardiomyopathy (DCM), which isoform of them plays the key role remains uncertain. The present study was designed to determine whether DCM could be prevented by selective inhibition of HDAC3 and the underlying mechanism. Male type 1 diabetic Ove26/268 mice were given the selective HDAC3 inhibitor (HDAC3i) for 2 months to prevent myocardial fibrosis and oxidative stress as a major cause of DCM. Serum glucose level and body weight were not significantly different among the normal control group, DCM group, and DCM+HDAC3i group. In the diabetic heart, HDAC3 was increased in the diabetic heart vs. the normal control heart, which was also confirmed by immunohistochemistry. Oxidative stress as a major cause of DCM was also inhibited by HDAC3i in the diabetic mouse. Immunoprecipitation shows that the binding of KEAP1 to HDAC3 and the underlying mechanism. Male type 1 diabetic Ove26 mice and age-matched wild-type mice were given the selective HDAC3 inhibitor (HDAC3i) for 2 months to prevent oxidative stress as a major cause of DCM. Serum glucose level and body weight were not significantly different among the normal control group, DCM group, and DCM+HDAC3i group. In the diabetic heart, HDAC3 was increased in the diabetic heart vs. the normal control heart, which was also confirmed by immunohistochemistry. Oxidative stress as a major cause of DCM was also inhibited by HDAC3i in the diabetic mouse. Immunoprecipitation shows that the binding of KEAP1 to HDAC3 and the underlying mechanism.
A Combined Basic Science and Population Science Approach Demonstrating the Potential for Simvastatin to Mitigate Cardiovascular Disease after Lower Hemi Body Radiotherapy

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Radiation is a cornerstone of successful cancer treatment, with one-half to two-thirds of all patients receiving radiotherapy. Survivors of cancer treated with radiation are at increased risk for cardiovascular disease (CVD). Understanding how radiation causes CVD will allow development of novel therapies. Irradiation of the lower hemi body, but not the upper hemi body, with 10 Gy in rats increases risk factors for CVD and results in cardiac fibrosis quantitatively similar to total body irradiation, suggesting radiation injury to the heart can be indirect. Simvastatin, an inhibitor of liver cholesterol synthesis administered continuously to rats (10 mg/kg/day) after 10 Gy lower hemi body irradiation mitigated against increased blood cholesterol and cardiac fibrosis. These findings indicate simvastatin limits transmission of a signal from the lower hemi body that decreases risk for the occurrence of CVD independent of any direct exposure of the heart to radiation. Bivariate examination of 3,607 patients following therapeutic lower hemi body irradiation using Chi-square, Wilcoxon rank-sum and t-tests was used to examine risk factors for CVD in patients diagnosed with congestive heart failure, myocardial infarction, atrial fibrillation, and cardiomyopathy before 80 years of age. We found that 47.4% of patients age 70–80 developed CVD compared to 29.7% who received simvastatin (p < 0.001, n = 293 and 361, respectively). Patients who were male, overweight, smokers, and had a diagnosis of chronic kidney disease and diabetes also had significantly higher risk of CVD. Race and hypertension were not indicative of increased risk for CVD. These clinical findings, taken together with the results from our animal studies, support a new research paradigm where radiation-induced heart disease can be indirect, with abdominal organs exporting factors that cause CVD. Simvastatin can be developed to mitigate and treat CVD after therapeutic radiation.

Conclusion:

This study analyzes how many patients with coronary heart disease (CHD) on it is unclear how many patients qualify for an anti-inflammatory therapy in everyday practice. This research has received full or partial funding support from the American Heart Association.

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Key Words: Endothelial function; Inflammation and inflammatory markers; Stem cell biology; Stem cells; Cell and atherosclerosis

Sacubitril/Valsartan Attenuates Fibrosis and Improves Left Ventricular Function in a Rabbit Model of HIFEF

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Background: Sacubitril/valsartan (SAC/VAL), a drug combining a neprilysin inhibitor and an angiotensin receptor blocker, was shown to reduce myocardial infarct size and left ventricular (LV) dysfunction in preclinical models of myocardial infarction (MI). In the PARADIGM-HF trial, SAC/VAL prevented the clinical progression of patients with heart failure (HF) more effectively than enalapril. Whether SAC/VAL attenuates cardiac fibrosis and improves LV function in a rabbit model of MI-induced HF with reduced ejection fraction (HFrEF) is unknown. Methods: Anesthetized adult male NZW rabbits (~2.5 kg) underwent left thoracotomy and the left anterior descending (LAD) coronary artery was identified and occluded for 45 min followed by reperfusion. Weekly echocardiography was performed to confirm reduced EF (~40%), which was uniformly achieved at 5 weeks post MI. Subsequently, rabbits were randomized to orally receive placebo (volume-matched water, BID), SAC/VAL (10 mg/kg, BID) or VAL (9.1 mg/kg/day) starting on week 6. At 10 weeks post MI, rabbits were sacrificed and hearts were harvested, fixed with 10% formalin and embedded in paraffin to assess myocardial fibrosis (Picrosirius red staining). Operations performed: echocardiography, Picrosirius red staining and analysis were blinded to treatment allocation. Results: Two weeks after treatment initiation, a significant improvement in LVEF was observed in the SAC/VAL group compared to both placebo and VAL, a benefit that lasted throughout the entire study (Fig. A). The functional improvement observed was associated with a significant reduction in LV scar size compared to placebo at week 10 (Fig. B). However, when compared to VAL, the decrease in scar size did not reach statistical significance despite a clear trend. Conclusion: Our results suggest that SAC/VAL may offer superior benefits compared to equivalent dose of stand-alone VAL in attenuating LV scar size and improving LVEF in a rabbit model of ischemic HFrEF.

Human iPSC-Derived Endothelial Cells Predict Predilection to Atherosclerosis by Endothelial Proinflammatory Activation

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Introduction: Coronary artery disease (CAD), the direct outcome of atherosclerosis, is the leading cause of death in the United States. Previous studies demonstrated that impaired function of aldehyde dehydrogenase 2 (ALDH2), a key enzyme for alcohol metabolism, is linked to increased susceptibility to CAD. A single-nucleotide polymorphism that generates E487K mutation (ALDH2*2) reduces enzymatic activity of ALDH2 to less than 40% of the wild type (WT) and is present in ~560 million people. However, it remains unclear how ALDH2 regulates atherosclerotic progression. Hypothesis: Recent studies suggest a critical role of ALDH2 in plaque development and endothelial activation. Therefore, we hypothesize that endothelial cells of ALDH2*2 carriers possess greater susceptibility to proinflammatory activation, whereby endothelial cells recruit immune cells, leading to increased risk of atherogenesis. Methods: To study the patient-specific effects of ALDH2*2 mutation on endothelial proinflammatory activation, we generated and characterized iPSC-derived endothelial cells (iPSC-ECs) from 5 WT subjects and 5 ALDH2*2 carriers. We exposed the iPSC-ECs to pro-inflammatory conditions and assessed the level of endothelial proinflammatory activation by gene expression analysis and monocyte adhesion assay. Results: Our preliminary data show ALDH2*2-iPSC-ECs exhibit impaired ALDH2 function resulting in metabolic dysregulation compared to WT. Presence of ALDH2*2 mutation resulted in enhanced inflammatory response in the iPSC-ECs when treated with proinflammatory cytokines such as TNF-α and IL-1β, as evidenced by up-regulation of cell adhesion molecules and augmented adherence to monocytes. The ALDH2*2-iPSC-ECs also exhibited an increased basal expression of vascular endothelial growth factor receptor 1 (FLT1) gene, which was further augmented upon inflammatory stimulation. FLT1 is a receptor for vascular endothelial growth factor ligands, playing a critical role in endothelial homeostasis and biology. Conclusion: Taken together, we elucidate the effects of impaired ALDH2 function on increased susceptibility to atherosclerosis by endothelial proinflammatory activation using patient-derived iPSC-ECs.

Need of Treating Residual Inflammatory Activity in Coronary Heart Disease: The Value of High Sensitive CRP and LDL in a Real World Cohort

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Introduction: Inflammation drives atherosclerosis and its complications. Thus, CANTOS as the first anti-inflammatory outcome trial in this population produced positive results. However, it is unclear how many patients qualify for an anti-inflammatory therapy in everyday practice. Hypothesis: This study analyzes how many patients with coronary heart disease (CHD) on...
Cardiac-Specific Overexpression Of Caveolin-3 Expedites Cardiac Relaxation After Adrenergic Stimulation

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Introduction: Caveolae are membrane localized signaling platforms that compartmentalize signal transduction proteins such as GPCRs. Previous studies from our laboratory have demonstrated that overexpression of caveolin-3 in cardiac myocytes (Cav3 OE) protects against pressure-overload induced heart failure. Additionally, Cav3 OE mice exhibit increased heart rate variability with lower nocturnal heart rates, but the mechanisms behind these phenotypes remain unclear. Hypothesis: Since Cav3 OE mice are protected from stressful cardiac stimuli, we tested the rationale that Cav3 OE hearts may show altered parasympathetic control of cardiac responses to adrenergic stimulation. Methods: Cav3 OE mice and transgene negative littermate controls (Ctrl) (12-16-week-old, n=10–11 each) were anesthetized with isoflurane and cardiac function measured by echocardiography at baseline. Isoprenaline (isoproterenol; Iso, 300 nM i.p.) was administered ~10 min after baseline recordings and cardiac function recorded at 2 min, 5 min, and 10–15 min after Iso. The same animal cohort received a single atropine injection (2 mg/kg i.p.) 20 min before isoflurane anesthesia and iso challenge. Echocardiography was performed as described above. Results: At baseline, no differences in cardiac function (ejection fraction, %EF;FS;SD) were detected between the two groups. After Iso injection, Cav3 OE mice demonstrated lower %EF at 2 min (p<0.001), lower %FS at 5 min (p<0.001), and higher %SD at 10–15 min after Iso (p<0.001). Importantly, Cav3 OE mice pre-treated with atropine no longer showed increased recovery at 5 min or 10–15 post Iso (p=0.007). A three-way ANOVA of the time-course after Iso injection found a significant effect of atropine on the responses of Cav3 OE versus Ctrl hearts to Iso (p=0.007). Conclusion: We show for the first time that Cav3 OE mice show a faster recovery from hypercontractility after isoproterenol stimulation. Since atropine abrogated this recovery, these data suggest that Cav3 OE mice exhibit increased parasympathetic tone that may be responsible for improved stress adaptation and heart rate variability.

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Key Words: Heart function tests; Adrenergic; Pharmacology; Heart rate/heart rate variability; Cardioprotection

24070

MFG-E8 Fragment Medin and Arterial Aging

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Background: Aging increases milk fat globule EGF-VIII (MFG-E8) expression in the rat, nonhuman primate and human aortic walls, facilitating the invasion, proliferation, and pro-inflammation of vascular smooth muscle cells (VSMCs). The MFG-E8 C-terminal fragment medin has been reported to be involved in the necrosis of VSMCs at the inflammatory sites of aortic dissection, however, its cleavage processes, aortic wall levels with aging and bio-role in VSMCs remains to be determined. Material and Methods: In this study, immunofluorescence, immunohistochemistry and western blot analysis demonstrate that MFG-E8 and its fragment medin (Figure), activated matrix metalloproteinase type II (MMP-2) and platelet derived growth factor receptor type-beta (PDGFR-β) protein levels were markedly increased in human grossly normal aortic walls from old (n=10, >50 yrs) vs. younger donors (n=10, <25 yrs). Importantly, exposure of medin peptide (20 to 100nM) to primary cultured VSMCs isolated from young (8 mo) and old (30 mo) FVBn rat aortae significantly increased MMP-2 activation, PDGFR-β expression, and migratory capacity measured by a modified Boyden chamber in a dose-dependent manner in both also. Separate exposure of activated MMP-2 to recombinant human MFG-E8 protein and also to MFG-E8 enriched old human aortic protein markedly increased the cleavage product medin in both, which was substantially inhibited by an MMP inhibitor, GM6001. Exposure of medin to VSMCs did not significantly affect the expression of cell cycle related proteins. In addition, PDGF-BB treatment markedly activated MMP-2 in both young and old VSMCs, which was substantially reduced by a PDGFR-β inhibitor, RTK. Conclusion: Taken together, targeting MFG-E8 or its cleavage product medin is a novel approach to the prevention or treatment of large arterial aging or age-associated disease.

Author Disclosures: Y. Wang: None.

Key Words: Aging; Arteries; Cardiovascular disease

24024

Utilizing Telemedicine to Construct Population-Based STEMI Systems of Care in Developing Countries

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Background: Major disparities exist between developed and developing countries in the outcomes of patients presenting with Acute Myocardial Infarction (AMI). Telemedicine has emerged as a powerful, cost-efficient, and scalable tool. Whether telemedicine can improve AMI results remains unclear. Methods: We incorporated a hub and spoke strategy for Latin America Telemedicine Infarct Network (LATIN) to expand access to care in regions in Brazil, Colombia, and Mexico that lacked AMI care. Small clinics and primary care health centers in remote areas (spokes) were strategically connected to hubs that could perform 24/7 primary PCI. Experts at 3 central sites in Uberlandia, Sao Paulo, and Bogota, provided urgent EKG diagnosis and tele-consultation for the entire LATIN network by triggering ambulance dispatch and implementing standardized AMI protocols. Results: A total of 257 LATIN centers (Brazil 95, Colombia 113, Mexico 48) were networked using similar telemedicine protocols. In Colombia, LATIN coverage was established to cover 31% of the nation’s 48 million population. With this expanded geographic reach, 4,894 (1.2%) of the 401,095 screened patients were diagnosed as having STEMI. A total of 2,041 (43.5%) STEMI were urgently reperfused. Primary PCI was performed in the majority of patients - 1,578 (77.3%) that were referred for urgent perfusion. The major reasons for non-treatment included insurance denials, lack of ICU beds and chest pain >12 hours. Time to Telemedicine Diagnosis (TTD) was 5.1 minutes, and tele-ECG accuracy was 98%. D2B time for the cohort was 53 minutes, but chest pain to treatment time was >6 hours. Overall, in-hospital mortality was 5.8%. Conclusion: LATIN demonstrates the feasibility of creating a population based and telemedicine-guided AMI management strategy that can hugely expand access to reperfusion strategies. Telemedicine has important public health implications as a global approach to urgent AMI care in developing countries.
Genome-Wide Association Study of Vasodilator Response
in Pulmonary Arterial Hypertension

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Introduction: Pulmonary arterial hypertension (PAH) is a rare and fatal disease associated with variable therapeutic response, suggesting a genetic contribution. Vasodilator-responsive PAH accounts for a minority of PAH cases and is associated with dramatically improved survival over vasodilator-nonresponsive PAH. The objective of our study was to identify genetic influences on vasodilator drug response in PAH. Methods: Two cohorts of patients with Group I PAH confirmed by right heart catheterization were derived from the PAH Biobank (NHLBI R24HL105333), representing over 40 US institutions. Differences between hemodynamics at rest (baseline mean pulmonary arterial pressure, mPAP) and after vasodilator (nitric oxide, prostacyclin) administration were determined to define acute vasodilator drug response (with unchanged cardiac index) as a continuous measure. All cases were genotyped using HumanOmni5 with single nucleotide polymorphism (SNP) call rate >99%, minor allele frequency (MAF) >3%, and Hardy-Weinberg p-value >0.01. Analysis was restricted to cases with European ancestry. We performed linear regressions in an additive model for acute vasodilator drug response with adjustment for baseline mPAP, age, gender, and first 3 principal components. A Bonferroni-corrected alpha=5x10-8 was used in the discovery cohort and alpha=0.05 was used in the replication cohort. Results: The discovery cohort included 434 PAH cases and the replication cohort included 49 less severe PAH cases. QQ-plots showed no evidence of genomic inflation (lambda=1.00). Association of the intronic SNP rs8057488 (MAF=0.05) in the sorting nexin 29 gene (SNX29) with acute vasodilator drug response reached genome-wide significance in the discovery cohort (beta=-7.03 mmHg, p=3.39x10-8). In addition, calpain-2 depletion significantly attenuated AngII-induced expansion of ex-vivo maximal diameter of abdominal aortas in obese mice (Cre+ compared to non-Cre littermates). Mice were fed a high fat diet (60% Kcal) for 20 weeks. After 16 weeks of diet feeding, mice were infused with AngII (1,000 ng/kg/min) by osmotic minipumps for 4 weeks. Depletion of calpain-2 had no effect on high fat diet-induced body weight gain, fat mass, glucose and insulin tolerance. Interestingly, calpain-2 depletion significantly attenuated AngII-induced expansion of ex vivo maximal diameter of abdominal aortas in obese mice (Cre+: 1.4 ± 0.14, Cre+/-: 0.9 ± 0.04 mm; P<0.001). Conclusion: These findings suggest that calpain-2 plays a critical role in AngII-induced AAA development in diet-induced obese mice.

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