Heart Institute Retreat

7:30-8:15	**Breakfast**	
8:15-8:45	Executive Co-Directors	State of the Heart Institute
8:45-9:00	Scott Hamlin	T-Building Update

Session 1 Cardiomyopathy and Heart Failure **Moderator: Stephanie Ware**

9:00-9:30	David Morales	The potential for creating functional myocardium and tissue engineered valves: Near or Distant Future
9:30-10:00	Burns Blaxall	Exploring new targets for heart failure with small molecules
10:00-10:30	Jeff Towbin	Final Common Pathways Responsible for Cardiomyopathies

10:30-11:00 Coffee Break

Session 2 Valve & Vascular Disease **Moderator: Michael Taylor**

11:00-11:30	Robert Hinton	Early angiogenesis due to elastic fiber fragmentation in aortic valve disease
11:30-12:00	Elaine Urbina	Mechanisms for Increased Arterial Stiffness with CV Risk Factors
12:00-12:30	Katherine Yutzey	Molecular mechanisms of aortic valve disease: lessons from pediatric and adult patients applied to therapeutic studies in mouse models

12:30-1:30 Lunch

1:30-2:45 HI Resources **Moderator: Jeff Robbins**

Tiffany Khodadad	HI Research Core (HIRC)
Robert Hinton	HI Biorepository (HIBR)
Bradley Marino	HI Data Registry (HIDR)
Christopher Learn	Adult Congenital Heart Disease
Mehdi Keddache	Genetic Variation and Gene Discovery Core Facility

2:45-3:00 Coffee Break

Session 3 Brain Growth and Morbidities with Cardiovascular Malformations
Moderator: Cliff Chin

3:00-3:30	Jim Cnota	Intrauterine growth and development in babies with cardiovascular malformations: research questions and clinical implications
3:30-4:00	Bradley Marino	Neurodevelopmental outcomes in CHD survivors: research questions and clinical implications

4:00-6:00 Poster session, judging, social hour

2012

Heart Institute Research Retreat

Poster Abstracts

Listed alphabetically by first author.

1. Bex1, a novel pivotal player in the translational control of cardiac hypertrophy

Federica Accornero[1], Jop Van Berlo[1] and Jeffery D. Molkentin[1, 2]

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Objective. The development of cardiac hypertrophy requires an increase in protein synthesis by individual cardiac myocytes. Although significant progresses have been achieved in the understanding of the transcriptional control of cardiac hypertrophy, the regulation of translation in the cardiomyocytes is still poorly elucidated. Here we examined the role that Brain-Expressed X-linked protein 1 (BEX1) plays in the heart as a regulator of translation during cardiac hypertrophy and its influence on cardiac adaptation to stress stimulation using BEX1 knockout mice.

Methods and results. We identified BEX1 as an upregulated factor in heart failure models, which is predominantly produced in the heart by cardiac myocytes. We studied BEX1 knockout mice. While these mice did not have a baseline phenotype, they showed a decreased hypertrophic response to pressure overload stimulation and preserved cardiac function. Similarly, BEX1 knockout mice were protected toward cardiac remodeling and the development of fibrosis induced by isoproterenol infusion. By a proteomic approach we found 60S ribosomal protein L22 (Rpl22), ATP-dependent RNA helicase DDX1, ATP-dependent RNA helicase DDX3x and glutamyl-prolyl-tRNA synthetase (EPRS) as novel BEX1-interactors. Due to the known role for those interactors in mRNA metabolism and translation we hypothesize a role for BEX1 in the post-transcriptional control of protein synthesis. Specifically, we predict that BEX1 function in the heart by promoting the translation of a subset of pro-hypertrophic mRNAs that contribute to the pathological cardiac hypertrophy during stress conditions.

Conclusion and perspective. BEX1 is a novel regulator of cardiac hypertrophy through a post-transcriptional mechanism. The next challenge will be the understanding of the subset of mRNAs directly controlled by BEX1 and the development of strategy to therapeutically target BEX1 in the heart.

2. Notch and Wnt signaling pathways regulate heart valve cell proliferation and extracellular matrix gene expression

Christina M. Alfieri, Ming Fang, Timothy J. Mead, and Katherine E. Yutzey

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In the developing heart, the extracellular matrix (ECM) of the valve leaflets becomes stratified into fibrosa, spongiosa, and elastin-rich layers, oriented to blood flow, but the process by which this occurs has not been defined. Notch and Wnt signaling pathways are differentially active in the remodeling valve leaflets, but the requirements and functions of these pathways in valve leaflet maturation are not known. Activated Notch signaling is apparent on the endothelial surface surrounding the endocardial cushions at mouse embryonic day (E)12.5 and then becomes localized to the flow side of the valve primordia at E14.5. The TOPGAL Wnt reporter transgene is active in mice throughout the developing AV and semilunar valves at E16.5, with more localized expression in the stratified valve leaflets after birth. In cultured avian embryonic aortic valve interstitial cells (VICs), increased Wnt signaling promotes cell proliferation and expression of *periostin* and *matrix gla-protein* ECM genes. Induction of Notch signaling also promotes cell proliferation while inducing stabilization of the Wnt pathway intermediate b-catenin. Likewise increased Wnt signaling induces expression of Notch pathway target genes *Hey1* and *Hes1*, supporting crosstalk between the pathways. Notch and Wnt pathways were conditionally manipulated in vivo in mice using *Col2a1Cre*, which is expressed on the fibrosa surface layer of the developing valves. In these mice, limited valvular expression and severe defects in skeletal development leading to perinatal lethality precluded the detailed analysis of valve stratification mechanisms. *PeriostinCre* mice have been obtained for more complete manipulation of Notch and Wnt signaling in heart valve primordia. Together, these analyses provide initial evidence for Notch and Wnt signaling in regulation of cell proliferation and ECM gene expression in the remodeling valves.

3. Induced basal autophagy in Atg7 mice ameliorates cardiac proteinopathy

Md. Shenuarin Bhuiyan, J. Scott Pattison, Jeanne James, James Gulick, Hanna Osinska, and Jeffrey Robbins

Division of Molecular Cardiovascular Biology, The Heart Institute, Department of Pediatrics, The Cincinnati Children's Hospital Medical Center, Cincinnati, OH 45229, USA

Background: Basal autophagy is a cellular homeostatic mechanism that can eliminate damaged and potentially toxic proteins and organelles. We hypothesized that cardiac proteinopathies, such as desmin-related cardiomyopathy (DRC) that lead to accumulations of misfolded proteins, can trigger alterations in cardiomyocyte autophagy as an adaptive process. However, a mouse model of cardiac specific-induced basal autophagy is lacking and the effects of acute or chronic increased basal autophagy remains unclear.

Hypothesis*:* To determine whether induction of basal autophagy in the cardiomyocyte population in transgenic mice can ameliorate a genetic model of cardiac proteinopathy.

Methods and Results: The protein Atg7 is essential for autophagy and to induce high levels of basal autophagy we generated an inducible transgenic (Tg) mouse that can express this protein and thus, potentially upregulate autophagy. This mouse is able to undergo cardiac specific overexpression of the Atg7 gene, where the transgene can be switched on or off by doxycycline. The Atg7-Tg mouse showed 10 fold overexpression of Atg7 protein with normal heart morphology and function being maintained even after 10 months. Overexpression of Atg7 had no detectable effect on other proteins involved in the autophagy-lysosomal pathways. However, the hearts show increased basal autophagy, as measured by increased autophagic flux activity and cardiomyocyte ultrastructural examination. We showed earlier that autophagy is down-regulated in cardiac proteinopathy as observed in a mutant αB-crystallin ($CryAB^{R120G}$) mouse model of DRC. Therefore, we crossed the $CryAB^{R120G}$ mice to Tg mice with cardiac specific overexpression of Atg7. Sustained

Atg7-induced autophagy in CryAB^{R120G} hearts prolonged CryAB^{R120G} survival by 40% and led to maintenance of cardiac function and decreased cardiac hypertrophy.

Conclusions: Cardiac-specific Atg7 inducible-Tg mice with increased basal autophagy may serve as a novel model to study the regulatory role of autophagy in the heart. Induced basal autophagy in Atg7-Tg mice can extend the life-span in a model of cardiac proteinopathy. Modulating the overall autophagic state may be useful therapeutically in heart disease.

4. Tcf21 is activated during cardiac fibrosis in multiple mouse models of heart disease

Caitlin M. Braitsch, Onur Kanisicak, Jop H. van Berlo, Jeffery D. Molkentin, and Katherine E. Yutzey

Division of Molecular Cardiovascular Biology, CCHMC, Cincinnati, Ohio 45229

Coronary heart disease is the leading cause of death worldwide. During embryonic heart development, the bHLH transcription factor Tcf21/Pod1 promotes epicardium-derived cell differentiation into fibroblasts and inhibits smooth muscle (SM). Its role in the adult heart, however, is unknown. In order to define the role of Tcf21 in cardiac fibrosis, its expression was examined in different types of heart disease induced in mice harboring a *Tcf21*^{LacZ} knock-in reporter allele. The hypothesis is that **Tcf21 is reactivated following cardiac injury and promotes cardiac fibrosis in diseased adult hearts.** Mice subjected to ischemia/reperfusion (I/R) have increased subepicardial cells positive for WT1, RALDH2, and bGal, indicative of *Tcf21*^{LacZ} expression. Interstitial fibrotic regions in I/R hearts also actively express bGal and WT1, indicating reactivation of gene networks important during heart development. *Tcf21*^{LacZ} mice subjected to transverse aortic constriction (TAC) have extensive coronary perivascular fibrosis but limited epicardial activation. *Tcf21*^{LacZ} mice treated with chronic Angiotensin II (AngII) infusion also have perivascular and interstitial fibrosis but not epicardial activation. These data indicate that localized cardiac fibrosis is differentially activated depending on the type of cardiac injury. *Tcf21*^{LacZ} expression is activated in fibrotic regions of I/R, TAC, and AngII-treated mouse hearts, consistent with our previous report that Tcf21 is required for development of cardiac fibroblasts in the prenatal heart. Interestingly, robust perivascular bGal expression in TAC and AngII hearts is observed coincident with increased fibroblast and decreased SM markers. These data are consistent with Tcf21 promotion of fibroblasts and inhibition of SM cell fates in adult cardiac fibrosis similar to embryonic cardiac development. The long-term goal of these studies is to define potentially diverse molecular mechanisms of cardiac fibrosis dependent on localization and cell type activation in adult heart disease of distinct pathologic origins.

5. The Na+/Ca2+ exchanger 1 (NCX1) exacerbates dystrophic pathology of the hindlimb while rescuing the diaphragm

Adam R. Burr[1], Douglas P. Millay[1], Sanjeewa Goonasekera[1], and Jeffery D. Molkentin[1]

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Recent evidence from mouse models suggests that increased sarcolemma Ca2+ influx contributes to dystrophic pathology in skeletal muscle. Thus, one potential therapeutic strategy would be to increase sarcolemmal Ca2+ efflux or clearance. Expression of NCX1 is increased in the mdx and Sgcd mouse models. We initially hypothesized that such upregulation in NCX1 would be compensatory by helping increase sarcolemmal Ca2+ efflux, hence reducing myofiber necrosis and pathology. To mechanistically evaluate this hypothesis we generated transgenic mice that overexpress the NCX1 under the control of the human skeletal a-actin promoter. Surprisingly, NCX1 transgenic mice developed progressive hindlimb pathology. When NCX1 transgenic mice were crossed with the mdx and Scgd-/- dystrophic models, hindlimb pathology was dramatically exacerbated. However, NCX1 overexpression rescued pathology in the diaphragm of both dystrophic models. This suggests that NCX1 overexpress ion increased reverse mode exchange activity and net Ca2+ influx in the hindlimb while increasing forward mode activity and Ca2+ clearance in the diaphragm. These results suggest that inhibition of reverse mode activity could represent a novel treatment approach. DPM generated the transgenic mouse and performed initial phenotypic analysis. ARB performed photometry, biochemical experiments, and later mouse crosses included in this work.

This work was supported by grants from the NIH, Howard Hughes Medical Institute, and the Jain Foundation.

6. Increased Tbx20 expression promotes cardiomyocyte cell cycle progression in adult mice

Santanu Chakraborty, Arunima Sengupta and Katherine E. Yutzey

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During heart development cardiomyocytes (CMs) are highly proliferative, but adult CMs are relatively nonproliferative, thus contributing to the severity of myocardial injury and restricted cardiac repair *in vivo*. The ability of the T-box transcription factor Tbx20 to promote CM proliferation in neonatal and adult CMs was examined using a conditional gain-of-function approach in mice. We hypothesize that increased Tbx20 maintains differentiated CMs in a primitive state with increased fetal gene expression and proliferative capacity in the adult heart through activation of multiple signaling mechanisms. In neotnatal (N) hearts, βMHC-Cre mediated overexpression of Tbx20 results in increased phospho-histone H3 (pHH3) positive CMs compared to littermate controls (2.3% versus 1.3% at N1; 0.56% versus 0.25% at N8). Likewise, in adults, βMHCCre-mediated overexpression of Tbx20 results in an increased percentage of small, cycling, mono-nucleated CMs, marked by persistent expression of fetal cardiac genes, ssTnI and βMHC, and decreased expression of the senescence marker $p16^{INK4A}$ in the absence of cardiac hypertrophy. Adult hearts overexpressing Tbx20 are also protected against ischemia/reperfusion (IR) injury, but the protection mechanism has not yet been identified. In addition, adenovirus-mediated Tbx20 overexpression in neonatal rat CM leads to increased expression of both karyokinesis and cytokinesis markers. Increased activation of Bmp2-Smad1/5/8 signaling and elevated expression of PI3-AKT-GSK3β kinase signaling is also detected relative to β-gal infected controls. Together, these data demonstrate that Tbx20 overexpression promotes CM cell cycle progression and activates fetal cardiac proliferative pathways in adult hearts *in vivo*. Thus the manipulation of Tbx20 and downstream affected signaling pathway(s) has potential as a new therapeutic approach to promote myocardial regeneration or repair.

7. Overexpression of the Na^+/K^+ ATPase α2 but not α1 isoform attenuates pathological cardiac hypertrophy and remodeling

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[1]These authors contributed equally

The Na^+/K^+ ATPase (NKA) directly regulates intracellular Na^+ levels, which in turn indirectly regulates Ca^{2+} levels by proximally controlling flux through the Na^+/Ca^{2+} exchanger (NCX1). Elevated Na^+ levels have been reported during heart failure, which permits some degree of reverse mode Ca^{2+} entry through NCX1, as well as less efficient Ca^{2+} clearance. To determine if maintaining lower intracellular Na^+ levels by NKA overexpression in the heart would enhance forward-mode Ca^{2+} clearance and prevent reverse-mode Ca^{2+} entry through NCX1 as a protective and anti-hypertrophic measure, we generated cardiac-specific transgenic mice overexpressing either the NKA-α1 or α2 isoform and subjected them to pressure overload hypertrophic stimulation. We found that while increased expression of the NKA-α1 isoform had no protective effect, overexpression of NKA-α2 significantly decreased cardiac hypertrophy after pressure overload in mice at 2, 10 and 16 weeks of stimulation. Remarkably, total NKA protein expression was not altered in either of these 2 transgenic models, as increased expression of one isoform led to a concomitant decrease in the other endogenous isoform. While total NKA ATPase activity and intracellular Na^+ levels were unchanged in either overexpression model, and both showed reduced Ca^{2+} transient amplitudes and sarcoplasmic reticulum Ca^{2+} load, only NKA-α2 overexpression led to faster removal of bulk Ca^{2+} from the cytosol in a manner requiring NCX1 activity. This increased NCX1 activity, though correlated with improved outcome after pressure overload, did not affect signaling through Ca^{2+}-sensitive signaling pathways such as calcineurin/nuclear factor of activated T-cells (NFAT), Ca^{2+}/calmodulin-dependent kinase II (CaMKII), or protein kinase Cα

(PKCα). Our results suggest that the protective effect produced by increased expression of NKA-α2 after pressure overload is likely due to Na^+ regulation in a unique signaling microdomain distinct from NKA-α1, leading to preservation of forward-mode NCX1 activity during disease, in association with optimized cardiac function.

8. Functional analysis of novel ZIC3 mutations identified in patients with heterotaxy

Jason Cowan, Muhammad Tariq, Stephanie M. Ware

Loss of function mutations in the zinc finger in cerebellum 3 (ZIC3) transcription factor result in heterotaxy, a condition characterized by abnormal left-right positioning of thoraco-abdominal organs and a wide variety of additional congenital anomalies, particularly of the cardiovascular system. Mutations in *ZIC3* have been reported in approximately 75% of all familial and 1% of all sporadic heterotaxy cases and have been associated with VACTERL, a constellation of malformations phenotypically overlapping heterotaxy. The presence of a polyalanine expansion in a patient with VACTERL is of particular interest as pathogenic expansions have been identified in several other developmentally critical transcription factors, including *ZIC2.* To further define the incidence and functional significance of *ZIC3* mutations in heterotaxy, *ZIC3* coding regions and splice junctions were screened in 200 unrelated heterotaxy patients. Ten mutations (8 novel) were identified, including a single alanine expansion (c.insGCC159-160). Functional analyses were supplemented by 4 additional, recently reported *ZIC3* mutations. Aberrant ZIC3 cytoplasmic localization was observed for mutations spanning multiple nuclear localization signals and correlated with decreased transactivation of a luciferase reporter. A missense mutation within zinc finger 5 (p.A447G) surprisingly increased luciferase transactivation, despite promoting aberrant ZIC3 localization. Neither polyalanine tract expansion differed significantly from wild-type with respect to either luciferase transactivation or ZIC3 subcellular localization. These analyses collectively indicate a higher than expected percentage of *ZIC3* mutations in patients with sporadic heterotaxy and suggest alternative pathogenesis of some *ZIC3* mutations, notably those within the polyalanine tract.

9. RARαb1 deficient embryos have increased RA signaling that promotes cardiomyocyte specification in zebrafish

Enrico D'Aniello and Joshua S. Waxman

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One function of retinoic acid (RA) signaling during development is to restrict cardiomyocyte (CM) specification. Understanding the transcriptional mechanisms RA signaling uses to restrict CM specification will aid our ability to precisely direct CM differentiation in vivo and in vitro. In vertebrates, however, the specific RA receptors (RARs) required to restrict CM specification have not been reported. Here, we find embryos deficient for RARαb1, a previously unrecognized conserved zebrafish RARα splice variant, have enlarged hearts with increased CM number. Surprisingly, we find that expression of *hoxb5b,* which RA signaling positively regulates and was found to restrict atrial cell number, was increased in RARαb1 deficient embryos. Importantly, depletion of *hoxb5b* is able to rescue the enlarged hearts found in RARαb1 deficient embryos, suggesting that the increase of *hoxb5b* at least in part causes the increase in the CM number. In contrast to inhibiting RA signaling components using other methods, examination of additional RA responsive genes and RARs indicates that their expression is also increased in RARαb1 deficient embryos, suggesting that the loss of RARαb1 results in a general increase of RA signaling. Although effects were not observed on the development of the heart size or CM number, similar effects on RA signaling components were found in RARαb2 deficient embryos. Altogether, our results suggest an intriguing model where depletion of RARαb1 results in a feedback loop that induces modest increases in RA signaling, which ultimately promote increased CM specification.

10. A TRPC6-dependent pathway for myofibroblast transdifferentiation, fibrosis and infarct healing in vivo

Jennifer Davis[1], Adam R. Burr[1], Gregory F. Davis[1], Lutz Birnbaumer[3], & Jeffery D. Molkentin[1,3]*

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With injury or cytokines fibroblasts become activated and transdifferentiate into myofibroblasts, cells that secretes high levels of extracellular matrix (ECM) proteins and develop contractile properties necessary for wound repair and tissue remodeling. Here we conducted a genome-wide screen in transformed mouse embryonic fibroblasts (MEFs) to identify novel genes that directly program myofibroblast transformation, which identified the transient receptor potential canonical member 6 (TRPC6). We show that TRPC6 expression is both necessary and sufficient to drive myofibroblast transformation through a Ca^{2+}-calcineurin-nuclear factor of activated T-cell (NFAT) signaling pathway. TRPC6 overexpression in cardiac, dermal, and embryonic fibroblasts fully induced myofibroblast transformation independent of the profibrotic ligand transforming growth factor b (TGFb), but dependent on calcineurin and p38 mitogen-activated protein kinase (MAPK) signaling through serum response factor (SRF). Genetic ablation of the *Trpc6* gene prevented myofibroblast transformation by TGFb and angiotensin II and significantly impaired dermal and cardiac wound healing after injury. *Ppp3cb*$^{-/-}$ (calcineurin Ab) mice were also impaired in dermal wound healing. Myofibroblast function and wound healing was restored in *Trpc6*$^{-/-}$ mice by reconstituting TRPC6 expression or by activating its downstream effector calcineurin using adenoviral gene transfer. Our data define a comprehensive pathway whereby profibrotic ligands (TGFb, AngII, etc) promote p38 MAPK signaling to induce SRF transcriptional activity that then upregulates TRPC6 expression to promote Ca^{2+} entry and calcineurin-NFAT signaling for programming transdifferentiation of fibroblasts into myofibroblasts.

11. *Coup-tf1a* acts downstream of RA signaling to predominantly restrict ventricular cell number in zebrafish

Tracy Dohn and Joshua Waxman

The regulation of cardiac cell specification and differentiation plays an essential role during vertebrate development. A better understanding of the molecular regulatory pathways that direct cardiac development is required to generate novel stem cell therapeutics. Retinoic acid (RA) signaling is one of the few pathways found to restrict cardiac specification in vertebrates. However, downstream effectors of this signal are not yet well understood. In a screen for RA target genes in zebrafish, *coup-tf1a* which is expressed in the lateral plate mesoderm adjacent to the cardiac progenitors, was identified as a candidate effector of heart size. In order to determine if *coup-tf1a* is required to limit heart size, we used splice blocking morpholino oligonucleotides. We found that *coup-tf1a* deficient embryos exhibit enlarged hearts with an increase in cardiac cell number similar to what is found in RA signaling deficient embryos. However, while RA signaling affects both cardiac chambers, Coup-tf1a is required to predominantly restrict ventricular cell number. Additional analysis of *coup-tf1a* deficient embryos found an increase in the expression of ventricular cardiac progenitor and ventricular differentiation markers suggesting a role for Coup-tf1a in restricting ventricular cell specification. Together, our data nicely complement previous studies of Hoxb5b, which is required to restrict atrial cell number, and suggest that Coup-tf1a functions downstream of RA signaling to allow for the proper allocation of cells within each cardiac chamber through restricting ventricular cell specification.

12. The Role of BMP Signaling in the Progression of Calcific Aortic Valve Disease (CAVD)

M. Vicky Gomez, Jonathan D. Cheek, Elaine E. Wirrig & Katherine E. Yutzey

Calcific Aortic Valve Disease (CAVD) is a significant cause of mortality affecting >2% of the US population, and its prevalence increases to 10% in the aged population. The current standard of care is valve replacement, which is the second most common open-heart surgery with ~100,000 replacements every year. CAVD is a progressive disease and despite its clinical significance, the specific pathogenic mechanisms regulating its development are not well understood. Human studies of diseased valves suggest that the BMP signaling network plays a critical role in the progression of CAVD. In addition, BMP signaling has essential functions during osteogenic gene induction, a key feature of CAVD. Our hypothesis is that activation of BMP signaling promotes CAVD progression. Our goal is to determine the components of the BMP pathway involved in CAVD and the particular time-points at which activation occurs. The *Klotho*-null mouse is a model of premature aging that develops aortic valve calcification at the hinge region similar to human CAVD. In addition, there is an induction of osteogenic genes localized to the calcified region. In these mice, calcification occurs independent of inflammation and valve thickening, supporting an intrinsic mechanism for calcification. Ongoing studies show increased phosphorylation of Smad1/5/8, downstream effectors of the BMP pathway, in both the *Klotho*-null mice and human adult calcified valves. Moreover, increased pSmad1/5/8 precedes osteogenic gene induction and its activation is localized with calcification in the *Klotho*-null mouse. Thus, we hypothesize that adult aortic valve calcification occurs by a BMP-dependent osteogenic mechanism. Future studies include treatment of the *Klotho* mice with LDN-193189, a small molecule inhibitor of BMP receptor activity, to determine its potential pharmacological role in the prevention or inhibition of CAVD and thus provide an effective therapy for CAVD.

13. STIM1 Overexpression in Skeletal Muscle Reproduce Some of the Hallmarks of Dystrophic Muscle

Sanjeewa A. Goonasekera, Robert T. Dirksen and Jeffery D. Mollkentin

STIM1, an ER/SR resident Ca^{2+} sensing protein together with sarcolemmal Orai1 channels is believed to orchestrate Ca^{2+} entry in skeletal muscle following SR Ca^{2+} store depletion. Genetic mouse models with loss of function of STIM1 and Orai1 demonstrate impaired skeletal muscle growth and perinatal lethality suggesting STIM1-Orai1 mediated Ca^{2+} entry is critical for skeletal muscle growth and function. Interestingly, both STIM1 and Orai1 protein expression is also elevated in muscular dystrophy with a concomitant increase in store operated Ca^{2+} entry. Although Ca^{2+} overload can precipitate the dystrophic phenotype, whether STIM1-Orai1 mediated increase in store operated Ca^{2+} entry is compensatory or deleterious needs to be established. To specifically assess if increased STIM1 expression is detrimental to skeletal muscle function, we generated a mouse model of STIM1 overexpression in a skeletal muscle. At 6 weeks, STIM1 overexpression in a number of skeletal muscle groups was confirmed. Photometry experiments in acutely isolated flexor digitorum brevis myofibers were used to determine if STIM1 overexpression resulted in enhanced store operated Ca^{2+} entry. Histological analysis demonstrated that increase in STIM1 expression resulted in a large percentage of myofibers with centrally localized nuclei, infiltration of inflammatory cells and enhanced interstitial fibrosis and elevated serum creatine kinase levels. Electron microscopic analysis exhibited swollen mitochondria and myofilament disarray. However, properties of electrically evoked Ca^{2+} transients and exercise capacity as assessed by treadmill running was only modestly affected in STIM1 tg mice compared to Ntg littermates at 6 months of age. Taken together, our data demonstrates that STIM1 overexpression by itself can reproduce some of the hallmarks of muscular dystrophy suggesting increased expression of STIM1 can be responsible for the deleterious phenotype associated with dystrophic muscle.

14. Functional Dissection of Myosin Binding Protein C Phosphorylation

Manish K Gupta[1], James Gulick[1], Hanna Osinska[1], Jeanne James[1], Valerie Lasko[2], Michelle Nieman[2], John N. Lorenz[2] and Jeffrey Robbins[1]

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Rationale: Phosphorylation of cardiac myosin binding protein C (cMyBP-C) plays an important role in cardiac function and is actively regulated both in the normal heart and during the development of cardiac disease. Hence it is critical to understand the mechanistic and functional consequences of cMyBP-C phosphorylation.

Objective: To examine the non-equivalency of the group of three phosphorylatable sites (Ser-273, Ser-282, and Ser-302) whose phosphorylation states have been defined as being essential when ablated in concert in a transgenic mouse model. However, the distinct role, if any, of each individual phosphorylation site is not established.

Methods and Results: Three transgenic lines were generated: DAA that expressed cMyBP-C containing Asp273-Ala282-Ala302, AAD, in which a charged amino acid was placed at residue 302 (Ala273-Ala282-Asp302) and SDS (Ser273-Asp282-Ser302). The effects of these replacements were determined at the whole animal and organ levels. The DAA and AAD mice showed pathology that was more severe than either the cMyBP-C null animals or animals that expressed cMyBP-C that was unable to be phosphorylated at any of the three sites. Both the DAA and AAD hearts exhibited atrial enlargement, left ventricular chamber dilation, extensive interstitial fibrosis, irregular cardiac rhythm and sudden cardiac death. Ultrastructural studies of DAA mice confirmed myocyte disarray. Yeast two hybrid analyses were used to determine the effects of altering the charge at these sites with respect to the ability of cMyBP-C to bind to the S2 domain of myosin or cardiac actin. While phosphorylation at either Ser-273 or Ser302 did not affect myosin S2 interaction, substitution of Ser 282 with a

charged residue prevented interaction of cMyBP-C with both S2 myosin and actin.

Conclusion: Our results confirm the non-equivalency of these three sites in terms of the effects of their post-translational modifications on cMyBP-C functionality in the heart. Ser-282 appears to be the key residue in controlling S2 interaction with the protein.

15. Readmission within 30 days of congenital heart surgery: incidence, risk factors, and resource utilization

Samuel Hanke MD, Catherine Krawczeski MD, Bradley Marino MD, Samir Shah MD, Edmund Berry PhD, Eileen King PhD, Lynn Darbie MS, James Cnota MD

Background: Hospital readmission is a widely accepted measure of healthcare quality. Children who have undergone congenital heart surgery (CHS) have high resource utilization during the surgical hospitalization and are at risk for increased resource utilization from readmissions. Given the unique characteristics of CHS, readmission research from other populations is not generalizable. Our objective was to identify the incidence of hospital readmissions within 30 days of discharge after CHS, identify risk factors for readmission and describe the resource utilization of these readmissions.

Methods: Children age 0-17 years who underwent CHS (ICD-9 procedural codes *35.xx-39.xx*) between January 1, 2006 and September 30, 2011 were identified using the Pediatric Health Information System (PHIS), an administrative database from 43 children's hospitals in the United States. Readmission was defined as any admission within 30 days of discharge from index hospitalization. Patient demographics, index hospitalization factors and readmission details were analyzed. A Zero Inflated Poisson regression was used to model the relationship between readmissions and patient specific factors. Generalized estimating equations were used to model hospital charges at readmission.

Results: Out of the 53,105 patients that had a CHS discharge; 10,667 (20.1%) were readmitted within 30 days. Total readmission hospital charges were $498,539,570 over the study time period. Readmission occurred a median of 9 days after discharge (IQR 4-16), with a median of 1 day duration (IQR 1-4) and incurred a median of $4,216 in charges (IQR $534-$25,504). Independent risk factors for readmission included longer initial hospitalization (total and ICU), more charges at initial hospitalization, higher RACHS-1 score, heart transplantation, Medicaid insurance and the presence of gastrointestinal, respiratory or neuromuscular co-morbidities.

Independent risk factors for increased utilization at readmission included younger age at initial hospitalization, RACHS 5-6 and heart transplantation.

Conclusions: Hospital readmissions following CHS are frequent and associated with significant resource utilization. Identification of at-risk patients for readmission provides an opportunity for targeted interventions to reduce readmissions.

16. Myopalladin nonsense mutation causes restrictive cardiomyopathy via ERK1/2 and CARP down-regulation

Anne-Cecile Huby, Ph.D., Ken Takagi, M.D., Nan Gong, M.D., Jeanne James M.D., Ruben Martherus, Ph.D., Kristen Kramer, B.S., Zaza Khuchua Ph.D., Jeffrey A. Towbin, M.D., Enkhsaikhan Purevjav M.D., Ph.D.

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Introduction: Left ventricular (LV) diastolic dysfunction, dilated atria, and restrictive physiology (RP) despite normal systolic function, normal LV size and thickness are the characteristics of restrictive cardiomyopathy (RCM). Histology reveals increased myocardial stiffness and fibrosis. A variety gene abnormalities has been identified in familial RCM (FRCM); including our recently identified mutations in the Z-disk gene myopalladin (*MYPN*). Here, a novel nonsense *MYPN* mutation (Q529X-MYPN) in FRCM was analyzed for causative mechanisms.

Hypothesis: MYPN is a nodal messenger molecule that transmits stretch-signaling from Z disk to nucleus. Q529X-MYPN truncates the rod and C-terminus of MYPN consisting of three Ig-residues, the nebulin/nebulette-SH3 domain and α-actinin binding regions. We hypothesized that truncation of MYPN disrupts the stretch-sensing and mechano-transduction chain connecting the Z-disk with titin and the nucleus, resulting in RCM.

Methods: A knock-in $MYPN^{WT/Q529X}$ mouse model was created *via* gene targeting. Functional, morphohistological, molecular and gene expression studies were performed.

Results: Heterozygous $MYPN^{WT/Q529X}$ mice developed echo signs of RP, as noted by increased E/A ratios with preserved systolic function at 12 weeks of age. Interstitial and perivascular fibrosis was observed histologically, without overt hypertrophic remodeling. Protein analysis revealed down-regulation of CARP and reduced phosphorylation of MEK1/2 and ERK1/2. Transcriptional analysis showed no significant changes in CARP-target genes (ANP, BNP and β-MyHC) consistent with down-regulated CARP. No significant changes were noted in ECM genes (CTGF, Col1a1, Postn and

OPN) or hypertrophic and inflammatory molecules (TGFβ1, MPEG, IL-6, and TNFα). Fibrosis was not associated with activation of p38 or JNK.

Conclusions: MYPN$^{WT/Q529X}$ recapitulated the human RCM phenotype and demonstrated disturbed cardiomyocyte mechano-sensing due to gene mutation in the Z-disk protein MYPN responsible for the development of cardiac fibrosis and diastolic dysfunction. The data suggest presence of specific ERK1/2- and CARP-dependent signal transduction for cardiac stiffness and fibrosis due to Q529X-MYPN mutation.

17. In vivo characterization of murine cardiac fibroblasts within normal and pathological heart

Onur Kanisicak, Jop Van Berlo, Suh-Chin Lin, Michelle Sargent, Federica Accornero, Jeffery D. Molkentin

Cardiac fibroblasts (CFs) are potential therapeutic targets in treating or preventing heart failure since they play a critical role in maintenance of normal cardiac function as well as in cardiac remodeling and fibrosis upon myocardial injury.

Heterogeneity among cardiac fibroblasts in normal and pathological conditions has been noted by many studies both in terms of function and origin. However, studying these aspects of cardiac fibroblasts has been challenging due to the lack of appropriate genetic markers that are specific to the fibroblast. Here In this study, we utilize two novel fibroblast-specific inducible Cre mouse lines to both identify and directly investigate CFs autonomous role in regulating fibrosis and heart failure in vivo.

Recent studies have shown that a small population of epicardial cell express the gene TCF21, which populates the developing heart and are suggested to give rise to possibly the entire resident CFs in adult heart. A similar marker, periostin, has also been discovered and shown to have a unique expression profile where it is either restricted to embryonic stages or to fibroblasts that are activated with injury but not expressed in static fibroblasts.

To investigate these differences and possible fibroblasts labeled with these lineages we have obtained the TCF21-MerCreMer and developed the Periostin-MerCreMer knock-in mouse lines, a ligand-dependent inducible Cre-expressing mouse, to control the timing of recombination of either TCF21 or Periostin positive cells. Using this method we have labeled and compared sub-populations of fibroblasts both at homeostasis and during the pathological remodeling and found novel populations of cardiac fibroblasts that were not identified before whose functions are yet to be determined to either be beneficial or detrimental.

Results of this study will lead in to investigations of fibroblast biology and the signaling pathways that control their trans-differentiation, their ECM modulating activity, and their proliferation and ability to modulate cardiac disease as well as how we can possibly use this knowledge to develop novel therapies for cardiac fibrosis.

18. Bax and Bak Are Required for Mitochondrial Permeability Transition Pore-dependent Programmed Cellular Necrosis

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During myocardial ischemia-reperfusion (I-R) injury the heart undergoes massive amounts of necrotic cell death in the affected area. The opening of the mitochondrial permeability transition pore (MPTP) is a critical event in programmed cellular necrosis. The MPTP is thought to span across the inner and outer mitochondrial membranes where it regulates mitochondrial swelling, energy production, and initiation of necrotic cell death in response to high Ca^{2+} and/or ROS stimulation. However, the molecular identity of the outer and inner membrane components of the MPTP remains elusive. Here we determined that the Bcl-2 family members Bax and Bak, which are central regulators of apoptosis, are also required for programmed necrosis induced by Ca^{2+}, ROS, DNA alkalyation and TNFα + caspase inhibition. Cardiac specific deletion of Bax/Bak significantly protected the heart from I-R injury and reduced lethality in mice subjected to permanent myocardial infarction injury. Isolated mitochondria deficient in Bax/Bak were resistant to MPTP dependent mitochondria swelling accompanied with greater Ca^{2+} uptake capacity, similar to cyclophilin D deficient mitochondria. Mechanistically, loss of Bax/Bak reduced outer mitochondrial membrane conductance and permeability, but had no effect on the inner mitochondrial membrane. Non-oligomerized Bax could also directly enhance permeability of reconstituted liposomes. Reconstitution with mutants of Bax that cannot oligomerize and form apoptotic pores permitted MPTP formation and restored necrotic cell death. Collectively, our results suggest that Bax/Bak are required for MPTP dependent mitochondrial dysfunction and hence are necessary integrators of both apoptotic and programmed necrotic cell death.

19. Cardiac neural crest specific vascular smooth muscle cell dysregulation results in regional proteoglycan misexpression and biomechanical dysfunction in a mouse model of aortopathy

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Aortopathy is a subclinical disease associated with sudden cardiac death that is characterized by vascular smooth muscle cell (VSMC) and elastic fiber abnormalities. Elastin insufficient (*Eln+/-*) mice demonstrate latent aortic root dilation, increased VSMCs and elastic fiber fragmentation. We hypothesized that aortopathy originates in the cardiac neural crest (CNC) regions of the aorta. Aorta tissues from adult and aged wild type (WT) and *Eln+/-* mice were examined by anatomic (root vs. ascending) and developmental (CNC vs. non-CNC) regions. Tissue was analyzed for proteoglycans (aggrecan, biglycan), ECM remodeling enzymes (MMP-2, Cathepsin L), CNC-derived cells (Sonic Hedgehog), VSMCs (α-SMA, SM22), elastic fiber architecture (Hart's stain) and regional morphometrics. Spatial distribution of VSMC and CNC markers was determined using a novel 3D reconstruction approach. Aortic distensibility and circumferential cyclic strain were quantified using magnetic resonance imaging. Regional aorta tissue tensile stiffness was determined using micropipette aspiration and a half space model adjusted for tissue thickness. The CNC side of WT ascending aorta was significantly thicker and stiffer than non-CNC ($p<0.05$). Similar differences were observed in *Eln+/-* mice, but were more pronounced at the aged stage ($p<0.01$). Aggrecan was localized to CNC aorta and in *Eln+/-*, but absent in WT. Further, biglycan, MMP-2 and Cathepsin-L were increased in the aortic root only. The stiffness of ascending aorta CNC and non-CNC regions was significantly higher than the aortic root in WT and *Eln+/-* mice ($p<0.0001$), and increased with age ($p<0.05$). While tissue stiffness values were not different among specific WT aortic root sinuses, the *Eln+/-* non-coronary sinus (non-CNC) paradoxically demonstrated increased stiffness when compared with CNC

sinuses (p<0.0001). These findings demonstrate that CNC-derived aorta tissue is spatiotemporally associated with VSMC abnormalities, maladaptive matrix remodeling and biomechanical dysfunction in *Eln+/-* mice, suggesting a primary role in the development of aortopathy.

20. Does Peritoneal Dialysis Improve Outcomes after Cardiopulmonary Bypass in Infants with Acute Kidney Injury?

David Kwiatkowski MD, Yu Wang, MS, Stuart Goldstein MD, Catherine Krawczeski MD

Acute kidney injury (AKI) after cardiopulmonary bypass (CPB) in infants is common and associated with poor outcomes. Peritoneal dialysis (PD) and furosemide have been used to attain negative fluid balance due to AKI induced oliguria, but have not been compared prospectively. We will prospectively compare outcomes of infants with oliguria after CPB **randomized** to PD vs. furosemide with the hypothesis that infants receiving PD have superior outcomes.

Infants < 6 months with planned PD catheter placement during CPB are enrolled. Infants with urine output <1 mL/kg/hr for 4 hours during the first postoperative day (POD) are randomized to a standardized regimen of PD or furosemide. If furosemide does not result in urine output >1mL/kg/hr over 16 hours, PD is initiated as necessary. The primary outcome is the mean fluid balance on POD2. Secondary outcomes are times to negative fluid balance, extubation and chest closure; duration of ICU and hospital stays, and 24/48 hour serum NGAL concentrations. Outcomes are compared with chi-square, Wilcoxen rank-sum, and two-sample t-testing as appropriate.

Our current randomization goals are 64 patients, although the study is currently undergoing adaptive design repowering. At the time of writing, 21 of 33 enrolled infants developed oligura and were randomized. To date, baseline demographics and characteristics (age, weight, sex, RACHS-1 surgical severity, baseline serum creatinine, CPB time) are similar between PD and furosemide groups. Two subjects on furosemide had late PD started due to persistent oliguria. Interim outcome analysis is pending. The only complication was a hemodynamically insignificant peritoneal bleed in a patient that later used the catheter for uncomplicated PD.

Initial results suggest that PD is safe. We expect to prove our hypothesis that PD is associated with improved outcomes when used for treatment of oliguria among infants after CPB and thus an important component of postoperative management.

21. Identification of a novel evolutionarily conserved regulator of the mitochondrial permeability transition pore and cell death

Jennifer Q. Kwong, Jason Karch, Adam R. Burr and Jeffery D. Molkentin

Cardiomyocyte death often engages a common pathway of pathogenesis stemming from impaired calcium homeostasis which triggers the formation of the mitochondrial permeability transition pore (MPTP). Activation of the MPTP leads to a sudden increase in permeability across the mitochondrial inner membrane, resulting in mitochondrial dysfunction, impaired respiration, decreased energy production, and ultimately, death.

Despite the importance of the MPTP to cardiomyocyte death, the molecular regulators linking calcium overload to MPTP are largely undefined. In this study, we constructed a *Drosophila* model of calcium overload mediated cell death by expressing the murine plasma membrane calcium channel Trpc3 in the wing disc. Following a systematic genome-wide in vivo screen for novel death mediators, we found that RNAi mediated knockdown of the fly gene CG8323 strongly repressed the aberrant Trpc3 mediated death. Interestingly, RNAi mediated knockdown of CG8323's mammalian homolog SLC25a35 in cultured mouse cells also conferred protection against calcium overload-induced cell death, mirroring the effects seen in the fly. Further, SLC25a35 depleted mitochondria were protected against calcium overload induced mitochondrial permeability transition. We believe that SLC25a35 represents a new evolutionarily conserved mitochondrial control point that integrates calcium overload with the MPTP and cell death. We are currently further studying the interaction between SLC25a35 and the MPTP.

22. MCTP2 is a Novel Regulator of Cardiac Development

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Left Ventricular Outflow Tract (LVOT) Defects are a subset of congenital heart defects that include bicuspid aortic valve, aortic stenosis, coarctation of the aorta, and hypoplastic left heart syndrome. LVOT defects demonstrate high levels of heritability, with elevated relative risk of disease for siblings of affected individuals. Despite a strong indication of genetic contribution, only a few causative genes, such as *Notch1*, have been identified. Screening a cohort of patients with LVOT defects, our lab previously identified copy number variations and point mutations in the gene encoding MCTP2 (Multiple C2 Domains Transmembrane Protein 2), a transmembrane Calcium-binding protein. Little is known about the cellular or developmental function of this gene. To identify a potential role for MCTP2 in cardiac development, we analyzed the developmental expression pattern, subcellular localization, and functional role of MCTP2. Immunohistochemistry demonstrates that MCTP2 is expressed in the developing mouse heart at time points critical for left ventricular outflow tract development. Further, we demonstrate that MCTP2 localizes in vitro to the plasma membrane and intracellular vesicles. The cellular distribution of MCTP2 overlaps with Rab5, an endosomal marker, and with extra-nuclear Notch1. Notch1 signaling plays a critical role in epithelial to mesenchymal transition during outflow tract development. Using two MCTP2 splice site morpholinos, we demonstrate that knockdown of MCTP2 in *Xenopus laevis* disrupts heart development and endocardial cushion formation in the developing outflow tract . These findings implicate *MCTP2* as a crucial gene in heart development, and suggest that MCTP2 may interact with Notch trafficking to regulate left ventricular outflow tract formation.

23. Pediatric non-syndromic thoracic aortic aneurysm: an opportunity for early intervention?

Benjamin J. Landis, Stephanie M. Ware, Lisa J. Martin, Amy Garrison, Jonathan Arthur, Robert B. Hinton

Introduction: Thoracic aortic aneurysm (TAA) is a subclinical disease, and dissection is estimated to cause 50,000 deaths per year in the US. Connective tissue disorders such as Marfan syndrome (MFS) are associated with TAA, and angiotensin receptor blocker (ARB) or beta blocker (BB) therapy reduces disease progression. The timing of and response to medical therapy in non-syndromic TAA is not well characterized in children. We hypothesized that ARB therapy slows TAA progression in early-onset non-syndromic TAA.

Methods: This is an observational study of pediatric patients (age < 21 years) with TAA. TAA was defined as aortic root or ascending aorta z-score greater than +2 by echocardiography. Early-onset TAA was defined as a z-score between +2 and +3 while late-onset TAA was defined as a z-score > +3. Patients with a clinical or molecular diagnosis of a well characterized genetic syndrome associated with TAA were excluded.

Results: A total of 72 patients were identified. The median age was 14.5 years (range 4 months to 21 years), and 62 (86%) were male. TAA was isolated to the aortic root in 24 subjects (33%) and the ascending aorta in 5 subjects (7%). Mitral valve prolapse or bicuspid aortic valve was present in 12 (17%) and 13 (18%) subjects, respectively. The most common non-cardiovascular features were malar hypoplasia (n=25), pes planus (n=24), tall stature (n=20), myopia (n=16), and pectus excavatum (n=15). There were 21 subjects treated with ARB (8 “early”) and 22 subjects treated with BB (7 “early”).

Conclusions: Deep phenotyping of pediatric patients with non-syndromic TAA identified frequent non-cardiovascular features, suggesting that nonspecific primary connective tissue disorders may contribute to disease pathogenesis in these patients. Statistical analyses of early vs. late medical therapy will provide insight on the impact of medical therapy on TAA progression.

24. Gene Regulation by Twist1 Homodimers and Heterodimers During Heart Valve Development

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Heart valves develop from extracellular matrix rich endocardial cushions (ECCs) populated by proliferative, migratory, and undifferentiated mesenchymal cells in the atrioventricular canal and outflow tract. Twist1, a basic helix-loop-helix (bHLH) transcription factor, is highly expressed in ECCs and is downregulated in the remodeling valves. Interestingly, it is re-expressed in pediatric and adult valve disease. Twist1 binds E-box consensus sites (CANNTG) within the genome as a homodimer or heterodimer with other bHLH factors. Twist1 homo and heterodimers differentially regulate genes in cranial suture and *Drosophila* mesoderm development. We hypothesize that Twist1 homo and heterodimers differentially regulate gene expression during heart valve development. Our previous research has identified direct transcriptional targets of Twist1 *Tbx20, Sema3C, Cadherin-11, Collagen2a1, Rab39b*, and *Gadd45a* expressed in ECCs. Whether these targets are differentially regulated by homodimers or heterodimers in heart valve development has yet to be explored. Through chromatin immunoprecipitation (ChIP) assays, differential binding of Twist1 and dimerization partners E12/E47 has been observed at known enhancers. Furthermore, ChIP experiments performed throughout heart valve development (E10.5-E14.5) reveal differential binding temporally at these enhancers. ChIP-sequencing (ChIP-seq) experiments are being performed to further identify gene targets likely to be regulated differentially by Twist1 homo and heterodimers during heart valve development. Additional experiments are focused on identifying other bHLH factors that may be dimerization partners for Twist1, and the genes regulated by each dimer pair. These studies will aid in understanding the mechanism through which Twist1 promotes functions such as proliferation and migration during heart valve development and disease.

25. Differing cardiac phenotypes between PP1α and PP1β heart-specific gene-deleted mice

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Background: PP1 is a major serine/threonine phosphatase that regulates a variety of biological processes such as cardiac muscle excitation and relaxation. The three PP1 catalytic subunits (α,β, and γ) have ~90% homology in the catalytic domains and unique N and C terminal sequences. A recent study has shown that pp1β is the major isoform involved in sarcoplasmic reticulum Ca^{2+} cycling in adult cardiac cells, suggesting each isoform might play different role in the heart. In this study, we created heart-specific PP1 deletion mice and assessed the cardiac performance. Based on the fact that increased PP1 activity is associated with progressed heart failure, while overexpression of constitutively active inhibitor I and 2 improves cardiac function, we hypothesized that deletion of each PP1 isoform will benefit the heart.

Methods: Each PP1 isoform was conditionally deleted in the mouse heart using a Cre-loxP approach. LoxP sites were introduced into intron 1 and 3 of each PP1 isoform. Both loxP-targeted lines were bred with mice expressing β-myosin heavy chain promoter driven Cre to achieve isoform specific gene deletion in the heart. Echocardiography was performed in these mice at different ages. We also investigated protein phosphorylation status of selected PP1 targets that underlie cardiac contraction and calcium handling in neonatal cardiac myocytes.

Results: We found that heart-specific deletion of PP1α caused a reduction of fractional shortening and worsening of cardiac function. Two weeks after transaortic constriction (TAC), PP1α deleted mice had greater increases in heart-weight to body-weight ratio compared with control mice, suggesting that PP1α was important for proper cardiac compensation. Interestingly, however, combined deletion of both PP1α and PP1β rescued the cardiac performance defects observed in PP1α deleted mice. Mechanistically, overexpression of PP1α or PP1γ decreased phosphorylation of

phospholamban and myosin binding protein C. In conclusion, we showed that PP1 isoforms play distinct roles in the heart in regulating contractility and compensation after TAC.

26. Effect of Endurance Exercise on Transgenic ARVC Mice

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Introduction: Arrhythmogenic right ventricular cardiomyopathy (ARVC) is an inherited disorder that causes sudden death and right ventricular heart failure in the young. Clinically, it has been suggested that competitive sports may accelerate ARVC pathogenesis in susceptible persons. The goal of this study was to investigate the cause and effect of exercise stress in the development of ARVC using a transgenic (Tg) mouse model with cardiac-restricted overexpression of human mutant R2834H desmoplakin (DSP).

Methods: DSP^{R2834H} Tg (Tg-mut), non-transgenic human wild type DSP^{WT} (Tg-wt) and control non-transgenic (Ntg) mice were divided into groups, sedentary and exercise. Mice in the exercise group were subjected to graded increase in intensity of exercise on a treadmill from the age of 4 weeks. Every 4 weeks, echocardiograms (echo) and ECGs (single lead) were recorded. Mice were dissected for tissue sampling and gross morphology.

Results: Initial echo data results are as shown in Table 1. When adjusted for age, number of days exercised, gender, interaction of genotype and exercise status, Tg-mut RV was bigger than Ntg mice (P=0.0005). Within the Tg-mut group, the RV diameter was not statistically significantly different between exercise and sedentary mice when adjusted for same variables. Heart weight/body weight ratio was higher in Tg-mut as compared to Ntg {sedentary (= 0.001) and exercise group (P = 0.0060} and Tg-wt. Tissue staining showed early development of fibrosis in Tg-mut mice. The only significant ECG finding occurred in the exercise group: Tg-mut had increased P wave amplitude and decreased R wave amplitude (P = 0.02 and P =0.001, respectively) compared to Ntg mice.

Conclusions: We report significant early histopathologic, and ECG changes with exercise in mutant DSP^{R2834H} Tg mice compared to Ntg and Tg-wt mice.

Table 1 : Echo Parameters of Non-transgenic (Ntg), DSP^{R2834H} Tg (Tg-mut), and DSP^{WT} (Tg-wt)

	Parameter	Ntg	Tg-mut	Tg-wt	P value
Exercise	RV diameter	1.33 ± 0.48 (N= 12)	1.81 ± 0.37 (N=30)		**0.002**
	RV diameter	1.33 ± 0.48		1.55 ± 0.35 (N=12)	0.175
	RV diameter		1.81 ± 0.37	1.55 ± 0.35	0.512
Sedentary	RV diameter	1.59 ± 0.53 (N=13)	1.95 ± 0.40 (N=15)		0.172
	RV diameter	1.59 ± 0.53		1.49 ± 0.55 (N=8)	0.107
	RV diameter		1.95 ± 0.40	1.49 ± 0.55	1
Exercise	LV diameter	3.85 ± 0.55 (N=14)	3.42 ± 0.35 (N=28)		**0.007**
	LV diameter	3.85 ± 0.55		3.44 ± 0.35 (N=13)	**0.04**
	LV diameter		3.42 ± 0.35	3.44 ± 0.35	1
Sedentary	LV diameter	3.86 ± 0.38 (N=12)	3.59 ± 0.24 (N=15)		0.063
	LV diameter	3.86 ± 0.38		3.42 ± 0.28 (N=8)	**0.009**
	LV diameter		3.59 ± 0.24	3.42 ± 0.28	**0.711**
Exercise	LA diameter	1.98 ± 0.32 (N=13)	2.14 ± 0.37 (N=28)	2.01 ± 0.37 (N=11)	0.37
Sedentary	LA diameter	2.01 ± 0.24 (N=10)	2.13 ± 0.36 (N=15)	2.41 ± 0.52 (N=7)	0.09
	Age	108 ± 46 days	92 ± 44 days	95 ± 47 days	0.3
	No of days of exercise	31 ± 21.63	36 ± 23.47	33 ± 24.63	0.8

27. Unlocking the role of histone deacetylases in modulating pathogenic protein aggregation in cardiomyocytes

Patrick M. McLendon and Jeffrey Robbins

Background: Cardiac proteinopathy can cause cardiac disease and heart failure but the pathological processes remain understudied. Mutated aB-crystallin ($CryAB^{R120G}$) expression is sufficient to cause desmin-related cardiomyopathy, a protein conformational disorder in which toxic misfolded protein species accumulate as perinuclear aggregates, leading to cardiac hypertrophy and heart failure. Histone deacetylases (HDACs) have roles in modulating gene expression via deacetylation of histone and non-histone proteins. Substantial crosstalk between HDACs and other pathways involved in maintaining protein quality control, such as autophagy and the heat shock response pathways, has recently been demonstrated. HDAC inhibition can also be beneficial in cardiac hypertrophy and reduces aggregates in neurodegenerative disease. Therefore, we hypothesize that inhibiting HDAC activity will reduce aggregate content in $CryAB^{R120G}$ hearts, which may lead to decreased cardiomyocyte toxicity and improved heart function.

Methods and Results: HDAC inhibition in $CryAB^{R120G}$-infected rat neonatal cardiac myocytes (RNCs) resulted in substantially reduced aggregate formation, and this effect was conserved using several different broad-spectrum HDAC inhibitors. These inhibitors were able to clear pre-existing aggregates as well as prevent misfolded species from aggregating. Overexpression of HDAC6, a known mediator of protein trafficking towards aggresomes, accelerated aggregate formation in $CryAB^{R120G}$–expressing RNCs. HDAC6 knockdown led to smaller, cytoplasmic aggregates with less coalescence in the perinuclear space, corresponding to decreased aggresome formation. Autophagic flux assays demonstrate that inhibitors of HDAC6 can increase autophagic degradation, which provides clues towards the mechanism of aggregate reduction. The data herein demonstrate that HDACi may represent a viable therapeutic strategy in cardiac proteinopathy.

28. Toward Evidence-Based Guidelines for Cardiac Screening in Pediatric Hypertrophic Cardiomyopathy

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Individuals at risk of familial hypertrophic cardiomyopathy (HCM) require routine cardiac surveillance. Published guidelines recommend initiation of cardiac screening between 10 and 12 years of age but also suggest that the timing and frequency may be modified by genetic mutation and/or family history status. The aim of this study was to determine whether current guidelines adequately address surveillance needs in the pediatric population. The population comprised 34 probands with HCM diagnosed at 12 years of age or younger. Twenty-one probands (62%) were asymptomatic at diagnosis. Twenty-four (71%) had positive genetic HCM testing with the majority of mutations occurring in *MYBPC3* (n=11) or *MYH7* (n=11). The average age of diagnosis was 5.8 years (range 2 weeks to 12 years) among mutation carriers and 4.7 years (range 2 months to 12 years) in probands without an identifiable mutation. In the cohort with positive genetic testing, there was marked intra-familial variability in disease severity and age of onset in the probands and their 88 affected relatives. Despite previous case reports showing multiple mutations in patients with early onset HCM, only 6% (n=2) of this cohort of children had more than one pathogenic mutation, and there was no correlation with age or severity. These data indicate that many children at risk for familial HCM develop disease prior to the age of current recommended screening. Furthermore, in our cohort, severity of disease within a family was not a good predictor of age of onset or presentation, indicating that current guidelines do not sufficiently address the goals of surveillance in childhood.

29. Assessment of Mechanical Dyssynchrony in Patients with Single Ventricle Morphology at Various Stages of Palliation by Cardiac Magnetic Resonance using Myocardial Tissue Tagging by Feature Tracking

Ryan A. Moore M.D.; Kan Hor M.D.; Michael Taylor M.D.

Background: Cardiac Magnetic Resonance (CMR) is increasingly being utilized to diagnose and manage congenital and acquired heart disease in pediatrics [1, 2]. The ventricular morphology of some of these defects prohibits the use of traditional ventricular global functional measurements obtained by non-invasive imaging. Recent techniques, such as echocardiography speckle tracking and magnetic resonance tissue tagging, have looked at regional ventricular function as a biomarker for patients with congenital and acquired heart disease [3, 4]. CMR has been shown by our group to provide detailed and accurate analysis of ventricular mechanics at the regional level based on timing of CMR-derived circumferential strain (e_{cc}) in patients with normal ventricular morphology but known degenerative myocardial disease [5]. Patients with single ventricle morphology who have undergone staged palliation have been showed to exhibit ventricular mechanical dyssynchrony by speckle-tracking echocardiography [6]. In addition, strain and rotation mechanics in patients with single left ventricles have shown marked dyssynchrony at the apical level after Fontan staged palliation with CMR-derived images evaluated by echocardiographic speckle-tracking software [7]. With the development of CMR-specific feature tracking (FT) techniques, ventricular mechanics can easily be calculated and reliably reproduced on patients with the complex congenital and acquired heart defects [8, 9]. There has never been a study to assess the degree of dyssynchrony at multiple ventricular levels in patients with single ventricle morphology by CMR-specific techniques. The aim of this study is to provide a comprehensive analysis of the ventricular mechanics in individualized groups of patients with single ventricle morphology by using CMR feature tracking techniques.

Hypothesis: With the development of a ventricular mechanics biomarker by CMR feature tracking techniques, physicians will be able to identify early myocardial dysfunction and better risk stratify patients with single ventricle morphology.

Methods: Single ventricle patients (n=100) will be stratified into groups based on age, type of ventricular morphology, and stage of surgical palliation. Age-matched controls will be defined as patients who obtained a clinical CMR that was read as normal without evidence of current disease (n=100). CMR-specific feature tracking software (TomTec Imaging Systems, Munich, Germany) will be utilized to obtain circumferential strain and dyssynchrony indices. Dyssynchrony indices will be calculated based on timing of CMR derived circumferential strain (e_{cc}). The calculated indices will include cross-correlation delay (XCD), uniformity of strain (US), regional vector of variance (RVV), time to maximum strain (TTMS) and standard deviation (SD) of TTMS. Abnormal XCD value will be defined as > normal + 2SD. US, RW, TTMS, and SD will be calculated for -patient with abnormal XCD.

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30. Emilin1 deficiency is associated with elastase dependent pERK1/2 activation and aberrant angiogenesis in aortic valve tissue

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Emilin-1 (Elastin Microfibril Interface-Located protein) is an elastin binding protein that regulates elastogenesis and inhibits TGF-β signaling. *Emilin-1* is expressed in the developing and mature heart valves, and *Emilin-1* deficiency results in elastic fiber assembly defects. Aortic valve disease (AVD) is characterized by elastic fiber fragmentation, fibrosis and aberrant angiogenesis. We hypothesized that *Emilin-1* gene ablation *(Emi1$^{-/-}$)* is associated with dysregulation of TGF-β signaling that leads to MAPK/Elastase activation that further results in fibrosis and aberrant angiogenesis respectively in the aortic valve tissue. The expression of *Emilin-1* binding proteins elastin and fibulin-5 (an angiostatic factor) was decreased in the aged stage. In addition, the *Emi1$^{-/-}$* aortic valve displays a marked increase in angiogenic factors (VEGF-A, B, Flt-1) and decrease in angiostatic factors (Endostatin and Chm) at the juvenile stage and fibrosis at the aged stage only. Interestingly, pSmad2/3, pSmad1/5/8, p-Erk1/2, and elastase expression were increased at early stages, and macrophage expression was increased at the late stage**.** In-addition, aged *Emi1-/-* mice treated with doxycycline (non-specific elastase inhibitor) displayed attenuation of p-Erk1/2 activation in aortic valve tissue. Although aged *Emi1$^{-/-}$* valve tissue did not show calcification, cultured *Emil1$^{-/-}$* valve interstitial cells do calcify faster and more abundantly than control cells in response to osteogenic stimuli. Echocardiography showed normal ventricular function in *Emil1$^{-/-}$* adults, and marked ventricular dysfunction in aged mutants. These findings implicate elastase dependent p-Erk1/2 activation and TGF-β signaling dysregulation in the fibrocalcific and inflammatory responses in aortic valve tissue in *Emilin1-/-* deficient mice.

31. Early Aberrant Angiogenesis due to Elastic Fiber Fragmentation in Aortic Valve Disease

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Purpose: Elastic fiber fragmentation (EFF) is a hallmark of aortic valve disease (AVD), and neovascularization has been identified as a late finding due to atherosclerosis. EFF stimulates angiogenesis, and genetic syndromes due to mutant elastic fiber components are associated with valve disease. We hypothesized that angiogenesis would be an early AVD finding, preceding atherosclerosis, due to EFF.

Methods: To examine disease progression, regional anatomy and pathology of valve tissue was examined using histochemistry, immunohistochemistry and electron microscopy from early-onset (<40yo) and late-onset (≥40yo) non-syndromic AVD specimens. To assess the effects of elastic fiber dysregulation, Williams and Marfan syndrome aortic valves were also analyzed. Angiogenesis (VEGF-A, CD-34 and chondromodulin), EFF (elastin, fibrillin-1, emilin-1, fibulin-4 and -5), and atherosclerosis (CD-68, LRP-5) were assessed.

Results: Bicuspid aortic valve was more common in early-onset AVD (n=21), and cardiovascular comorbidities were more common in late-onset AVD (n=11). Early-onset AVD specimens demonstrated angiogenesis without atherosclerosis. A distinct pattern of elastic fiber components surrounded early AVD neovessels, including increased emilin and decreased fibulin-5. EFF was present in both WS (n=6) and MFS (n=4) valves, but was different. WS but not MFS aortic valves demonstrated angiogenesis. Distinct matrix differences were observed by anatomic region between groups.

Conclusions: Aberrant angiogenesis is an early mechanism in AVD pathogenesis preceding inflammation, implicating elastic fiber dysregulation as an inciting factor. Elucidation of the underlying mechanisms may inform the development of new pharmacologic therapeutics and durable bioprostheses.

32. Fun 2B Fit Exercise and Diet Intervention Reduces BMI Z-Scores of Overweight and Obese Children at Community YMCAs

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Background: Pediatric obesity is a growing problem in the United States. To combat this issue, the Center for Better Health and Nutrition partnered with 4 YMCAs in the greater Cincinnati area to create an intervention, Fun 2B Fit, for children with BMIs ≥ 85th percentile. Fun 2B Fit is open to children aged 9 to 13 years referred by their primary care providers and to children who participate in the pediatric weight management program *Healthworks!.*

Methods: Study participation was offered to all Fun 2B Fit participants. Data collected included number of sessions attended, height, weight and demographics. Results were compared for *Heatlhworks!* and community children.

Results: Of the 146 Fun 2B Fit children, 22 were consented and had measurements. Mean age was 10.2 years with 4.6 sessions attended over 3 months. There was a mean reduction in BMI z-score of 0.085 over the study period ($p<0.05$). There was no significant difference between *Healthworks!* or community children in BMI z-score change or number of children who decreased their BMI.

Conclusions: Participation in the Fun 2B Fit program had a significant impact on lowering BMI Z-scores. There was no significance in number of successes based on whether the child was a *HealthWorks!* patient or not.

33. Exercise intolerance and impaired energy metabolism in mouse model of Barth syndrome

Corey Powers, Chonan Tokunaga, Yan Huang, Zaza Khuchua.

Background: Barth syndrome (BTHS) is an X-linked mitochondrial myopathy caused by mutations in tafazzin (Taz) gene. BTHS is characterized by reports of exercise intolerance. Taz is a crucial enzyme in cardiolipin (CL) biosynthesis pathway and is essential for the maintaining the inner mitochondrial membrane. Cardiolipin is deficient in the cardiac and skeletal muscles of BTHS patients and Taz-knockdown (Taz-KD) mice. Cardiolipin deficiency causes significant structural abnormalities in mitochondria and striated muscles of Barth patients and Taz-deficient mice.

Hypothesis: We sought to determine whether the CL-deficiency affects mitochondrial energy production and exercise capacity in Taz-KD mice.

Methods: Mitochondrial respiratory capacity was assessed in neonatal cardiomyocytes using Seahorse XF24 Bioanalyzer. Exercise capacity, O_2 consumption and CO_2 production rates, were measured in a metabolic treadmill at different exercise intensities. Two-dimensional blue-native gel electrophoresis (2D-BNGE) was employed to assess the integrity of mitochondrial energy-producing enzymes.

Results: Respiratory capacities of cardiolipin-deficient mitochondria were reduced two-fold compared to wild type controls. Tafazzin-deficient mice demonstrate normal rates of energy expenditure at basal resting conditions. When subjected to moderate-intensity workload, Tafazzin-deficient mice exhibit severely limited energy expenditure and fail to adapt to high-energy demands. Our results suggest that fatty acid oxidation is impaired in Taz-KD mice. Using 2D-BNGE we demonstrated that in CL-deficient mitochondria, physical interaction between mitochondrial electron transport chain (ETC) complex-I and the trifunctional protein (TFP) is destabilized. Evidence suggests that cardiolipin is required for interaction of TFP with ETC complex I.

Conclusion: Impaired FAO in CL-deficient may be a contributing pathogenic factor in human Barth syndrome.

34. Pathogenicity of naturally-produced fragments of cardiac myosin binding protein C and their effects on heart function and failure

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Background: Myosin binding protein C (MyBP-C) is a thick filament protein consisting of 1274 amino acid residues (149kD) and mutations in the cardiac isoform (cardiac MyBP-C; cMyBP-C) are responsible for a substantial proportion (20-35%) of identified cases of familial hypertrophic cardiomyopathy (FHC). Recently we found a 40kD fragment is produced from cMyBP-C when the heart is stressed, using a stimulus such as ischemia reperfusion injury. This fragment can be detected in both the mouse and human heart and appears to be stable. Its ability to interfere with normal cardiac function is unexplored.

Methods and Results: To understand the potential pathogenicity of the 40kd fragment in vivo, we generated cardiac myocyte-specific transgenic mice (TG) using a Tet-Off inducible system to permit controlled expression in cardiomyocytes. When 40kD protein expression is induced by crossing the responder animals with tetracycline transactivator (tTA) mice, the double TG mice show protein expression and, subsequently, sarcomere dysgenesis and altered cardiac geometry. The double transgenic heart fails between 3 to 17 weeks of age. Expression, the fragment in cardiomyocytes led to development of significant cardiac hypertrophy with myofibrillar disarray and fibrosis. Subsequent analyses showed that MEK-ERK hypertrophic signaling pathways were activated. To determine the role of this pathway in the pathogenic response being generated, we subjected an experimental cohort of animals to treatment with the MAPK/ERK kinase inhibitor U0126 during pregnancy. The drug effectively improved heart function and prolonged survival as compared to the untreated control cohort.

Conclusions: The data show that a 40kD fragment of cMyBP-C, which is generated during the development of heart disease in both the mouse and

human, is a pathogenic fragment whose presence leads to hypertrophic cardiomyopathy and heart failure. Blockade of the MEK-ERK pathway was effective therapeutically in decreasing morbidity and increasing lifespan in the face of continued synthesis of the fragment.

35. Further defining circumferential strain characteristics in young Duchenne muscular dystrophy patients

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Background There is a known correlation between n-terminal mutations in the dystrophin gene and earlier onset of cardiac disease. We have previously demonstrated abnormal circumferential strain, global and segmental, measured by transthoracic echocardiography (TTE) in young patients with DMD in the setting of normal shortening fraction. In the current study we evaluated our patient group to determine whether there was segregation of strain based on location of the gene mutation.

Methods TTE from DMD patients ≤8 years (n=63) recorded during 2009-2010 were compared to TTE of an age-matched control group with no cardiovascular disease (n=61). Feature tracking analysis software (Image Arena, TomTec, Germany) was used to measure global circumferential strain (ε_{cc}) and segmental ε_{cc} based on a modified AHA/ASE 16-segment model. Patients were further segregated into those with mutations in exons 1-45, 45+, null mutations, or other mutations.

Results We were previously able to demonstrate a significant difference in strain between normal controls and patients with DMD. However, when patients with DMD were segregated by location of mutation on the dystrophin gene there were no differences between groups.

Conclusion In the present study we were unable to demonstrate differences in strain between groups based on location of dystrophin mutation despite previous evidence that mutations located on the n-terminal portion of the gene develop cardiac disease earlier than those at the c-terminal end. However, this patient group was older and had abnormalities in standard measures of function such as shortening fraction. While the mutation was related to overall changes in function compared to normal controls, it may be too early to distinguish between genotypes when the measured difference is still small. Following strain in different genotypic groups as they age may allow differentiation.

36. Cyp26 enzymes are required during two distinct phases for proper patterning of zebrafish heart chambers

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Normal heart development requires appropriate levels of retinoic acid (RA) signaling, with too much or little being teratogenic. One way the amount of RA is moderated in the embryo is by Cyp26 enzymes, which metabolize RA into easily degraded derivatives. However, the role Cyp26 enzymes play during heart development has not yet been addressed. In zebrafish, we found that two Cyp26 enzymes, Cyp26a1 and Cyp26c1, are expressed in the anterior lateral plate mesoderm marginally overlapping with the early cardiac progenitors. While singular knockdown of Cyp26a1 or Cyp26c1 does not overtly affect heart development, the hearts of the Cyp26a1 and Cyp26c1 deficient embryos are linearized with smaller ventricles and dilated atria by 48 hours post fertilization (hpf). Interestingly, the phenotype observed in double morphants does not replicate the teratogenic effects seen when embryos are treated with high concentrations of RA, which can eliminate cardiomyocytes. Closer examination of when cardiomyocytes are affected revealed two phases of Cyp26 enzyme requirement. We find an earlier role where loss of Cyp26 enzymes leads to increased atrial cardiomyocyte differentiation, which translates into more atrial cells at 48 hpf. Although we do not find an earlier effect on ventricular cardiomyocyte differentiation, we find ventricle cardiomyocyte number is reduced by 48 hpf and progressively lost through 72 hpf. Therefore, our results suggest a previously unappreciated model where Cyp26 enzymes are required to prevent increases in RA signaling at two distinct phases to allow for proper heart development.

37. Identification of novel mediators of the unfolded protein response

Tobias G. Schips, Marjorie Maillet, Jeffery D. Molkentin

Accumulation of unfolded or misfolded proteins in the endoplasmic reticulum (ER) lead to the activation of a process called the unfolded protein response (UPR). The basic leucine zipper transcription factor ATF6α functions as a master regulator of the UPR. ATF6α is constitutively expressed as an ER transmembrane protein. Upon ER stress, ATF6α undergoes proteolytical processing and the cytoplasmic N-terminus translocates to the nucleus where it activates ER stress response genes.
To identify novel activators of the ATF6α mediated ER stress response, we performed a genome wide over- expression screen in Hela cells. A luciferase reporter, consisting of five repeats of the ATF6α consensus binding side was cotransfected and luciferase was measured as a marker for ATF6α activity.

The initial screen identified 60 genes which showed an increase in ATF6α activity. After validation of these hits, we selected four proteins, which showed highest ATF6α activity, for further characterization.

1. Fukutin is a putative transmembrane protein with highest expression in skeletal muscle, heart and brain. Fukutin is localized to the Golgi apparatus where it functions as a glycosyltransferase. It was shown to be necessary for the maintenance of muscle integrity by glycosylating α-dystroglycan.
2. Tmed3 (transmembrane emp24 protein transport domain containing 3) is a GOLD domain containing transmembrane protein. The emp24 protein family is important for efficient and selective shuttling of proteins from the ER to the Golgi.
3. Rad51-like1 is part of the Rad51 protein family which is highly conserved in most eukaryotes and involved in homologous recombination after DNA double strand breaks.
4. Nell2 (neural epidermal growth factor (EGF)-like molecule-like 2) is a thrombospondin-1-like secreted glycoprotein, it contains six epidermal growth factor-like domains and an N-terminal thrombospondin-1 domain. Nell2 was initially described as a neuron specific protein but it is also expressed in the murine heart, with high expression in cardiomyocytes.

38. Head and Neck Vessel Size by Angiography Predicts Neo-aortic Arch Obstruction After Norwood/Sano Procedure for Hypoplastic Left Heart Syndrome

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Background Neo-aortic arch obstruction (NAAO) after the Norwood/Sano procedure (NS) for hypoplastic left heart syndrome (HLHS) occurs in one third of patients. This leads to increased morbidity and mortality and there is no objective method to predict occurrence. Distal obstruction from aortic coarctation leads to increased wall shear stress and vessel dilation in proximal head and neck vessels, so we hypothesized that a ratio between these vessel sizes could predict future NAAO.

Methods We identified patients with HLHS who underwent NS and at least one subsequent cardiac catheterization. Diameters of each head and neck vessel were measured and the sum divided by the diameter of the aortic isthmus, deemed the Head and Neck Index (HNI). For comparison, we calculated the established measure Coarctation Index (CI) by dividing isthmus diameter by descending aortic diameter. Patients who never developed NAAO (NoObs) were compared to those with NAAO at first catheterization or on subsequent catheterization (Obs).

Results There were 27 patients in the NoObs group and 17 in the Obs group. Using a CI value ≤ 0.5 to identify patients who developed NAAO, ROC analysis showed an AUC of 0.871 ($p < 0.001$), a sensitivity of 47% and specificity of 89%; PPV was 73% and NPV was 73%. HNI value > 2.65 gave an AUC of 0.81 ($p = 0.001$), sensitivity 77% and specificity of 93%; PPV was 87% and NPV was 86%. Three patients did not have NAAO on initial catheterization and subsequently developed it; all had CI > 0.5, which

would not predict NAAO, and HNI >2.65, which correctly predicted future NAAO.

Conclusions HNI is superior to CI at identifying children with HLHS who develop NAAO after NS and could help prognosticate future occurrences.

39. FOXO and FOXM1 transcription factors have antagonistic functions in neonatal cardiomyocyte cell cycle withdrawal and *IGF1* gene regulation

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Fetal cardiomyocytes (CM) are highly proliferative, whereas neonatal CM withdraw from the cell cycle. The transcriptional regulatory network that regulates cell cycle withdrawal in neonatal CM is not well known. Here, we investigate the role of the forkhead family transcription factors FOXO and FOXM1 in regulating CM proliferation in neonatal cardiomyocytes. CM-specific deficiency of FOXO1 and FOXO3 results in increased CM proliferation in hearts of mice at 1 and 3 days after birth. Conversely, CM-specific deficiency of FOXM1 results in decreased neonatal CM cell proliferation. Inhibition of AMP-activated protein kinase (AMPK), an upstream activator of FOXO, increases proliferation in rat neonatal CM and this increase is attenuated with overexpression of FOXO1. This suggests that AMPK-mediated activation of FOXO is required to promote cell cycle withdrawal in neonatal CM. In order to determine a mechanism by which FOXOs control CM cell cycle withdrawal, we examined the regulation of *FOXM1* and *IGF1* genes, which are associated with increased CM proliferation. CM-specific loss of both FOXO1 and FOXO3 results in increased expression of both *FOXM1* and *IGF1*. In contrast, CM-specific loss of FOXM1 results in decreased expression of *IGF1*. Interestingly, we identified *IGF1* as a direct downstream target of cardiac FOX transcription factors, which is negatively regulated by FOXOs and positively regulated by FOXM1, dependent on AMPK activation status. These data support a regulatory mechanism in neonatal cardiomyocytes whereby the balance of FOXO and FOXM1 transcription factors controls cell cycle regulation through *IGF1* expression. Understanding the molecular mechanisms by which FOXO/FOXM1 regulates CM proliferation could be harnessed in adults for the treatment of cardiac injury.

40. Global Longitudinal Peak Strain Detects Early Cardiac Damage in Hypertensive Children

P Shamszad, JD Knudson, DK Feagin, TC Slesnick, DI Feig

Background: Childhood hypertension is increasingly common and likely contributes to the premature development of cardiovascular and renal disease. Early detection of hypertensive cardiac damage may allow for prompt therapy and reduce the risk of late morbidity and mortality. While left ventricular mass index (LVMI) has been used as a surrogate of hypertensive cardiac damage in children, its prognostic and diagnostic value is not completely understood in this population.

Objective: To test the hypothesis that strain assessment using automated function imaging (AFI) detects cardiac changes in hypertensive children earlier than LVMI.

Methods: A cross-section observational study of hypertensive children < 19 years old between October 2010 and May 2012 was performed. Any patient who was newly diagnosed with hypertension and underwent echocardiographic examination including standard 2D, Doppler, tissue Doppler, and automated function imaging (AFI) modalities was included. Global longitudinal peak systolic strain (GLPSS) was measured using a 17 segment model of the left ventricle. All echocardiographic measures were compared to normative reference values for age.

Results: Twenty four patients (mean age 14.6 ± 2.7 years, 67% male) were identified. Mean systolic and diastolic blood pressure index was 1.1 ± 0.1 and 1.0 ± 0.1, respectively. Mean LVMI did not significantly differ from 95th-percentile normative values (41.6 ± 11.2 g/m2.7 vs. 39.3 ± 1.5 g/m2.7, p = 0.328). Mean GLPSS was significantly lower than 5th-percentile normative values (-17.6 ± 2.5 vs. -19.5 ± 0.42, p = 0.001) and were decreased across all myocardial segments. Left atrial volume index was also significantly higher than reported normal values in children (27.6 ± 7.3 ml/m2 vs. 22.0 ± 6.0 ml/m2, p = 0.002). No patient exhibited an abnormal shortening or ejection fraction, mitral E-wave to A-wave ratio, or mitral E-wave to lateral mitral e'-wave ratio.

Conclusions: A population of unselected, newly diagnosed hypertensive adolescents had atypically low GLPSS and increased left atrial volume. The use of GLPSS measured by AFI may be a more sensitive marker of early myocardial remodeling in hypertensive children and routine measurement of GLPSS should be considered.

41. Right Ventricular Functional Indices Precede Overt Ventricular Dysfunction in an Ovine Model of Maternal-Fetal Anesthesia

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Objectives: Fetal surgery has become a treatment option in a number of fetal conditions. During surgery, fetal cardiac depression related to anesthesia with high dose desflurane (HD-DES) can develop. Fetal echocardiography is typically used to assess cardiac function during intervention. The purpose of this study was to evaluate changes in cardiac function parameters that occur prior to overt ventricular dysfunction in an ovine model of maternal-fetal anesthesia.

Study Design: Six pregnant ewes were exposed to HD-DES. Fetal echocardiography was assessed at baseline and every 30 minutes until overt ventricular dysfunction, defined as right (RV) or left ventricular (LV) shortening fraction (SF) < 28%, or fetal death. RV and LV SF, Doppler myocardial performance index (MPI) and heart rate were recorded. RV and LV stroke volume was assessed as pulmonary (PVVTI) and aortic (AVVTI) valve velocity time integral. RV long-axis function was assessed by tricuspid annular plane systolic excursion (TAPSE). Baseline data was compared to the data collected prior to the development of RVSF or LFSF < 28% using paired t-tests.

Results: All lambs developed overt ventricular dysfunction at a mean of 164 ± 55 minutes, with 3/6 (50%) with isolated RV dysfunction, 1/6 (17%) with isolated LV dysfunction and 2/6 (33%) with biventricular dysfunction. There was a significant decrease in PVVTI, an increase in RV MPI, and a trend toward a decrease in TAPSE from baseline to follow-up (Table). There was no significant change in heart rate, AVVTI, or LV MPI prior to onset of overt ventricular dysfunction.

Discussion: Right ventricular functional indices decreased prior to overt ventricular dysfunction in this ovine model of maternal-fetal anesthesia. There was no change in left ventricular indices prior to the development of overt ventricular dysfunction. Detailed monitoring of RV functional status

may lead to earlier identification of fetal cardiovascular dysfunction during fetal surgical intervention.

Parameter	Baseline (mean)	Prior to Dysfunction (mean)	p Value
PVVTI (cm)	6.4	5.3	0.03
AVVTI (cm)	7.3	7.2	0.89
RV MPI	0.29	0.44	0.06
LV MPI	0.47	0.45	0.63
TAPSE (mm)	6.5	5.2	0.09
Heart Rate	172	172	0.99

AVVTI, aortic valve velocity time integral; LV, left ventricle; PVVTI, pulmonary valve velocity time integral; RV, right ventricle; TAPSE,tricuspid annular plane systolic excursion

42. *Zic3* is required in the migrating primitive streak for node morphogenesis and left-right patterning

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In humans, loss of function mutations in *ZIC3* cause X-linked heterotaxy, a disorder characterized by abnormal left-right asymmetry of organs and frequent cardiovascular malformations. *Zic3* is expressed ubiquitously during critical stages for left-right patterning but its later expression in the developing heart remains controversial and the molecular mechanism(s) by which it causes heterotaxy are unknown. *Zic3* null mice recapitulate the human heterotaxy phenotype but also have early gastrulation defects, complicating an assessment of the role of *Zic3* in cardiac development. To define the temporal and spatial requirement for *Zic3* in left-right patterning, we generated conditional *Zic3* mice and *Zic3*-LacZ-BAC reporter mice. The latter provide compelling data indicating that *Zic3* is expressed in the mouse node. We hypothesized that *Zic3* expression in node cells is required for proper left-right asymmetry. To address this question, *Zic3* was deleted in each cell type of the node using a conditional loss of function approach. Surprisingly, *Zic3* deletion in the node results in viable, phenotypically normal mice. However, immunohistochemistry and scanning electron microscopy indicate abnormal node morphology in *Zic3* null mice. We observed similar node dysplasia when *Zic3* was deleted from the migrating mesoderm and primitive streak at E7.5. At later stages, abnormal molecular markers of left-right patterning and heart looping defects were observed. Interestingly, mice were viable when *Zic3* was deleted from heart progenitors or the cardiac compartment. These results contrast with the accepted paradigm that left-right asymmetry is initiated at the node in mice and implicate *Zic3* in earlier signaling events affecting node formation and subsequent node function.

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43. Characterization of dystrophin (DMD) mutations and correlation with cardiac function markers

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Duchenne muscular dystrophy (DMD), Becker muscular dystrophy (BMD), and X-linked dilated cardiomyopathy (XLCM) are diseases caused by mutations in the dystrophin (*DMD*) gene and comprise the dystrophinopathies.[1,2] DMD has a more severe skeletal muscle phenotype in comparison to BMD, and XLCM has an exclusive cardiac phenotype.[3] In almost all cases the dystrophinopathies manifest dilated cardiomyopathy, but the time of onset and the progression of cardiac impairment are not uniform.

It is not clear whether there is a correlation between a patient's specific dystrophin mutation (genotype) and the clinical severity of his dystrophinopathy (phenotype). Initially it was hypothesized that the phenotype of DMD/BMD was related to the size of the dystrophin protein that was created from a patient's specific *DMD* sequence. However, this maxim is not universally true, and there are examples of DMD patients with in-frame mutations and BMD patients with out-of-frame mutations.[4-7] Several studies have examined the correlation of dystrophin mutations to the various DMD phenotypes (e.g. skeletal muscle, cardiac, neurologic), but they have produced mixed results.

We will investigate potential correlations of genotype and cardiac phenotype using cardiac MR (CMR), which has been shown to be more sensitive in the detection of DMD-associated cardiac impairment.[8] We will examine 328 individual patients with a total of 842 CMR studies, and relate their specific genotypes with left ventricular (LV) ejection fraction (EF), LV mass, LV volume, LV circumferential strain, and delayed enhancement (a fibrosis surrogate). The CMR data will also be evaluated in a serial manner, where available, for correlation of genotype and changes in cardiac function over time. We hope to identify genotypes that have more or less severe cardiac impairment, as this may help guide medical management of dystrophinopathies in the future.

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44. Whole-exome sequencing identifies autosomal recessive heterotaxy

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Heterotaxy is a rare multiple congenital anomaly syndrome resulting from abnormal left-right (LR) embryonic organ patterning with human prevalence of ~1 in 10,000 newborns. Although heterotaxy is the most highly heritable cardiovascular malformation, the genetic basis is most frequently multifactorial and autosomal recessive inheritance has been described only rarely. In this study, we analyzed whole-exomes of 19 probands with heterotaxy-spectrum disorders. This includes 4 probands belonging to families with obvious autosomal recessive inheritance. Exome analyses identified approximately ~8000 coding variants in each individual which were subsequently filtered using 1000 Genome, dbSNP135 and NIH exome sequencing project (ESP) databases. The remaining variants were further analyzed using regions of homozygosity (ROH) revealed by SNP genotyping and/or inheritance-based exome analysis. This identified suspected disease-causing variants in novel candidate genes in 7 probands as well as in previously known heterotaxy or related phenotype genes in 4 probands. The majority of variants were homozygous and located in ROH (~2Mb-44Mb), supporting the autosomal recessive inheritance in 6 familial cases. Sanger sequencing of probands and available family members confirmed these homozygous mutations and validated their recessive segregation with phenotype. RT-PCR analysis showed expression of novel selected candidates (*C21ORF59, CXORF41, DNAJB7, MIA3, INTS7, JMJD1C*) in early mouse development (E7-9.5) suggesting a possible role in LR pattering and early heart development. Further expression and functional analyses of these genes are in progress. This study proves the importance of exome analysis complemented with ROH segregation revealed by SNP arrays for identification of unknown causes of human lateralization defects and these causes will provide insights to understand new mechanisms involved in embryonic LR patterning.

45. Autonomic Dysfunction Found in Pediatric DMD Patients by Heart Rate Variability Analyses

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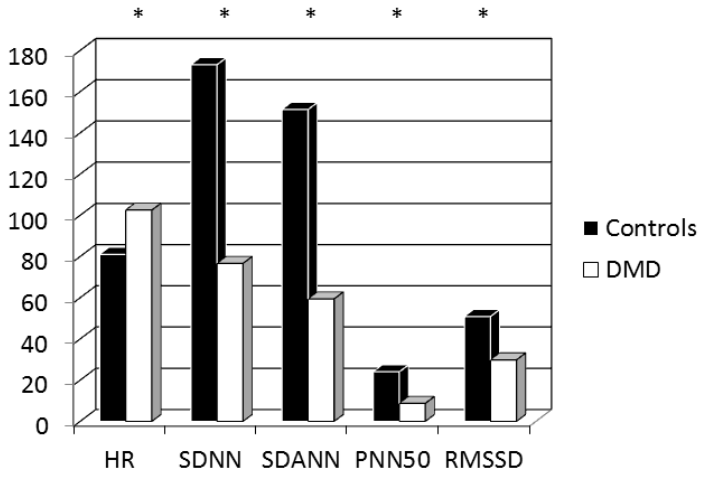
Introduction: An elevated resting heart rate (HR) is frequently observed during the natural history of Duchenne Muscular Dystrophy (DMD). We hypothesize that the elevated resting HR reflects autonomic dysfunction that can be identified by performing heart rate variability (HRV) analyses.

Methods: DMD patients (N=20) and controls (N=6) had anthropometric, BP, HR data collected and underwent HRV analyses via Holter monitoring. Time and frequency domain HRV parameters were calculated. T-test was used to perform pair-wise comparison between groups. A p-value of <0.05 was used for statistical significance.

Results: DMD cases did not differ from controls in systolic blood pressure, diastolic blood pressure, and average age (12.1 $\pm$ 3.6 years controls vs 12.5 $\pm$ 3.5 years cases). The controls were lighter (BMI 11.0 $\pm$ 9.5 controls vs 22.9 $\pm$ 5.0, $p \leq 0.0007$). DMD cases had higher resting HR (81.0 $\pm$ 9.3 bpm controls vs 102.5 $\pm$ 8.3 cases, $p \leq 0.001$). Among HRV variables collected, decreases were seen in: standard deviation of R to R intervals (SDNN, $p<0.0001$), the percent NN intervals differing by >50 ms from previous NN interval (pNN50, $p<0.0001$), the root-mean-square of successive differences of NN intervals (RMSSD, $p<0.0001$), the standard deviation of the means R to R segment (SDANN, $p<0.0001$) (see Table 1). Low frequency (0.04-0.15Hz) to High frequency (0.15-0.4) ratio did not differ statistically.

Conclusion: In conclusion, DMD patients have increased resting HR, and decreased time domain HRV values indicating parasympathetic withdrawal. This type of autonomic dysfunction in adults is associated with increased mortality. HRV analyses in DMD patients may be helpful in risk stratification.

Table 1: Decreased Time Domain HRV in DMD Patients



*P $\leq$ 0.0001

46. Novel model of stochastic cardiomyocyte apoptosis to activate cardiac stem cells

Jop H. van Berlo, Allen York and Jeffery D. Molkentin

Introduction. Recently, it has become evident that the heart can renew itself. Despite much debate on the exact level of endogenous renewal rates, after injury the heart only displays limited regeneration. One reason for the limited regeneration in response to a myocardial infarction could be the ischemic environment is not conducive to stem cell mediated repair. Here, we developed a new mouse model of myocardial injury that does not rely on a large scar. Instead, we induce cardiomyocyte apoptosis stochastically throughout the heart as a means of activating cardiac stem cell regeneration.

Methods. Inducible cardiomyocyte specific MerCreMer mice were bred to the Rosa26-DTA reporter. We induced stochastic cardiomyocyte apoptosis by administering raloxifene at various doses. Immunohistochemistry was done using standard techniques.

Results. In response to raloxifene mice displayed dose dependent cardiac dysfunction within 4 days. Cardiac function however, was restored to normal after 4 weeks. Evans Blue dye injections showed about 5% cardiomyocyte dropout due to apoptosis. In response to injury there was widespread activation/influx of c-kit positive cells. We injected EdU to assess proliferation of c-kit positive cells and of cardiomyocytes and showed EdU, c-kit double positive cells suggestive of cardiac stem cell proliferation. We next tested whether some EdU positive cells could become adult cardiomyocytes. Indeed, 4 months after the initial injury and injection of EdU we could find adult cardiomyocytes that were positive for EdU.

Conclusion. Using a novel model of inducible stochastic cardiomyocyte apoptosis, we were able to generate a dose dependent cardiac dysfunction that restored to normal in 4 weeks. There was activation of c-kit positive cells that proliferated and may have differentiated into adult cardiomyocytes. We are currently comparing the level of c-kit activation to

the level in response to ischemia reperfusion and assessing to what extent restoration of cardiac function is due to regeneration.

47. Genetic deletion of RhoA and RhoC promotes stress induced hypertrophy and failure in the mouse heart

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Background. The role of the small GTPases RhoA/C in vivo as regulators of the cardiac hypertrophic response is currently unknown. Therefore, this study investigated the *in vivo* role of both RhoA and RhoC in the development of pathological cardiac hypertrophy and failure.

Methods and results. Endogenous RhoA and RhoC protein and activity levels significantly increase in the heart after pressure overload induced by transverse aortic constriction (TAC). Cardiomyocyte-specific deletion of RhoA-loxP with β-myosin heavy chain-cre ($RhoA^{\beta\text{-}MHC\text{-}cre}$) and RhoC total gene deleted mice produced viable adults, without altering the basic architecture and function of the heart at 2 and 8 months of age. However, $RhoA^{\beta\text{-}MHC\text{-}cre}$ mice subjected to 2 weeks of TAC or angiotensinII/phenylephrine infusion developed greater cardiac hypertrophy and fibrosis compared to wild-type$^{\beta\text{-}MHC\text{-}cre}$ and RhoA-loxP controls. Moreover, 12 weeks of TAC resulted in decreased cardiac contractility, greater ventricular dilation, lung edema and a significant induction of the cardiac fetal gene program in absence of RhoA as compared to control hearts. Ablation of RhoC resulted in greater increases in cardiac hypertrophy after 2 weeks of TAC compared with controls, as well as cardiac dysfunction. Remarkably, cardiac deletion of RhoA resulted in a compensatory upregulation of RhoC protein levels and *vice versa*. Hence, $RhoA^{\beta\text{-}MHC\text{-}cre}$-$RhoC^{-/-}$ mice were generated, showing no obvious morphometric or functional abnormalities at 2 months of age. However, at 8 months of age, $RhoA^{\beta\text{-}MHC\text{-}cre}$-$RhoC^{-/-}$ spontaneously developed mild cardiac hypertrophy. Two weeks of TAC in young $RhoA^{\beta MHC\text{-}cre}$-$RhoC^{-/-}$ hearts displayed an even more exaggerated phenotype than either single null mouse model, such as massive cardiac hypertrophy, lung edema and left ventricular dysfunction. We are currently analyzing all known RhoA and C interacting and signaling proteins for alterations in these double null hearts

that might explain why they normally serve as negative regulators of cardiac hypertrophy.

Conclusions. Taken together, our data identify RhoA and RhoC as important molecular switches that reduce cardiac hypertrophy and prevent transition to heart failure in the mouse heart.

48. Cardiovascular Disease in Twin-Twin Transfusion Syndrome: Comparison of Cardiovascular Assessment Systems

Chet Villa, Mounira Habli, Jodie Votava-Smith, James Cnota, Foong-Yen Lim, Allison Divanovic, Yu Wang, Erik Michelfelder

Objective: The Cincinnati Staging System, one of several systems used to characterize cardiac dysfunction in twin-twin transfusion syndrome (TTTS), assesses severity of cardiac dysfunction by grading ventricular hypertrophy, atrio-ventricular valve regurgitation and myocardial performance index (MPI). These systems, which also include the Children's Hospital of Philadelphia (CHOP) and cardiovascular profile scores (CVPS), have been correlated to outcome and used to follow recovery following laser therapy. This study sought to evaluate the relationship between these staging systems in TTTS.

Study design: We retrospectively reviewed 100 fetal echocardiograms divided into 4 equal cohorts with none, mild, moderate or severe recipient twin cardiomyopathy by Cincinnati Stage. Each echocardiogram was also graded using the CHOP and CVPS scores. We assessed correlation among each of the scoring systems. We also assessed correlation between the right ventricular (RV) and left ventricular (LV) MPI, a Doppler based index of myocardial function, and CHOP and CVPS scores. Correlation was assessed by Spearman correlation coefficient.

Results: Cincinnati Stage has modest correlation with CHOP ($r=0.41$, $p<0.0001$) and CVPS ($r= -0.45$, $p< 0.0001$) scores. There was modest correlation between RV ($r=0.35$, $r=-0.45$, $p\leq 0.0003$) and LV ($r=0.32$, $r=-0.41$, $p\leq 0.001$) MPI and the CHOP and CVPS scores, respectively. However, 48% of patients with minimally elevated CHOP (0-1) and 46% of patients with minimally depressed CVPS (9-10) scores had significant elevation in RV or LV MPI (z-score >3) (see Figure 1).

Conclusions: There is modest correlation between the Cincinnati Staging system, MPI and the CHOP and CVPS scores. However, almost half of all patients with normal to near-normal CHOP and CVPS scores display significant elevation of RV or LV MPI. Quantitative assessment of cardiac function using MPI may identify patients with cardiac dysfunction not

identified by qualitative variables included within the CHOP and CVPS scores.

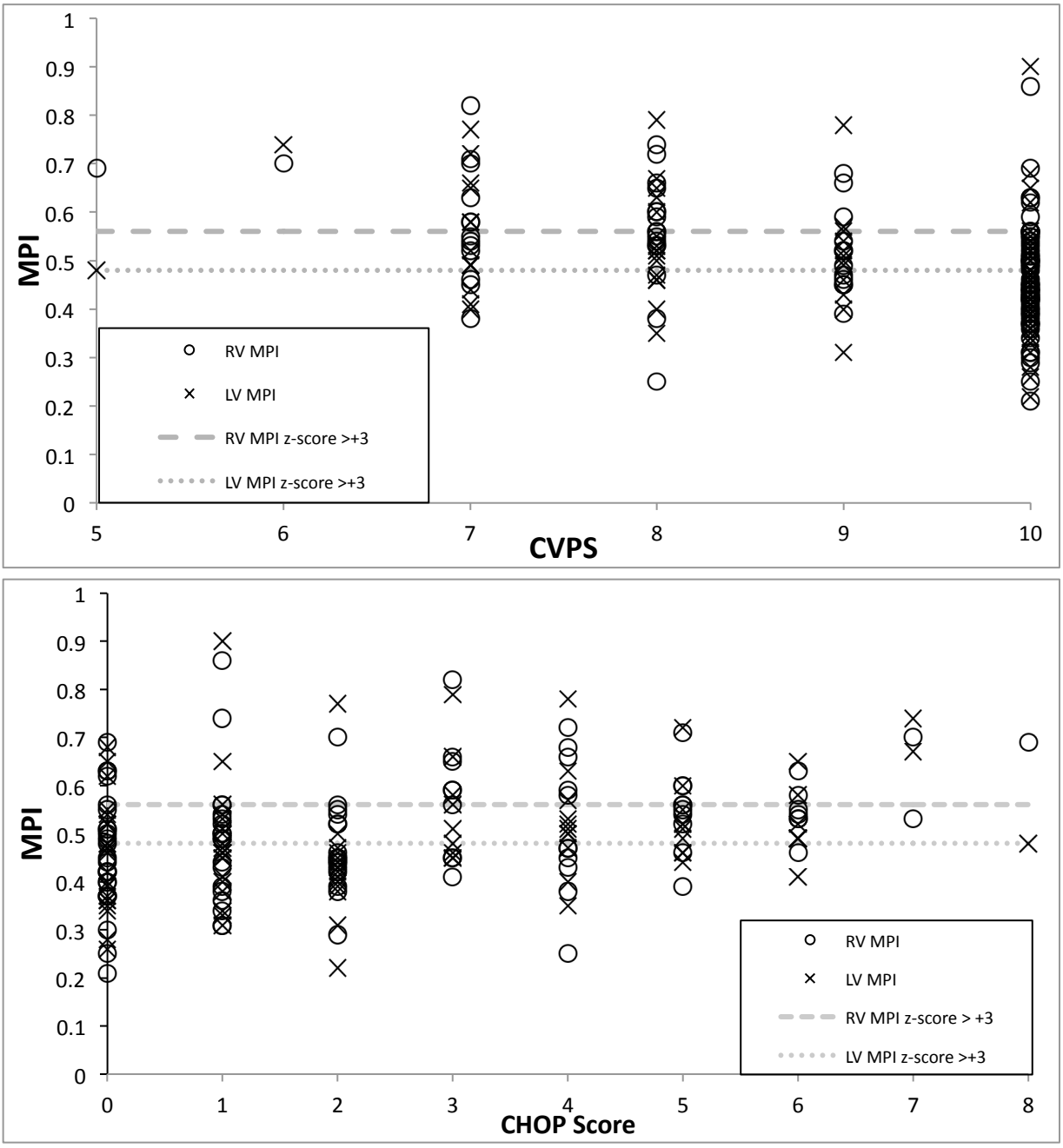

Figure 1. Children's Hospital of Philadelphia (CHOP) score and cardiovascular profile score (CVPS) versus myocardial performance index (MPI).

49. COX2 expression is induced in calcific aortic valve disease

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Aortic valve disease affects approximately 2% of people in the United States. In calcific aortic valve disease (CAVD), deposition of calcification has been compared to the osteogenic process of new bone formation, and many osteogenic factors are expressed in calcific diseased AoVs. The current study uses the *klotho*-deficient mouse model of CAVD, which develops valve calcification with concomitant induction of osteogenic gene expression. Notably, there is no evidence of increased inflammatory cell infiltration in the calcified AoV lesions of *klotho*-deficient mice, suggesting that calcification in response to inflammation is not the primary mechanism underlying the AoV calcification in these mice. Microarray expression analysis reveals that cyclooxygenase 2 (COX2) gene expression is increased in the AoVs of *klotho*-deficient mice compared to wild type littermates. COX2 synthesizes prostaglandins and is implicated in osteoblast differentiation and bone repair, in addition to its well-known role in inflammation. We hypothesize that increased expression of COX2 contributes to osteogenic-like AoV calcification in *klotho*-deficient mice. By immunohistochemistry, we demonstrate that COX2 is expressed in the region of AoV calcification in *klotho*-deficient mice but is not apparent in control valve interstitium. This finding is corroborated in human CAVD specimens, where COX2 expression is increased in calcified diseased valves, whereas minimal expression is observed in controls. Furthermore, expression of the prostaglandin receptor EP2 is increased in AoV tissues of *klotho*-deficient mice consistent with increased prostaglandin signaling. Currently, *klotho*-deficient mice are being treated with oral Celecoxib (selective COX2 inhibitor), in order to determine if inhibition of COX2 function will prevent or reduce AoV calcification in these mice. Together these data provide initial evidence for the potential use of COX2 inhibitors to pharmacologically prevent or delay AoV calcification.

50. Inhibition of p38α reduces pathology in multiple models of muscular dystrophy

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The muscular dystrophies are a group of inherited diseases that are characterized by progressive muscle weakness and wasting, with cycles of degeneration and regeneration of muscle fibers. Identification of the molecular effectors underlying myofiber degeneration and death, as well as the compensatory influx of inflammatory cells and fibrotic replacement, might suggest novel treatment targets. p38α mitogen-activated protein kinase (MAPK), which is a highly potent inflammatory reactive signaling factor, has been implicated in skeletal muscle development, although very little is understood about its potential role in muscular dystrophy. Here we specifically deleted p38α in the skeletal muscle of δ-sarcoglycan (*sgcd)* null mice and *mdx* mice using a floxed p38α allele and the *MLC1f-cre* knock-in allele to examine its role in MD. At three and six months of age we observed a significant reduction in pathologic indices and an increase in muscle endurance during forced running. A similar reduction in pathology in skeletal muscle of *sgcd-/-* and *mdx* mice was observed when treated with a p38α/β pharmacologic inhibitor over 9 weeks. Dystrophic hamsters were also tested to determine if the inhibitor effects were present in a non-mouse model of muscular dystrophy. *F1B* (wild type) and *TO-2* (*Sgcd-/-)* dystrophic hamsters were treated for 9 weeks with inhibitor and prednisone, the standard of care for patients with dystrophy. At the end of the treatment period, we observed a similar reduction in pathology in the skeletal muscle of the dystrophic hamsters in addition to an increase in muscle endurance during forced running. Finally, in order to determine if over activation of p38 is sufficient to cause a dystrophy-like pathology in skeletal muscle, we used a transgenic approach in mice. Skeletal muscles from constitutively active MKK6-transgenic mice, which had constitutive p38 activation, exhibited severe muscle wasting phenotype with the hallmarks of dystrophic disease. Taken together, these data suggest a detrimental role of p38α in the progression of muscular dystrophy and

suggest a novel therapeutic approach that could be employed quickly in humans.