

Heart Institute Research Retreat
September 12, 2011 * Kingsgate Marriott Conference Center

8:00-8:30 Breakfast and Welcome: Jeff Robbins, Jeff Towbin

Session 1 Moderator: Linda Cripe

8:30-9:00 Jeff Molkentin Inhibition of p38 MAPK reduces muscular dystrophy in mice

9:00-9:30 Kan Hor Assessment of Duchenne Muscular Dystrophy Beyond Squeeze

9:30-10:00 Bob Siegel The Heart Institute: A Pound of Prevention

10:00-10:30 Coffee Break

Session 2 Moderator: Enkhe Purevjav

10:30-11:00 Jeff Robbins Cardiomyopathy: what mice can tell us

11:00-11:30 Lynn Jefferies Current Diagnostic and Therapeutic Strategies in Cardiomyopathy: A Novel Approach

11:30-12:00 Stephanie Ware Genotype-phenotype correlations in pediatric cardiomyopathy

12:00-1:00 Lunch

1:00-2:15 HI Resources

Moderator: Jeff Robbins

Jeanne James	In Vivo Imaging of Small Animals
Robert Hinton	Heart Institute Biorepository (HIBR)
Stephanie Ware	Heart Institute Diagnostic Laboratory (HIDL)
Carolyn Lutzko	The Use of iPS cells in Cardiac Research
Brad Marino	Heart Institute Research Core (HIRC)

2:15-2:30 Break

Session 3 Moderator: Bing Hinton

2:30-3:00 Josh Waxman Interaction of cardiac and forelimb progenitor fields

3:00-3:30 Catherine Krawczeski Pediatric Heart Network Single Ventricle Reconstruction Trial: Results and Follow-up

3:30-4:00 Bradley Marino PCQLI Research Program

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

2011
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Poster Abstracts
Listed alphabetically by first author.

1. Placental growth factor regulates cardiac adaptation and hypertrophy through a paracrine mechanism

Federica Accornero¹, Jop Van Berlo¹, Matthew D. Benard¹, Peter Carmeliet² and Jeffery D. Molkentin^{1,3}

¹Department of Pediatrics, University of Cincinnati, Division of Molecular Cardiovascular Biology, ²Vesalius Research Center, K.U.Leuven (P.C.), Leuven, Belgium, ³Howard Hughes Medical Institute, Cincinnati, OH, USA.

Objective. Paracrine growth factor-mediated crosstalk between cardiac myocytes and non-myocytes in the heart is critical for programming adaptive cardiac hypertrophy in which myocyte size, capillary density, and the extracellular matrix function coordinately. Here we examined the role that placental growth factor (PGF) plays in the heart as a paracrine regulator of myocyte to non-myocyte communication and its influence on cardiac adaptation to stress stimulation using overexpressing and PGF knockout mice.

Methods and results. We identified PGF as a secreted factor that is predominantly produced in the heart during pressure overload. We studied mice with conditional post-natal PGF overexpression (PGF DTG). While these mice did not have a baseline phenotype, except increased fibrosis with aging, they responded to pressure overload stimulation induced by transverse aortic constriction (TAC) by an increase in hypertrophy (VW/BW (mg/g): 6.6 ± 0.2 for PGF DTG versus 5.6 ± 0.2 for controls; $n \geq 6$ per group; $p < 0.01$), capillary density (vessels/myocyte: 1.6 ± 0.05 for PGF DTG versus 1.4 ± 0.03 for controls; $n \geq 7$ per group; $p < 0.01$) and fibrosis. Despite a mild increase in fibrosis, cardiac function remained intact even after 12 weeks of pressure overload. On the other hand, PGF knockout mice (*Pgf*^{-/-}) succumbed to heart failure within a week of pressure overload (fractional shortening (%): 20.2 ± 2.3 for *Pgf*^{-/-} versus 30.4 ± 1.1 for controls; $n \geq 7$ per group; $p < 0.01$). These hearts displayed dilation and capillary rarefaction (vessels/myocyte: 1 ± 0.05 for *Pgf*^{-/-} versus 1.2 ± 0.04 for controls; $n \geq 7$ per group; $p < 0.01$). Mechanistically we show that PGF has no direct effect on the cardiomyocytes but works through its direct actions on endothelial cells and fibroblasts by inducing capillary growth and fibroblasts proliferation.

Conclusion. PGF is a secreted factor that supports hypertrophy and cardiac function during pressure overload by affecting endothelial cells and fibroblasts.

2. Determination of the cMyBP-C's binding sites for actin and their regulation by phosphorylation state of cMyBP-C

Md. Shenuarin Bhuiyan, James Gulick, Manish Gupta, Jeffrey Robbins

Molecular Cardiovascular Biology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH

Despite very early demonstrations of myosin binding protein C's (MyBP-C) interaction with the actin thin filament, different investigators using biochemically-based assays have come to different conclusions regarding cardiac MyBP-C's (cMyBP-C) N-terminal actin binding region, necessitating an independent method of confirming binding and identifying the responsible domain(s) and specific residues. In the present study, we dissect the unique regulatory role of cMyBP-C's N-terminal domains via interaction with actin and/or S2-MyHC and help to establish the detailed structure-function relationships needed to fully understand cMyBP-C's ability to impact on myofilament contraction. We set up a genetic, yeast 2-hybrid (Y2H) screen to systemically define the binding site(s). Using Y2H and co-sedimentation experiments, we confirmed that cMyBP-C's C1 and m domains (as well as domains containing them as observed in C1-C2, L-C2, C0-C2 and C1-m) are putative actin binding sites. We also distinguished the cMyBP-C's S2-MyHC binding site from that of the actin binding site by Y2H experiments. We identified the critical actin binding residues in the C1 region based on similarities to a consensus actin binding site and subsequently mutated specific residues in this region in an effort to abolish actin binding. We confirmed the actin binding ablation by Y2H and co-sedimentation experiments. Ablation of cMyBP-C/actin binding did not interfere with cMyBP-C/S2-MyHC binding. In order to understand the regulatory role of phosphorylation on cMyBP-C/actin interaction, we generated cMyBP-C domains where the Ser-273, Ser-282 and Ser-302 sites in the m domain were mutated to either a nonphosphorylatable residue (alanine) or a charged phosphorylation mimetic (aspartate). Y2H and co-sedimentation studies indicated that the phosphomimetic cMyBP-C regulatory motif, or m domain cannot bind with actin. Phosphomimetic C1m and C1mC2 also showed reduced actin binding while phospho-ablated fragments had no effect on actin/cMyBP-C interaction. These results suggest that the N-terminus of cMyBP-C interacts with F-actin through fragment C1 and m binding sites and that binding at these sites is reduced by phosphorylation.

3. Pod1/Tcf21 is regulated by retinoic acid signaling and is required to inhibit smooth muscle differentiation in epicardium-derived cells

Caitlin M. Braitsch, Michelle D. Combs, and Katherine E. Yutzey

Division of Molecular Cardiovascular Biology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH

During embryonic development, epicardial cells undergo epithelial-to-mesenchymal transition to form epicardium-derived cells (EPDCs), which invade the myocardium and differentiate into fibroblasts, smooth muscle, and endothelial cells. Immunofluorescence studies demonstrate that the transcription factors (TF) Pod1(Tcf21), WT1, Tbx18, and NFATc1 are expressed in overlapping and distinct epicardial and EPDC subpopulations in chicken and mouse hearts. The upstream regulation of TF expression in EPDCs was examined in isolated chick embryonic day 7 (E7) EPDCs. Expression of *Pod1* and *WT1*, but not *Tbx18* or *NFATc1*, is upregulated with all-*trans*-retinoic acid (RA) treatment, as determined by quantitative RT-PCR. Similar induction of *Pod1* and *WT1* expression in EPDCs occurs with RA treatment of intact CE7 hearts. In addition, RA treatment inhibits smooth muscle differentiation, as indicated by SM22 α expression, while treatment with the RALDH1 α inhibitor DEAB increases SM22 α in CE7 hearts. In differentiating EPDCs, Pod1 is downregulated as EPDCs differentiate into smooth muscle; thus Pod1 and SM22 α have mutually exclusive expression in chick E7 and mouse E17.5 EPDCs. These data led to our hypothesis that Pod1 inhibits EPDC differentiation into smooth muscle. In order to determine if loss of Pod1 affects EPDC differentiation into smooth muscle *in vivo*, *Pod1*^{LacZ} knock-in mice were obtained, and cell fate specification was analyzed by qRT-PCR and immunohistochemistry in embryonic hearts and lungs. In E17.5 *Pod1*^{-/-} hearts, Pod1-deficient EPDCs robustly express SM22 α in the entire subepicardium covering the myocardium. In heterozygous littermate control hearts, SM22 α is not expressed in the subepicardium; rather, SM22 α expression is activated in smooth muscle cells surrounding coronary vessels after downregulation of Pod1. These data indicate that loss of Pod1 results in premature differentiation of EPDCs into smooth muscle, and that Pod1-deficient smooth muscle cells in the subepicardium are unable to migrate into the myocardium. Previous studies have shown that Pod1 is expressed in the lung mesenchyme, and loss of Pod1 results in hypoplastic lungs. *Pod1*^{-/-} lungs have significantly increased SM22 α expression at E18.5, compared to heterozygotes, which indicates that loss of Pod1 leads to premature differentiation of lung mesenchyme into smooth muscle. Together these data support our hypothesis that RA signaling promotes *Pod1*, thereby inhibiting EPDC differentiation into smooth muscle. Overall this work will help to define the mechanism regulating differentiation of progenitor cells into smooth muscle in the developing heart and lungs.

4. The role of sodium calcium exchanger 1 (NCX1) in muscular dystrophy

Adam Burr, Doug Millay, Sanjeewa Goonasekera, Jeffery Molkentin

Duchenne muscular dystrophy (DMD) is caused by mutation of the protein dystrophin. Without dystrophin the membrane becomes less stable and calcium influx increases through tears or stretch activated channels. Increased calcium influx can activate calpains and induce necrosis leading to muscle damage. The process of sodium calcium exchange could increase calcium efflux and decrease muscle necrosis. We *hypothesized* that increased NCX1 expression would increase calcium efflux and decrease dystrophic pathology. To test this hypothesis we generated muscle specific NCX1 transgenic mice. Surprisingly, the upregulation of sodium calcium exchange induced hindlimb but not diaphragmatic muscle pathology. When we crossed the NCX1 transgenic mouse to *mdx* (dystrophin deficient mouse model) and *sgcd*^{-/-} (delta sarcoglycan deficient model of limb girdle muscular dystrophy) mice we found that the pathology in the hindlimb was exacerbated while the pathology in the diaphragm had been rescued. These results suggest that NCX1 acts primarily to mediate calcium influx in the hindlimb and in calcium efflux mode in the diaphragm. In isolated FDB muscle fibers we found that sodium calcium exchanger function was increased. We also investigated the role of endogenous NCX1 in muscular dystrophy by deleting it in *sgcd*^{-/-} muscular dystrophy. We found that NCX1 deletion rescued pathology at early time points, but that this protection was lost at 6 months as endogenous NCX1 expression waned. Thus, NCX1 activity contributes to early pathology in muscular dystrophy and sustained expression is itself pathologic.

5. An essential and highly conserved role for Zic3 in left-right patterning, gastrulation and convergent extension morphogenesis

Ashley Cast, MS¹, Chunlei Gao, PhD², Jeffrey Amack, PhD², and Stephanie Ware, MD, PhD¹

¹Division of Molecular Cardiovascular Biology, Department of Pediatrics, Cincinnati Children's Hospital Medical Center, ²Department of Cell and Developmental Biology, State University of New York Upstate Medical University

Mutations in ZIC3 result in X-linked heterotaxy in humans, a syndrome consisting of left-right (L-R) patterning defects, midline abnormalities, and cardiac malformations. Zic3 null mice phenocopy human heterotaxy; however, they also exhibit defects in gastrulation, neural tube closure, and axial patterning, all of which require proper convergent extension (C-E) morphogenesis. Zic3 is highly expressed during gastrulation in the neuroectoderm and mesoderm of mouse, *Xenopus laevis*, and zebrafish, which suggests a conserved functional role in early development. Based on the phenotypes of Zic3 null mice and expression data, we hypothesized that Zic3 is necessary for proper C-E morphogenesis. To further investigate the mechanistic role of Zic3, we utilized two model systems, *Xenopus laevis* and zebrafish, to perform loss of function analyses. Assay of characteristic C-E events including blastopore closure, notochord elongation, somite and neural plate width, and anterior-posterior axis elongation demonstrated significant impairment in C-E of Zic3 morphants compared to controls. Interestingly, morphants with moderate to severe C-E defects exhibit an increase in laterality defects, suggesting that early C-E defects may underlie L-R patterning defects at later stages. The majority of *Xenopus* and zebrafish Zic3 morphants exhibit bilateral expression of left-sided molecular markers, followed by gut coiling and heart looping abnormalities at later stages, indicating a conserved role of Zic3 in L-R patterning. These results identify a previously unrecognized role for Zic3 in proper C-E morphogenesis during early vertebrate development and provide insight into a potential novel mechanism for human laterality disorders.

6. Different effects of Tbx20 on cardiomyocyte proliferation during early and late embryonic heart chamber maturation *in vivo*

Santanu Chakraborty and Katherine E. Yutzey

The Heart Institute, Cincinnati Children's Hospital, Cincinnati, OH 45229, USA.

Early embryonic lethality of *Tbx20*-null mice precludes the detailed assessment of *Tbx20* function in later stages of heart chamber and valve development. *TBX20* gain-of-function mutations in humans have been associated with a wide spectrum of cardiovascular abnormalities. Therefore, the effects of increased *Tbx20* during heart development were examined using *CAG-CAT-Tbx20* transgenic mice with Cre-dependent expression of *Tbx20*. Here, *Nkx2.5* and β MHCCre mice are used for early and late overexpression of *Tbx20* in cardiomyocytes of transgenic animals. *Nkx2.5*Cre mediated overexpression of *Tbx20* results in reduced proliferation and increased expression of a cell cycle inhibitor, p21 in cardiomyocytes at embryonic day (E) 9.5. In contrast, β MHCCre-mediated overexpression of *Tbx20* in differentiated cardiomyocytes results in increased thickness of compact myocardium with increased cardiomyocyte proliferation at E14.5. In β MHCCre;*Tbx20* hearts, initial chamber maturation is apparently unaffected at E10.5, but *Tbx2* expression is decreased in myocardium at E14.5. At E18.5, *Tbx20* overexpression leads to increased expression of *Tbx5*, *Cx40*, and *Cx43* throughout the ventricular myocardium. *Tie2*Cre-mediated overexpression of *Tbx20* results in reduced endocardial cushion (EC) formation with decreased proliferation. *Tbx20* overexpression in the *Tie2*Cre lineage also results in reduced expression of several endothelial genes, but expression of mesenchymal markers is maintained. Thus increased expression of *Tbx20* represses cell proliferation in early cardiomyocytes and EC endothelial cells but promotes proliferation and expression of *Tbx5* and Connexin genes during heart chamber maturation. Together, these analyses provide initial evidence that *Tbx20* has different functions in regulation of cell proliferation and lineage maturation at distinct stages of cardiomyocyte development.

7. The Role of Myopalladin in the Interactions of ANKRD1/CARP with the Co-Activator p53 in Hypertrophic Cardiomyopathy

Christopher H Cheng, MD, PhD, Jeffrey A Towbin, MD, Enkhsaikhan Purevjav, MD, PhD,

The Heart Institute, Division of Cardiology, Cincinnati Children's Hospital, 3333 Burnet Avenue, Cincinnati, OH 45229

Background: Myopalladin (MYPN) is a Z-disc protein that interacts with various Z-disc proteins, including the cardiac ankyrin repeat protein ANKRD1 (CARP). MYPN and CARP also interact with other sarcomeric proteins including titin and nebulin, which are involved in mechanosensing and sarcomeric integrity, as well as other pleiotropic functions in myocardial development and remodeling. Mutations in MYPN and CARP genes have been identified in human dilated and hypertrophic cardiomyopathies and studies in these cases have noted that both MYPN and CARP have dual distributions in the cytoplasm and nucleus. In the nucleus, CARP has been characterized as a transcriptional co-activator for p53, repressing gene expression of the cardiac proteins in an *in vitro* system. Ample evidence suggests a MYPN-dependent cytoplasmic/nuclear anchoring and regulating mechanism for CARP although the molecular basis of MYPN modulation of CARP gene transcription is unclear. Previously, our laboratory identified a novel MYPN variant (MYPN^{Y20C}) in dilated and hypertrophic cardiomyopathy patients and a transgenic murine model was created.

Methods and Results: A transgenic MYPN^{Y20C} cardiac-restricted overexpression mouse model was generated and resulted in hypertrophic cardiomyopathy. To gain insight into MYPN function, particularly in the regulation of CARP and its role in cardiomyopathy development, we evaluated these models for changes in binding of MYPN^{Y20C} and CARP using *in vitro* and *in vivo* systems. The MYPN^{Y20C} mutation resulted in perinuclear localization in myoblast cells while CARP retained its cytoplasmic/nuclear dual distribution; as did wild type MYPN. Co-immunoprecipitation demonstrated decreased association between CARP and MYPN^{Y20C} suggesting a dissociation of the cellular distributions of CARP and MYPN^{Y20C}. In addition, CARP was phosphorylated on its serine site(s) but coexpression with MYPN led to a suppression of serine phosphorylation of the CARP protein. In transgenic MYPN^{Y20C} hearts, peri-nuclear MYPN^{Y20C} had significantly increased association with p53, a co-activator for negative gene regulation of CARP. Nuclear localization of p53 is a critical element in the activation of its transcription function, and its sequestration in the cytoplasm renders the protein non-functional. Inactivation of p53 by MYPN^{Y20C} led to a loss-of-function of CARP inhibition on gene transcription, demonstrated by increased expression of the CARP-specific sarcomeric protein myosin heavy chain (MHC), and atrial natriuretic peptide (ANP), and brain natriuretic peptide (BNP) *in vivo*.

Conclusion: Taken together, our study demonstrates a novel role for myopalladin in the development of the hypertrophic cardiomyopathy. The perinuclear/cytoplasmic sequestration of p53 by MYPN^{Y20C} resulted in the inactivation of p53, which leads to loss-of-function of CARP gene regulation. Our data also suggests that myopalladin plays a pivotal and active role in relaying cardiac Z-disc signaling to those involved in CARP phosphorylation/activation.

8. Evidence of Trans-placental Antibody Transfer in Proposed Animal Model for Hypoplastic Left Heart Syndrome

Charles R. Cole¹, Mitali Basu¹, R. Scott Baker¹, Christopher T. Lam¹, Anoop Brar¹, Danielle Herbert¹, Adita Mascaro-Blanco², Katherine E. Yutzey¹, Madeline Cunningham, PhD² and Pirooz Eghtesady, MD, PhD³

¹Cincinnati Children's Hospital Medical Center, Cincinnati, OH, ²University of Oklahoma Health Sciences Center, Oklahoma City, OK, ³Washington University, St. Louis, St. Louis, MO

Background: The pathogenesis of Hypoplastic Left Heart Syndrome (HLHS), a congenital heart disease with significant morbidity and mortality, remains unknown. We previously hypothesized that a subset of HLHS represents a type of rheumatic heart disease in the fetus based on similarities between the diseases. Trans-placental passage of maternal anti-cardiac myosin (CM) antibodies is postulated to play a key role in the pathogenesis of disease. Anti-CM antibody titers are increased following streptococcal infection and have been shown to induce myocarditis and valvulitis in adults. Elevated anti-CM antibody titers have also been documented in mothers of HLHS babies. This study was performed to test our hypothesis in an animal model.

Methods: Female Lewis rats (~ 8 weeks old) were immunized with either streptococcal antigen M type 5 *S. pyogenes* (PepM5; n=6), rat CM (n=8) or saline (controls; n=5) with three booster injections administered at 2-week intervals. Serum titers of acquired PepM5 or CM antibodies were determined by ELISA assays every 7-14 days. No boosters were administered during gestation. Trans-uterine echocardiography was performed near term (E19-21) to determine fetal number and viability then cesarean section was performed under anesthesia to deliver the progeny. Maternal and fetal hearts were fixed in 4% paraformaldehyde, paraffin embedded, sectioned at 7 μ m intervals, and analyzed for histopathology.

Results: All rats immunized with PepM5 had elevated serum anti-PepM5 antibody titers (>1:12800) and two of these animals also had elevated CM titers (1:800). The offspring of these PepM5 immunized animals had elevated anti-PepM5 antibody titers (\geq 1:6400), but no anti-CM elevation. Rats immunized with CM had a variable response ranging from anti-CM titers of 1:1600 to >1:12800; there were two non-responders. Their fetuses had anti-CM titers that ranged from 1:100 to 1:800. None of the controls had detectable serum titers. 16 of 55 fetuses in CM treatment group had evidence of gross left-sided cardiac malformations; many developed hypoplastic left ventricular (LV) cavities. The pathologic hearts displayed thickened LV and septal myocardium with concurrent valvular abnormalities. Several animals had ventricular septal and atrioventricular cushion defects.

Conclusion: This animal model demonstrates that trans-placental transfer of anti-CM antibodies is associated with left-sided birth defects in the developing heart. These cardiac abnormalities occur along a variable spectrum that closely resembles a subset of HLHS phenotype.

9. Cardiac expression of the junctional complex protein Mg29 results in dilated cardiomyopathy and sudden death

Robert N. Correll, Jeffrey M. Lynch, Didier X.P. Brochet, Michelle A. Sargent, Allen J. York, Jeffery D. Molkentin

Mg29 is a 29-kD protein required for proper t-tubule maintenance and store-operated Ca^{2+} entry in skeletal muscle. Our lab recently demonstrated that overexpression of the transcription factor Mef2A in the mouse heart, which results in dilated cardiomyopathy at three weeks of age, was accompanied by 20-fold induction of Mg29 message. Subsequent analysis confirmed upregulation of Mg29 message and protein in a variety of cardiac disease models. To better understand the role of Mg29 in cardiac disease, we created a transgenic mouse with tetracycline inducible expression of Mg29 in the heart. Constitutive expression of Mg29 results in dilated cardiomyopathy shortly after weaning, accompanied by nonsustained ventricular arrhythmia and sudden death. Delaying Mg29 expression until after weaning resulted in a similar phenotype by six months of age. Cardiac myocytes isolated from Mg29 transgenic mice demonstrated spontaneous Ca^{2+} transients, and Mg29 co-immunoprecipitated with RyR2 in transgenic hearts, suggesting that Mg29 overexpression may cause disease by modifying RyR2 activation characteristics, promoting arrhythmia and diastolic Ca^{2+} leak.

10. Genetic and Functional Analysis of Copy Number Variation in Heterotaxy Spectrum Cardiovascular Malformations

Jason Cowan, Ashley Cast, Muhammad Tariq, John Belmont, Jeffrey Towbin, Teresa Smolarek, Seema Lalani, Stephanie M. Ware

University of Cincinnati

Submicroscopic genomic imbalances, also known as copy number variants (CNVs), are associated with both Mendelian and complex genetic disorders. Pathogenic CNVs are identified in 15-25% of patients with syndromic anomalies, but their prevalence in isolated birth defects is less clear. Heterotaxy spectrum cardiovascular malformations occur as syndromic or isolated defects and are genetically heterogeneous and highly heritable. Point mutations in genes currently linked to molecular pathways governing early left-right patterning are detected in less than 10% of patients with heterotaxy suggesting a potential for gene discovery with alternative testing methodologies. We hypothesize that identification of novel pathogenic CNVs in patients with heterotaxy spectrum malformations will identify novel genes/pathways important for cardiovascular development. We analyzed CNVs in 225 unrelated heterotaxy subjects using both Illumina SNP-genotyping and Agilent array-CGH technologies. Pathogenic CNVs were identified in 6%, with abnormalities ranging from large cryptic unbalanced translocations to small, single exon deletions. In addition, we identified novel, rare CNVs of potential clinical significance in 39% of patients. A subset contain strong candidate genes for heterotaxy including genes with roles in cilia/centrosomal structure, TGF-beta/Wnt/Shh signaling, ion transport, cell adhesion, and cardiovascular development. Functional analysis of candidate genes is ongoing using gene knockdown or overexpression in *Xenopus* and assessment of left-sided molecular marker expression and heart/gut looping. These results will identify novel molecular pathways underlying heterotaxy spectrum cardiovascular malformations. The data demonstrate that CNVs are an important cause of heterotaxy spectrum malformations and will guide development of genetic testing and recommendations for clinical diagnostic evaluation.

11. Retinoic acid receptor alpha-b variant 1 restricts cardiac specification during zebrafish development

Enrico D'Aniello and Joshua S. Waxman

Retinoic acid (RA) signaling regulates multiple aspects of vertebrate embryonic development. In the vertebrate heart, RA signaling is necessary for proper cardiomyocyte (CM) specification, as a lack or excess of RA signaling during embryonic development results in congenital cardiovascular malformations. Ligand-activated transcription factors, the RA receptors (RARs), mediate RA signaling. Although the functions of vertebrate RARs have been widely studied, currently the RARs required to restrict CM specification have not yet been identified. Understanding which RAR(s) function to restrict CM specification is critical to elucidate the precise transcriptional mechanisms required for proper CM specification. We have been able to identify a previously unrecognized RARalpha-b splice variant (RARabv1) in zebrafish. Interestingly, depletion of RARabv1, using morpholino oligonucleotides (MOs), results in zebrafish embryos with enlarged hearts, increased CM cell number and increased CM marker gene expression at 48 hpf. These phenotypes, which are reminiscent of loss of RA signaling using previously established genetic and pharmacological methods, suggest RARabv1 is required to restrict CM specification. In order to determine if the enlarged hearts in RARabv1 depleted embryos are due to earlier effects on CM specification and differentiation, we examined cardiac progenitor markers (*nkx 2.5*, *gata4* and *hand2*) and CM differentiation markers (*myl7*, *vmhc* and *amhc*). Consistent with a role in restricting CM specification and differentiation, we found that depletion of RARabv1 induced an increase in the expression both the CM specification and differentiation marker genes. Together, our studies suggest that RARabv1 in zebrafish is required to restrict CM number through effects on CM specification. Therefore we have provided a basis for future studies aimed at dissecting the *in vivo* and *in vitro* transcriptional mechanisms that are required to limit CM specification.

12. TRPC6 promotes the fibroblast to myofibroblast transition

Jennifer Davis, Adam Burr, Gregory Davis, Jeffery D Molkenin

Molecular Cardiovascular Biology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, 45229

During wound healing and tissue remodeling fibroblasts become activated and transdifferentiate into myofibroblasts, a highly specialized cell that expresses contractile α -smooth muscle actin (α SMA), contracts a collagen matrix, and produces extracellular matrix to provide structural support for an injured tissue. Chronic activation and generation of myofibroblasts is thought to promote pathologic remodeling and a wide range of fibrotic diseases. Currently, the field lacks a comprehensive understanding of the regulatory networks or factors involved in myofibroblast activation. Thus, we conducted a genome-wide screen in transformed mouse embryonic fibroblasts (MEF) using the Mammalian Genome Collection (MGC) cDNA library to identify novel genetic activators of the myofibroblast program. Over 17,000 genes from the MGC library were cotransfected with an α SMA-luciferase reporter construct and luciferase activity was measured. Approximately 1% of all clones analyzed generated a positive result, one of which was the gene encoding transient receptor potential 6 (TRPC6), a membrane receptor/channel that provides a Ca^{2+} signal. Similar to results obtained from fibroblasts stimulated with a known myofibroblast agonist, TGF β , TRPC6 overexpression in primary neonatal rat cardiac, human dermal, and mouse dermal fibroblasts transitioned over 60% of the cells to a myofibroblast phenotype that is characterized by α SMA stress fiber formation and the functional ability to contract floating collagen gel matrices. Conversely, primary fibroblasts isolated from *Trpc6* $-/-$ mice were refractory to TGF β stimulated collagen gel contraction suggesting that *Trpc6* is required for myofibroblast formation. Myofibroblast function was also assessed *in vivo* by creating excisional full-thickness wounds in *Trpc6* $+/+$ and $-/-$ mice. By day 5 of the wound healing process TRPC6 $+/+$ mice had achieved $84 \pm 4\%$ closure of the wound while TRPC6 $-/-$ mice showed significantly less closure attaining only $33 \pm 5\%$ regression, impaired granulation tissue formation, and a significantly lower number of myofibroblasts in the wound's border zone. In a myocardial infarction model TRPC6 $-/-$ mice relative to their $+/+$ littermates also showed a significantly higher rate of mortality due to cardiac rupture. Interestingly, TGF β stimulation of primary fibroblasts increased TRPC6 expression but not TRPC3 and significantly elevated store operated Ca^{2+} entry (SOCE) by 23% relative to control fibroblasts, while TRPC6 overexpression elevated SOCE by 49%. Concomitant with the increased SOCE was an increase in calcineurin-NFAT transcriptional activity by both TGF β stimulation and TRPC6 overexpression. Similar to TRPC6, the overexpression of activated calcineurin transitioned 50% of fibroblasts into myofibroblasts, while inhibitors of calcineurin-NFAT signaling blocked TRPC6 mediated myofibroblast formation. *In vivo* adenoviral expression of both TRPC6 or activated

calcineurin rescued the impaired wound healing in TRPC $-/-$ mice, while calcineurin Ab knockout mice had significantly impaired early wound healing. Collectively, these data present a model in which tissue injury stimulates TRPC6 expression that through calcineurin-NFAT induces myofibroblast transdifferentiation, a vital feature in the early of wound healing process.

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13. Distinct phases of Wnt/ β -catenin signaling direct cardiomyocyte formation in zebrafish

Tracy E. Dohn and Joshua S. Waxman

Normal heart formation requires distinct phases of canonical Wnt/ β -catenin (Wnt) signaling. Understanding the mechanisms by which Wnt signaling drives correct cardiac formation *in vivo* is critical for understanding the formation of the heart as well as essential to studies developing stem cells into cardiomyocytes (CM) *in vitro*. Here, we investigate the roles of Wnt signaling using heat-shock inducible transgenes which allow us to increase or decrease Wnt signaling in the embryo. During the first 24 hours of development, we find three distinct phases during which Wnt signaling modulates CM formation. Wnt signaling has previously been implicated in mesoderm specification as well as regulating the pre-cardiac mesoderm, however, we have identified a later role during CM proliferation in which Wnt signaling is necessary and sufficient to promote the differentiation of atrial cells. This study also extends the previous studies on Wnt signaling during mesoderm specification and in the pre-cardiac mesoderm. Interestingly, we define a new role for Wnt signaling in the pre-cardiac mesoderm in which Wnt is sufficient to prevent cardiac cell differentiation leading to cell death as opposed to the previously proposed roles in inhibition of cardiac progenitor specification. Together with an even later role of Wnt signaling in restricting the proliferation of differentiating ventricular CMs, our results indicate that there are four distinct phases of Wnt signaling during the first 3 days of zebrafish development that allow for the proper formation of the heart.

14. Incorporating Genetics and Genetic Counseling in the Cardiac Intensive Care Unit (CICU)

A. Garrison¹, D. Nelson¹, M. Magness¹, E. Miller¹, A. Parrott¹, J. James, J.L. Jefferies, J.A. Towbin, R. Hinton¹, P. Goldenberg¹, S. Ware¹

¹*The Heart Institute, Cincinnati Children's Hospital Medical Center*

Congenital heart disease (CHD) is the most common birth defect, with an incidence of 8 in 1000 live births. Approximately 8–13% of CHD is due to chromosomal defects. The genetic contribution to CHD with or without extra-cardiac anomalies, cardiomyopathy, and arrhythmias has been described previously, but is likely underestimated. Early diagnosis of genetic abnormalities in patients with complex cardiac disease has been proven to optimize outcome. An algorithm incorporating cardiovascular (CV) genetic services in the Cardiac Intensive Care Unit (CICU) was developed to serve as a guide for CICU personnel. The algorithm is based on cardiac disease, complexity of CHD, and extra-cardiac features. A consult by a CV geneticist, cardiologist and CV genetic counselor is advised for patients with cardiac disease with extra-cardiac features; patients with isolated, non-syndromic cardiac disease receive a consult by a genetic counselor for family history and discussion of recommended genetic testing. Between March 2010 and May 2011 CV genetic consults were obtained for 127 patients in the CICU. Indications for CV genetics consultation included CHD, cardiomyopathy and arrhythmias. Of the 127 patients seen, 94 had microarray testing, of which 22 revealed a chromosomal imbalance (23.4%); 27 had FISH testing for Deletion 22q11.2, of which 7 were positive (21.2%); and 45 had single gene testing, of which 17 were diagnostic (37.7%). The overall detection rate for genetic abnormalities in this cohort was 34.6%. This algorithm has established collaboration between genetics and the CICU. Given the high yield of genetic testing in this patient population, early incorporation of genetic services are important to optimize evaluation, management, and outcome.

15. Decreased cardiac L-type Ca^{2+} channel activity induces hypertrophy and heart failure in mice

Sanjeeva A. Goonasekera, Karin Hammer, Mannix Auger-Messier, Ilona Bodi, Xiongwen Chen, Hongyu Zhang, Steven Reiken, John Elrod, Robert N. Correll, Allen York, Franz Hofmann, Sven Moosmang, Andrew R. Marks, Steven R. Houser, Donald M. Bers and Jeffery D. Molkentin

L-type Ca^{2+} channel (LTCC) antagonists have been a therapeutic focal point in human cardiovascular diseases for several decades. However, LTCC antagonists appear to have untoward effects in heart failure patients, and it remains uncertain how vasculature versus cardiomyocyte effects contribute to their therapeutic profile. To investigate this issue we examined heterozygous gene-deleted mice for $\alpha_1\text{C}$ ($\text{CaV}1.2$, pore forming subunit), as well as $\alpha_1\text{C}$ -loxP-targeted mice to achieve graded heart-specific $\text{CaV}1.2$ deletion. Adult cardiomyocytes from $\alpha_1\text{C}^{-/+}$ mouse hearts at 10 weeks of age showed a 25% decrease in LTCC current and a modest but significant decrease in cardiac function, which we initially hypothesized would be cardioprotective given beneficial effects previously reported in animal models of hypertrophy and failure with LTCC antagonists. However, $\alpha_1\text{C}^{-/+}$ mice subjected to pressure overload stimulation showed greater cardiac hypertrophy, greater reductions in ventricular performance, and greater ventricular dilation compared with $\alpha_1\text{C}^{+/+}$ controls. $\alpha_1\text{C}^{-/+}$ mice also showed signs of cardiac decompensation after 2 weeks of isoproterenol stimulation and exercise swimming, effects not seen in controls. The same detrimental effects and predisposition to decompensation with stress stimulation was observed in $\alpha_1\text{C}$ -loxP animals with a heart-specific deletion of one allele. More severe reductions in $\alpha_1\text{C}$ protein levels with combinatorial deleted alleles produced spontaneous cardiac hypertrophy (nearly 2.5-fold larger hearts) before 3 months of age. Mechanistically, we present data that suggests a reduction in LTCC current leads to a compensatory increase in intracellular Ca^{2+} to augment the gain in excitation contraction-coupling to preserve contractility. This state results in ryanodine receptor (RyR2) Ca^{2+} leak and increased calcineurin-nuclear factor of activated T-cells (NFAT) signaling to cause hypertrophy and disease.

16. Analysis of the cardiac Myosin Binding Protein-c interactome

Manish K Gupta, James Gulick and Jeffrey Robbins

Department of Pediatrics and the Heart Institute, Cincinnati Children's Hospital Medical Center, Cincinnati, OH 45229

Myosin binding protein C (MyBP-C) is a thick filament protein that is involved in maintaining sarcomeric structure and function. Mutations within the cardiac isoform of MyBP-C (cMyBP-C) are known to be a cause of cardiomyopathies worldwide, with more than sixty million people being affected. Emerging evidences indicate that cMyBP-C not only interacts with sarcomeric proteins (titin, actin myosin) but also with new partners responsible for regulating other cellular functions. In this study, we searched for novel protein interactions within the cMyBP-C interactome by using proteomic as well as yeast two-hybrid analysis. Our initial analysis centered on proteins that interact with the n-terminal end of cMyBP-C. Using a recombinant C0-C2 fragment as bait, samples were affinity purified from mouse cardiac lysate and separated by electrophoresis. Purified proteins were analyzed by LC-MS/MS coupled with high stringency bioinformatics. Additionally, the yeast two-hybrid system was used to screen a human cardiac cDNA library, using the full-length cMyBP-C as bait. Together, these screenings identified 87 probable interactors, which include cytosolic, sarcoplasmic reticulum, mitochondrial as well as nuclear proteins. Interactions with select candidate proteins were validated by co-localization and co-immunoprecipitation and supported by *in Silico* analysis using bioinformatic. The interacting proteins link cMyBP-C with several novel pathways involved in cell contractility, signal transduction, protein homeostasis, calcium signaling and energy metabolism. Our findings reveal additional roles that cMyBP-C may play in cardiac function.

17. A spectrum of heterotaxy-related heart malformations in *Zic3* hypomorphic mice

Allison M. Haaning, Malgorzata E. Quinn, Stephanie M. Ware

ZIC3 mutation in humans leads to X-linked heterotaxy, a developmental disorder characterized by abnormal organ arrangement and complex cardiovascular malformations. *Zic3* hypomorphic mice were created by targeted insertion of a neomycin cassette in the first intron of *Zic3*. The various complex heart and organ situs defects seen in *Zic3* hypomorphic mouse embryos reflect those seen in human heterotaxy patients. Whereas *Zic3* null mice often die prior to cardiogenesis secondary to gastrulation defects, *Zic3* hypomorphs typically survive until birth. Due to the increased survival rate and high penetrance of heart defects in *Zic3* hypomorphs, they are an ideal model for better understanding the underlying etiology of heterotaxy-related heart defects. Reversed or incomplete heart looping were the primary defects observed at early embryonic stages, and the effects of these early looping defects were later translated into complex cardiovascular defects, such as improper chamber specification and malposition of the great arteries. Whole-mount *in situ* hybridization to analyze expression of *Nppa*, *Nkx2-5*, and *Tbx5* revealed comparable levels in *Zic3* hypomorphic and wild-type embryos; however, the localization of these markers was abnormal when heart looping was abnormal. Previous publications have implied a direct role for *Zic3* within the cardiac compartment. To further examine this, *Zic3* was deleted in the heart and heart progenitors using *Wnt1-cre*, β MHC-cre, and *Mef2c-AHF-cre* mice. There was no lethality or heart abnormalities associated with heart-specific deletion of *Zic3*, implying that cardiovascular malformations in *Zic3* null and hypomorphic mice are secondary to defective heart looping caused by improper left-right specification.

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

Samuel Hanke, MD

Background: Early hospital readmissions have emerged as an indicator of health care quality in adults. Recent studies have evaluated the impact of readmissions in the general pediatrics population. Patients with congenital heart disease (CHD) have high resource utilization and were found to have high readmission frequency when compared with other children with chronic care conditions. Evaluation of the rate and predictors of readmission in patients who have undergone CHD surgery and the resource utilization of these readmission hospitalizations in the United States remains unknown.

Objective: Our primary aim is to determine the incidence of hospital readmissions within 30 days of CHD surgical discharge in patients less than 18 years old and to identify the predictors for readmission. Our secondary aim is to describe the resource utilization of hospital readmissions within 30 days of CHD surgical discharge in patients less than 18 years old and to identify the predictors for increased resource utilization.

Methods: The study will be a retrospective cohort study of patients in the Pediatric Health Information System (PHIS) database with hospital discharge dates between January 1, 2005 and November 30, 2008. Our study population will include all patients that have undergone congenital heart surgery excluding isolated patent ductus arteriosus closure in neonates and transcatheter interventions. Patient demographics, payer type, index hospitalization factors, readmission hospitalization factors and total hospital charges will be analyzed as potential predictors of both readmission and increased resource utilization.

Implications: Results of this study will be used to identify the subset of CHD surgical patients who are at increased risk for readmission and ultimately provide targeted quality improvement interventions to reduce the likelihood of readmission. Reduction of readmission will also lead to decreased resource utilization and ultimately decreased costs with increased value for the care of this population.

19. Novel BMP10 mutation identified in patients with left ventricular noncompaction induces impaired binding affinity between BMP10 and BMP receptors

Keiichi Hirono, MD, PhD, Christopher H. Cheng, MD, PhD, Enkhsaikhan Purevjav, MD, PhD, Jeffrey A. Towbin, MD

Background: Left ventricular noncompaction (LVNC) is a chronic myocardial disease characterized by a pattern of prominent trabecular meshwork and deep intertrabecular recesses communicating with the left ventricular (LV) cavity. LVNC has been considered to be a developmental failure of the heart to form fully the compact myocardium during mid-gestational period of cardiogenesis. Up to 40% of LVNC patients have evidence of familial disease and a significant genetic heterogeneity is notable. However, no evidence of an arrest in embryonic endomyocardial morphogenesis due to abnormalities in genes expressed in the mid-gestational period of cardiogenesis has been demonstrated. Bone morphogenetic protein 10 (BMP10) is cardiac specific ligand expressed in the late stages of cardiac development responsible for cardiomyocyte proliferation, differentiation and compaction.

Rationale: We studied whether p.V406I variant located in the β -domain of the mature BMP10 peptide alters receptor-binding properties of BMP10. Signal-mediating and signal-transducing ability of mutant BMP10 was compared WT BMP *in vitro*.

Methods: Cohorts of 232 patients with LVNC and 272 control individuals were screened using high resolution melting (HRM) assay and direct sequencing. HEK293 cells were co-transfected with WT or mutant BMP10 tagged GFP-chimeras and BMPR1a and BMPR2 tagged with FLAG. Protein purification by affinity chromatography, immunoprecipitation and cross-linking were performed.

Results: The p.V406I (c.5830G>A) variant in the BMP10 gene was identified in two members in the LVNC family. Both, WT or V406I mutant, rhBMP10 were detectable as a monomer, a dimer or a polymer suggesting that V406I mutation did not affect dimerization properties of BMP10. However, mutant BMP10 had a lower affinity to receptors, BMPR1a and BMPR2, compared to WT BMP10 suggesting impairment of ligand-binding ability in mutant, probably, due to domain-specific effects of the V406I mutation.

Conclusion: We report a novel mutation, V406I, in the BMP10 gene in patients with LVNC. Our results suggest significant changes in BMP10 protein function due to an impaired coupling of V406I mutant to BMPR1a and BMPR2 receptors, modulate the BMP10-regulated downstream pathways including MAPK, Smad and ERK pathways,

causing prominent trabeculation and disruption of myocardial compaction and lead to LVNC in humans.

20. Fas-Ligand induce cardiomyopathy via the activation of the ERK1/2 pathway

Anne-Cecile Huby¹, Enkhsaikhan Purevjav¹, Jeffrey A. Towbin¹

¹The Heart Institute, Department of Pediatrics, Cincinnati Children's Hospital Medical Center, Cincinnati, OH.

Fas receptor and its ligand, Fas-L, are the most well characterized co-stimulatory molecules playing an essential role in the induction of programmed cell death or apoptosis. Increase of circulating Fas-L in serum and in cardiomyocytes are described in different models of cardiac diseases such as myocarditis, pressure overload, AND myocardial infarction/ischemia. In addition, associated activation of inflammatory pathways has been observed. However, no studies have yet revealed mechanisms underlying the pathogenicity of FasL in the heart.

Transgenic mice (C57/Bl6) with cardiac-specific over-expression of Fas-L were investigated. We characterized cardiac function by echocardiography. Cellular and molecular studies were performed on heart tissue using genomic, histologic, immunological, and protein analysis techniques.

Fas-L transgenic mice had a significant rate of mortality ($p < 0.0001$) compared to WT due to heart failure. Histological studies of Fas-L hearts revealed predominant perivascular fibrosis associated with inflammation. Expression studies of secondary messengers of the activated Fas/FasL system demonstrated up-regulation of an active CASP-3 confirming induction of apoptosis in myocardium. Down-regulation of FLIP, NIK and NF- κ B was evident. Interestingly, RIP was up-regulated leading to the activation of ERK1/2 (4.27 ± 0.158 vs 10.086 ± 1.591 in WT and FasL mice hearts, respectively, $p = 0.022$). Analysis of inflammatory mediators revealed increase in secretion of interleukines (IL-17, IL-6, IL-1 β and TNF- α) and diffuse infiltration of CD3 positive cells. Studies of fibrotic pathways showed an increased expression of periostin and osteopontin, but not activation of TGF β 1, showing that periostin and osteopontin can be activated independently of TGF β 1 via the ERK1/2 pathway.

Our findings suggest that Fas-L induces cardiac failure, via an activation of the ERK1/2 pathway, which is an important player involved in the hypertrophic response in the heart. Targeting the ERK1/2 pathway in cardiac diseases associated with Fas-L expression may therefore be able to prevent progression to cardiomyopathy.

21. Bax and Bak are essential mediators of the programmed necrotic pathway by regulating the mitochondrial permeability transition pore

Jason Karch, Jennifer Q Kwong, Adam R Burr, Michelle A Sargent, Hanna Osinska, Jeffrey Robbins, Jeffery D Molkentin

Recent findings have suggested that necrotic cell death can be a programmed event. One crucial step in programmed necrosis is the opening of the mitochondrial permeability transition pore (MPTP). Once formed under conditions of high Ca^{2+} or ROS stimulation, the MPTP is thought to form between the inner and outer mitochondrial membranes where it regulates mitochondrial swelling, energy production, and initiation of cell death. However, the molecular identify of the inner and outer membrane components of the MPTP remains elusive, although cyclophilin D (CypD), a prolyl isomerase located in the matrix of the mitochondria, is known to be a critical component and the primary regulatory of the process. Here we determined that the Bcl-2 family members Bax and Bak, which are central regulators of apoptotic cell death, are also absolutely required for programmed necrosis. MEFs deficient in Bax and Bak were resistant to all forms of programmed necrosis, induced by Ca^{2+} , ROS, DNA alkylating agents, and TNF α with caspase inhibition. Mechanistically, Bax and Bak are required for CypD-regulated mitochondria swelling in isolated mitochondria from Bax/Bak deficient MEFs, livers, and hearts. Similarly, Bax/Bak deficient mitochondria have a greater calcium uptake capacity than the controls, similar to CypD deficient mitochondria. In addition, mutants of Bax that cannot form apoptotic pores in the outer mitochondrial membrane are still sufficient to mediate programmed necrosis and mitochondrial swelling. Moreover, the inhibition of necrotic cell death and mitochondrial swelling was reversed by any non-specific pore forming agent in the outer mitochondrial membrane. Collectively, these results suggest that Bax/Bak function as an essential part of the outer membrane component of the MPTP.

22. Maladaptive Matrix Remodeling and Regional Biomechanical Dysfunction in a Mouse Model of Aortic Valve Disease and Aortopathy

Varun K. Krishnamurthy,^{1,2} Amy M. Opoka,¹ Christine B. Kern,³ Farshid Guilak,⁴ Daria A. Narmoneva², Robert B. Hinton¹

¹Division of Cardiology, The Heart Institute, Cincinnati Children's Hospital Medical Center, Cincinnati, OH. ²Department of Biomedical Engineering, University of Cincinnati, Cincinnati, OH. ³Department of Regenerative Medicine and Cell Biology, Medical University of South Carolina, Charleston, SC.

⁴Departments of Surgery and Biomedical Engineering, Duke University Medical Center, Durham, NC.

Aortic valve malformation (AVM) and disease (AVD) are associated with aortopathy, typically manifesting as dilation of aortic root. Elastin haploinsufficiency causes both arteriopathy and aortopathy, and interestingly AVM/AVD is present in 20-45% of patients. *Eln*^{+/-} mice demonstrate early AVM with latent AVD and aortopathy. The mechanism of pathologic ECM remodeling and its impact on the mechanical microenvironment are unknown. Aortic valve and aorta tissue from juvenile, adult, and aged *Eln*^{+/-} mice were studied. ECM composition was examined by Movat's pentachrome stain. Immunohistochemistry was performed for MMP-2 and 9, ADAMTS-5 and 9 (remodeling), sox-9 and *crtl*-1 (cartilage), and intact and cleaved versican (proteoglycan processing). ELISA determined type I and III collagen, and intact and cleaved versican content. Gelatin zymography revealed MMP-2 and 9 enzyme activity. Regional valve (annulus and cusp) and aorta (root and ascending) tissue mechanical properties were tested using micropipette aspiration. Cartilage-like nodules with increased sox-9 and *crtl*-1 expression were identified within *Eln*^{+/-} valve annulus at all stages. Increased cleaved versican in aged *Eln*^{+/-} valves indicated abnormal versican processing. Juvenile *Eln*^{+/-} mice demonstrated AVM without AVD with increased valve MMP-2 and 9 expression and activity. These changes markedly increased with the manifestation of AVD and aortopathy in aged *Eln*^{+/-} mice ($p < 0.0001$). ADAMTS-5 but not ADAMTS-9 expression was increased in aged *Eln*^{+/-} mice, consistent with increased cleaved versican. Biomechanical properties were altered in aged *Eln*^{+/-} valve regions, shown by lower Young's modulus values ($p < 0.0001$). These findings demonstrate that maladaptive ECM remodeling occurs early in the context of AVM and progresses, ultimately resulting in abnormal biomechanics, latent AVD and aortopathy. These mechanistic insights may inform the search for durable bioprostheses and new therapeutic targets.

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

Jennifer Q. Kwong, Jason Karch, and Jeffery D. Molkenin

Uncontrolled cardiomyocyte death is the hallmark of many cardiovascular diseases ranging from ischemic injury to heart failure. Myocyte 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, cell death.

To date, 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 (transient receptor potential channel C3) in the wing disc. Following a systematic genome-wide *in vivo* screen for novel death mediators, we found that RNAi mediated knockdown of the *Drosophila* gene CG8323 strongly repressed the aberrant Trpc3 mediated death.

CG8323 encodes a mitochondrial protein that is highly homologous to mouse SLC25a35, a newly identified but uncharacterized member of the SLC25 mitochondrial carrier superfamily. Surprisingly, RNAi mediated knockdown of SLC25a35 in cultured mouse cells 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.

24. Twist1 directly regulates genes associated with cell proliferation and migration in developing heart valves

Mary P. Lee and Katherine E. Yutzey

Congenital heart defects, including valve malformations, are among the most common birth defects in the world. Heart valves develop from extracellular matrix (ECM) rich endocardial cushions (ECCs) populated by highly proliferative, migratory, and undifferentiated mesenchymal cells. The basic helix-loop-helix transcription factor Twist1 promotes cell proliferation and migration in cultured ECC mesenchymal cells. Although implicated as a regulator of essential mesenchymal functions, Twist1 direct transcriptional targets remain largely unknown. We hypothesize that Twist1 directly regulates transcription of genes promoting cell proliferation and migration in ECC mesenchymal cells. Two candidate genes, *Tbx20* and *Cadherin-11 (Cdh11)*, contain evolutionarily conserved regions (ECRs) with putative Twist1 binding E-box consensus sites. ECRs associated with *Tbx20* and *Cdh11* are transactivated by Twist1 in an E-box dependent manner and are directly bound by Twist1 in mouse ECC mesenchymal cells. Additional Twist1 candidate genes were identified through Affymetrix microarray analysis of MC3T3-E1 cells treated with control siRNA or Twist1 siRNA. Loss of Twist1 resulted in decreased expression of genes associated with cell proliferation, migration, and ECM components. E-box consensus sites were identified within ECRs associated with *Semaphorin3C*, *Gadd45a*, and *Rab39b*. All three ECRs are directly bound by Twist1 in mouse ECC mesenchymal cells. These studies identified Twist1 direct transcriptional targets promoting cell proliferation and migration in ECC mesenchymal cells. Therefore, these Twist1 transcriptional target genes likely contribute to maintenance and expansion of valve progenitor cells during early stages of valve development.

25. Thrombospondin-4 is a novel regulator of adaptive ER stress

Jeff Lynch, Marjorie Maillet, Davy Vanhoutte, Hanna Osinska, Scott Blair, Michelle Sargent, John Lorenz, Bruce Aronow, Jeff Robbins, Jeff Molkentin

Thrombospondin-4 (Thbs4) is a secreted protein upregulated in several mouse models of heart disease and whose function in the heart is unknown. More than 70% of Thbs4-deficient mice die within 48 hours of transaortic constriction (TAC) while Thbs4 transgenics are protected against stress. Electron microscopy showed that transgenics have an expanded endoplasmic reticulum (ER) and microarray analysis revealed an upregulation of numerous ER genes. These changes in the ER serve to enhance ER chaperone protein expression, protein clearance, and the secretory ability of the cell. Thbs4 was found to mediate these effects by directly interacting with the ER stress sensing *Atf6a* transcription factor and facilitating its transport to the Golgi for processing. As Thbs4-null mice have delayed expression of *Atf6a* and ER proteins following TAC, we conclude that a major role of Thbs4 in the heart is as an ER stress response factor providing beneficial ER adaptation and protection.

26. Predictors of Exaggerated Systolic Blood Pressures During Exercise in Young Patients after Coarctation Repair

Peace Madueme

Background: In normotensive subjects, an exaggerated blood pressure (BP) response to exercise is associated with the development of resting hypertension (HTN), a risk factor for morbidity. Whether an exaggerated BP response to exercise in normotensive youth with coarctation of the aorta can be predicted, is not known. We sought to, 1) determine the prevalence of elevated BP during exercise in post-operative coarctation patients with normal resting BP and 2) investigate associations with exercise-induced HTN in this population.

Methods: 38 subjects who had undergone coarctation repair via end to end anastomosis and were normotensive at rest were prospectively enrolled. Anthropometrics and resting BP were measured. 2D echocardiography for systolic and diastolic function was performed. Arterial stiffness was assessed by pulse wave velocity (PWV). A graded exercise test was performed to evaluate BP response to exercise. An exaggerated BP response to exercise was defined as a maximum systolic BP (maxSBP) $\geq 95^{\text{th}}$ %. Correlation analyses were performed to determine associations with maxSBP. Stepwise regression analyses were performed to determine independent predictors of maxSBP.

Results: Mean age was 12.7 ± 3.9 years, 79% male. Mean resting systolic BP (restSBP) was 111.3 ± 13.7 mmHg, mean maxSBP was 178.1 ± 26.1 mmHg. The prevalence of a maxSBP $>95^{\text{th}}$ % was 16.7%. With multivariate analysis, maxSBP correlated with body mass index (BMI), shortening fraction (SF) and PWV ($R^2 = 0.64$, $p = 0.0001$). Mean values for SF, indexed left ventricular mass (iLVM) and estimates of ventricular filling (E/Ea) were elevated compared to published normal values, suggesting cardiac compensation for increased cardiac afterload.

Conclusions: There is a risk of elevated SBP during exercise in normotensive patients after coarctation repair. Relying on resting blood pressures is not sufficient to identify at risk patients. Echocardiography demonstrated abnormalities with ventricular filling, iLVM and SF, suggesting a chronic burden on the heart despite resting normotension. Regular imaging may be necessary to appropriately manage these patients and improve long term outcomes. Current resting BP reference values may need to be amended to sooner identify patients requiring more comprehensive evaluation.

27. Mechanisms of intercalated disc disruption in cardiomyopathies due to genetic abnormalities in the Z-disc proteins

Ruben S.R.M. Martherus PhD, Ken Takagi MD, Nan Gong MD, Jeffrey A. Towbin MD, Enkhsaikhan Purevjav MD, PhD

The Heart Institute, Cincinnati Children's Hospital Medical Center, Cincinnati, OH.

Background: Transgenic mice expressing mutated Z-disc proteins including muscle LIM protein (MLP), myopalladin or nebulin (NEBL) develop dilated cardiomyopathy (DCM) and display disruption of cell-cell junctions at the intercalated discs (ICD). ICD-disruption is also often found in arrhythmogenic right ventricular cardiomyopathy (ARVC) as a result of malfunctioning desmosomes due to mutations in genes encoding desmosomal proteins such as desmoplakin (DSP) and its direct binding partners plakophilin and desmocollin. However, the exact mechanisms involved in the arising of ICD-disruption in DCM remained unknown.

Methods and Results: Using NEBL adenoviral vectors we reconstituted a NEBL^{Q128R} mutant disrupted ICD phenotype in cultures of neonatal rat ventricular cardiomyocytes and H9C2 cells. Immuno-staining showed well defined cell-cell junctions in the non-transduced and β -galactosidase expressing control cells, while cell-cell disruptions were observed in the junctions of the NEBL^{Q128R} mutants as shown by the localization of DSP. The disruption of cell-cell interface was shown to be correlated with a disorganization of the intermediate filament protein desmin that binds both NEBL and DSP. Interestingly overexpression of human NEBL^{WT} protein enhanced desmin organization.

Conclusion: We suggest that a primary mechanism of ICD disruption in NEBL^{Q128R} derived DCM originates from perturbed DSP dynamics through disruption of desmin filaments. In addition to a direct structural link, the desmosome protein dynamics might also be affected by other processes such as calcium signaling, which was found to be disturbed in the cardiomyocytes of NEBL transgenic mice. Further studies are underway to identify a possible role of this process and additional potential players involved.

28. Emilin Deficiency is Associated with TGF-beta Mediated Aortic Valve Fibrosis and Angiogenic Remodeling

Charu Munjal,¹ Amy M. Opoka,¹ Giorgio M. Bressan,² Robert B. Hinton¹

¹Division of Cardiology, Heart Institute, Cincinnati Children's Hospital Medical Center, Cincinnati, OH.

²Department of Histology, Microbiology and Biomedical Technologies, University of Padua, Padua, Italy

Introduction: Emilin-1 is an elastin-binding protein necessary for elastic fiber formation. Emilin-1 is expressed in the developing and mature heart valves. Aortic valve disease is characterized in part by elastic fiber fragmentation, fibrosis and angiogenic remodeling.

Hypothesis: Because Emilin-1 has been shown to regulate TGF-beta signaling in arterial tissue, we hypothesized that Emilin-1 deficient mice (*Emilin1*^{-/-}) would manifest viable valve fibrosis.

Methods: *Emilin1*^{-/-} mice were analyzed at neonatal, juvenile, adult and aged stages. ECM composition and organization were assessed using pentachrome and hart's stains. Immunohistochemistry was performed to assess TGF-beta signaling (p-Smad2/3), cell proliferation (p-Histone H3), activated interstitial cells (SM22), fibrosis (periostin, type I and III collagen) and angiogenic remodeling (CD31, LOX, VEGF, cleaved versican and chondromodulin).

Results: Histochemical analysis demonstrated normal *Emilin1*^{-/-} valve ECM organization and morphometrics at neonatal and juvenile stages. At the adult stage, elastic fiber fragmentation was observed, and the hinge dimension was increased. Interestingly, aged *Emilin1*^{-/-} mice demonstrated a dramatic increase in cell density, valve thickness, and annulus area with increased collagens (type I and III collagen). There was no calcification. TGF-beta signaling and cell proliferation were significantly increased at the neonatal stage, persisting through senescence. Activated interstitial cells demonstrated a SM22 positive myofibroblast phenotype beginning in the annulus (adult stage) and increasing dramatically throughout the valve (aged stage). CD31 positive interstitial neovessels were present in the aged mice only, and LOX expression was increased, consistent with angiogenic remodeling.

Conclusion: These findings identify the *Emilin1*^{-/-} mouse as a model of latent valve malformation, and implicate TGF-beta dysregulation resulting in maladaptive fibrosis and angiogenic remodeling. Elucidation of underlying TGF-beta mechanisms will facilitate identification of new therapeutic targets such as pharmacologic angiogenesis inhibitors in the treatment of AVD.

29. Increased Frequency of Dietitian Visits Improves BMI Outcomes in Obese Children Participating in a Comprehensive Pediatric Weight Management Program

Margaret S Neidhard, Robert M Siegel, MD, Shelley Kirk, PhD, RD, LD

Center for Better Health and Nutrition, The Heart Institute, Cincinnati Children's Hospital Medical Center, OH

Background: Pediatric obesity is a major health issue with 12% of children aged 2 to 19 years having a body mass index (BMI) greater than the 95th percentile for their age. Studies in children under 13 years show that while low calorie diets can lead to modest improvement in BMI short-term, long-term adherence is more difficult. In October 2009, our hospital-based pediatric weight management center instituted a protocol of introducing our "Healthy Eating Plan" (HEP) at each initial medical evaluation. HEP is a dietary intervention emphasizing intake of foods with a low glycemic index ($GI \leq 50$) and those that are "heart-healthy" (limits foods high in saturated fats), organized with a traffic-light approach. Previously, families were required to have a dietitian visit and a uniform approach of portion controlled staged approach was used. In this study we compare the outcomes of patients seen in our center before (Pre-HEP) and after implementation of the protocol to introduce HEP at all initial medical evaluations

Methods: The charts of all new children seen at the Center for Better Health and Nutrition from 10/1/2009 to 3/1/2010 were reviewed. Data were collected for age, sex, race, height, weight, BMI, and number of dietitian visits. 51 of these patients were introduced to HEP, seen by an RD at least once, and returned for a follow-up medical visit. These 51 children were matched by initial age and BMI with patients seen in our center initially between 1/1/2006 and 5/1/2009. None of these 51 matched patients had been introduced to HEP at the initial medical visit. These patients' charts were reviewed for the same data as the first group.

Results: Two of 51 HEP patients were outliers with respect to time between initial medical visit and reassessment. These patients were removed from the data set. Their matches were also removed for the paired *t* test analysis. Both the Pre-HEP and HEP groups had a similar decrease in BMI, -0.77 ± 0.9 versus -0.60 ± 0.2 units (N.S.). A multiple linear regression model shows that more RD visits are associated with a greater improvement in BMI, independent of Pre-HEP vs. HEP grouping. ($p=0.04$). A similar number of patients in the Pre-HEP and HEP group, 29 versus 37, had a reduction in BMI.

30. Early Angiogenic Remodeling due to Elastic Fiber Dysregulation in Aortic Valve Disease

Amy Opoka,¹ Amy L. Juraszek,⁴ Hanna Osinska,¹ Pirooz Egtesady,² Zsolt Urban,⁵ Robert P. Mecham,⁶ Kevin E. Bove,³ Robert B. Hinton¹

¹Divisions of Cardiology, ²Cardiothoracic Surgery, and ³Pathology, Cincinnati Children's Hospital Medical Center. ⁴Division of Cardiology, University of Texas Southwestern. ⁵Department of Genetics, University of Pittsburgh. ⁶Department of Cell Biology, Washington University

Angiogenic remodeling (AR) has been identified in late stage aortic valve disease (AVD), characterized as a secondary finding due to atherosclerosis. Elastic fibers are a primary component of the aortic valve, and elastic fiber fragmentation is a hallmark of AVD. Elastic fiber fragments stimulate AR. Genetic syndromes due to elastic fiber dysregulation, including Williams (WS) and Marfan (MFS) syndrome, are associated with valve disease in ~30% of cases. The role of AR and elastic fiber dysregulation in early AVD is unknown. We hypothesized that AR would be an early finding, preceding atherosclerosis, due to elastic fiber fragmentation. Specimens from four age-matched pediatric groups were studied: AVD, control, WS and MFS (n=6 per group). To assess early vs. late findings, valve tissue from adult calcific AVD was also studied. Regional anatomy was assessed using histo- and immunohistochemistry and electron microscopy. AR (VEGF-A, CD-34 and chondromodulin), elastic fiber fragmentation (elastin, fibrillin-1, emilin-1, fibulin-4 and -5), and atherosclerosis (CD-68, LRP-5) were assessed. AR was present in pediatric AVD and increased in adult AVD. Neovessel formation was present with increased VEGF and decreased chondromodulin expression in CD-68 negative pediatric AVD and CD-68 positive adult AVD. Elastin and fibrillin expression was decreased and dispersed, and elastin fragments were associated with neovessels. Emilin and fibulin-4 expression was markedly increased and fragments of both were associated with neovessels. Interestingly, AR was present in WS but not MFS aortic valves. Distinct matrix differences were observed between groups and regions. These findings identify AR as an early mechanism in AVD independent of atherosclerosis, and establish the role of elastic fiber dysregulation in AVD pathogenesis. Elucidation of the underlying mechanisms may inform the development of new therapeutics.

31. Genetic variants in SCN5A promoter predict phenotype severity in patients with heterozygous loss of function mutations

Ji Kwon Park, MD, PhD,¹ Lisa J. Martin, PhD,² D. Woodrow Benson, MD, PhD³

Department of Obstetrics and Gynecology, and Institute of Health Sciences, School of Medicine, Gyeongsang Nation University, Jinju, Republic of Korea¹; Division of Cardiology, Cardiovascular Genetics^{1,3} and Division of Human Genetics,² Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA.

Background Heterozygous mutations in SCN5A gene have been associated with heterogeneous arrhythmia phenotypes; further among mutation carriers, clinical severity of arrhythmia phenotypes may range from asymptomatic ECG changes (mild) to life-threatening arrhythmias (severe) among family members carrying the same mutation. While a consensus exists on the indication for device implantation in mutation carriers with symptomatic arrhythmias, risk-stratification schemes for asymptomatic mutation carriers remain uncertain. We used a family based approach to determine the role of SCN5A promoter variants in predicting phenotype severity in kindreds identified with a loss-of-function SCN5A mutation.

Methods and Results We identified SCN5A mutations in 6 families selected because of varied arrhythmia phenotypes considered mild or severe among members of the same family. During systematic survey of 2.8kb promoter region of SCN5A in a large kindred (22 mutation carriers), two SNPs in complete linkage disequilibrium (c.-194-1854 C>T, rs41310749; c.-194-865 T>C, rs41310239) were identified. The promoter variants were significantly associated with disease severity (mild vs. severe phenotype) ($p=0.0004$), as all three patients with severe phenotype carried the two-SNP variant on both mutant and wild-type alleles. We tested this relationship in five other families with SCN5A mutations and found one additional family where co-inheritance of the promoter variants increased the risk of a severe phenotype.

Conclusion These family-based genetic findings suggests that the presence of specific promoter variants on both wild-type and mutant SCN5A alleles increase the risk of a severe phenotype in heterozygous carriers of SCN5A loss-of-function mutations.

32. Genetic Counseling Services in a Fetal Heart Program

Ashley Parrott¹, Stephanie Ware¹, Erin Miller¹, James Cnota¹, Amy Garrison¹, Erik Michelfelder¹

¹The Heart Institute, Cincinnati Children's Hospital Medical Center

As the most common birth defect, congenital heart disease (CHD) impacts the pregnancies of many expectant parents. The American Heart Association published a review endorsed by the American Academy of Pediatrics on the genetic basis of CHD in 2007 that highlights the historic underestimation of the genetic contribution to CHD and the rapid emergence of the field of cardiovascular (CV) genetics. Beginning in July 2010 at Cincinnati Children's Hospital Medical Center (CCHMC), collaboration between the Fetal Heart Program (FHP) and CV genetics was established. This formal relationship involves integration of CV genetic counseling services for patients referred to the FHP for fetal echocardiography. In a five month time frame between December 7, 2010 and May 6, 2011, forty-six families seen in the FHP received CV genetic counseling services. Over 90% (42 of 46) of these patients had not previously been offered genetic counseling services for an indication of CHD. Indication for evaluation included family history of CHD (46%), pregnancies with known or suspected CHD (54%), teratogenic exposure (6.5%), or some combination of these indications. Known family history of cardiac lesions as well as fetal diagnoses ranged from isolated septal defects to severe outflow tract anomalies. Each of these cardiac lesions carries independent genetic associations, recurrence risks, and recommended genetic tests. The need for cardiac lesion-specific risk assessment resulted in a CHD counseling tool created by a CV genetic counselor. This tool is a useful resource for genetic counselors and other healthcare providers to generate CHD related differential genetic diagnoses and recurrence risk estimates. This collaboration established a specialized service previously not available in the region. The CCHMC experience identifies a need for genetic counselors with specialization in CHD and highlights a potential area for growth of the genetic counseling field into specialized cardiac prenatal programs.

33. Preaxial polydactyly caused by *Gli3* haploinsufficiency is rescued by *Zic3* loss of function

Malgorzata E. Quinn, Allison Haaning, Stephanie M. Ware

Preaxial limb anomalies are an important class of birth defects that are incompletely understood genetically and mechanistically. *GLI3*, a mediator of hedgehog signaling, is one known genetic cause of limb malformations including pre- and postaxial polydactyly, Pallister-Hall syndrome and Greig cephalopolysyndactyly. A closely related Gli-superfamily member, *ZIC3*, causes left-right patterning defects including X-linked heterotaxy syndrome in humans, but has not been investigated in limb development. Here, we demonstrate via *Zic3* reporter gene transgenic mice and in situ hybridization analyses that *Zic3* and *Gli3* expression domains overlap in developing limb buds. *In vitro* experiments indicate that *Zic3* converts *Gli3* from repressor to activator. *In vivo*, we analyzed the effect of *Zic3* loss of function on phenotype, gene expression level and *Gli3* processing in *Gli3* mutant mice (*Gli3*^{Xt-J}). Ectopic *Shh* expression in anterior limb buds and *Shh* overexpression in the zone of polarizing activity of *Gli3* mutants is normalized by *Zic3* loss of function. Increased *Gli3* repressor/*Gli3* activator ratio observed in *Gli3*^{+/-} embryos is also rescued in *Zic3* null; *Gli3*^{+/-} embryos, whereas *Zic3* null mice show increased expression of *Gli3* in limb buds. Finally, *Zic3* null; *Gli3*^{+/-} neonates show rescue of the polydactylous phenotype seen in *Gli3*^{+/-} animals. We document a previously unrecognized role for *Zic3* in regulating limb digit number via its modifying effect on *Gli3* and *Shh* expression levels. Together these results indicate that two Gli superfamily members that cause disparate human congenital malformation syndromes interact genetically and demonstrate the importance of *Zic3* in regulating *Shh* pathway in developing limbs.

34. Mutations in the I-band region of nebulin augment cardiomyocyte calcium signaling through modification of the thin filament in preclinical inherited dilated Cardiomyopathy

¹Kathryn M Rafferty, ¹Jeanne F James, ²Evangelia Kranias, ¹Jeffrey A Towbin, ¹Enkhsaikhan Purevjav

¹The Heart Institute, Cincinnati Children's Hospital Medical Center, Cincinnati, OH. ²Department of Pharmacology & Cell Biophysics, University of Cincinnati, Cincinnati, OH

Background: Approximately 30% of patients with dilated cardiomyopathy (DCM) exhibit inherited mutations in sarcomeric and cytoskeletal genes. Nebulin (NEBL) is a sarcomeric Z-disk protein that is involved in muscle force generation via the cardiac tropomyosin-troponin (cTpm-cTn) association as well as in force transmission via the Z-disk interactions. Transgenic mice with cardiac-restricted overexpression of human mutant G202R or A592E NEBL exhibit DCM at 6 months. In the NEBL^{G202R} mutant, the DCM phenotype was associated with disruption of cTpm-cTn, while the NEBL^{A592E} mutant exhibited Z-disk abnormalities.

Objectives: Our objective was to determine whether the NEBL mutations promote DCM-linked mechanical dysfunction by destabilizing the calcium (Ca)-cTn interaction in preclinical stage.

Methods: Cardiac function was evaluated by echocardiography in 3-month old WT or mutant NEBL and non-transgenic (NTg) mice. Contractile mechanics and calcium transients were measured in isolated cardiomyocytes (CM). Protein analysis was performed in total heart lysates.

Results: *In vivo* echocardiography revealed decreased heart function in NEBL^{A592E} compared to NEBL^{WT}. Baseline contractile mechanics measured in isolated cardiomyocytes (CMs) were the same in all groups and the rate of Ca flux into the sarcoplasmic reticulum was faster in NEBL^{G202R} CMs compared to NEBL^{WT}. Treatment of isolated CMs with isoproterenol (Iso, 50nM) increased fractional shortening in the NTg group and rates of contraction and relaxation in both NTg and NEBL^{G202R} groups. Iso treatment increased Ca flux in all groups, but to a lesser extent in the NEBL^{A592E} group. Protein analysis in heart lysates showed an increased ratio of phosphorylated cTnI to total cTnI in NEBL^{G202R} mutants compared to NEBL^{WT}.

Conclusion: These data suggest that in the preclinical phase, NEBL^{G202R} mutants preserve mechanical function via modified Ca-cycling through altered cTnI phosphorylation. In addition, as NEBL^{A592E} mutants lack stress-mediated mechanical and Ca-cycling augmentation, the data suggests that mutations in the Z-disc region of NEBL blunt mechano-sensing and force transmission within the CM. Thus our data

show that mutations in NEBL may alter its function in a domain-specific manner, affecting differential protein-protein binding and downstream signaling.

35. Pathogenic role of truncated Myosin binding protein C

Md. Abdur Razzaque, Manish Gupta, James Gulick and Jeffrey Robbins

Division of Molecular Cardiovascular Biology, Cincinnati, OH

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 that a stable 29kD fragment is produced from cMyBP-C when the heart is stressed, using stimuli 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. To understand its potential pathogenicity, we generated cardiac myocyte-specific transgenic mice (TG) using a Tet-Off inducible system to permit controlled expression of the 29kD fragment in cardiomyocytes. When 29kD 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 4 to 17 weeks of age. During the period of 29kD expression, the mice developed significant cardiac hypertrophy with myofibrillar disarray and fibrosis. We then examined whether hypertrophic signaling pathways are activated or not and found that the MEK-ERK pathways are highly activated in the double TG hearts, when compared with the NTG and noninduced TG mice. These results suggest that 29kD fragment of cMyBP-C is a pathogenic fragment and can be a causative agent of hypertrophic cardiomyopathy and heart failure.

36. Abnormal circumferential strain is present in young Duchenne muscular dystrophy patients

Thomas D Ryan, Michelle A Grenier, Michael D Taylor, Linda H Cripe, Jesse Pratt, Eileen C King, Wojciech Mazur, John L Jefferies, D Woodrow Benson, Kan N Hor

Background Advances in management of Duchenne muscular dystrophy (DMD) have improved such that DMD-associated cardiac disease has become the leading cause of death. Cardiac function as measured by standard transthoracic echocardiogram (TTE) methods, *e.g.* fractional shortening (FS), rarely detects global dysfunction during the first decade. In the current study we used TTE to assess strain (ϵ), both segmentally and globally, in young DMD patients.

Methods TTE from DMD patients ≤ 7 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.

Results While there were no differences in FS between groups, the global ϵ_{cc} was lower in DMD patients (-21.7 ± 3.8 vs. -19.8 ± 4.2 , $p < 0.0001$; normal vs. DMD). Further, segmental ϵ_{cc} was lower in the anterior segment ($-23.0 \pm 6.1\%$ vs. $-18.9 \pm 7.0\%$, $p = 0.001$; normal vs. DMD), inferior segment ($-20.7 \pm 5.16\%$ vs. $-17.7 \pm 6.1\%$, $p = 0.003$; normal vs. DMD), and inferolateral segment ($-18.3 \pm 6.2\%$ vs. $-15.9 \pm 6.7\%$, $p = 0.04$; normal vs. DMD).

Conclusion In the present study we demonstrate that TTE detects both global and segmental ϵ_{cc} abnormalities (anterior, inferior, and inferolateral segments) in young DMD patients when FS is normal. This is consistent with previous pathological studies in which fibrosis was seen in similar segments in DMD-associated cardiac disease. These novel findings substantiate that the disease process is present in the myocardium long before standard measures detect global dysfunction. Further investigation is needed to determine whether segmental strain can be used to guide early treatment.

37. Requirements for Cyp26 enzymes in heart development

Ariel B. Rydeen, and Joshua S. Waxman

Congenital heart defects occur in 1 out of every 150 live births making it the leading birth defect in the US. For the heart to develop normally a balance of retinoic acid (RA) signaling is needed and teratogenic effects can be seen when RA is in excess. One balancing factor is the Cyp26 class of p450 enzymes, which metabolize RA into easily degraded derivatives. However, the role Cyp26 enzymes play in patterning the heart during development is not yet understood. To determine the function of Cyp26 enzymes in heart development, we are using loss of function studies in zebrafish. Two Cyp26 enzymes, Cyp26a1 and Cyp26c1, are found in the anterior lateral plate mesoderm, which suggests they may be involved in balancing the RA levels to allow for proper patterning of the developing heart. Interestingly, Cyp26a1 deficient embryos have thin linearized hearts, yet the earliest cardiac differentiation markers were unaffected. Similarly, embryos that are Cyp26c1 deficient also have aberrant heart morphologies but no indication that Cyp26c1 is required for early patterning of the heart field. Together, these results are surprising in that neither of the Cyp26a1 or Cyp26c1 deficient embryos alone exhibited any overt heart defects that are indicative of early embryonic excess RA signaling. Therefore, our data leads us to hypothesize that Cyp26a1 and Cyp26c1 may be functioning redundantly to limit RA signaling in the early embryo. Alternatively, maternal contribution of these Cyp26 enzymes could be responsible for limiting RA signaling in the early heart field. We are testing these hypotheses through a combination of molecular, genetic and pharmacological methods. Ultimately, these studies will help us to better understand the roles of RA in patterning the vertebrate heart and could lead to novel therapeutics aimed at alleviating the teratogenic effects of excess RA.

38. FOXO transcription factors are critical regulators of neonatal cardiomyocyte proliferation

Arunima Sengupta and Katherine E Yutzey

The Heart Institute, Cincinnati Children's Hospital, Cincinnati, OH 45229, USA

Fetal cardiomyocytes (CM) are highly proliferative, whereas neonatal CM withdraw from the cell cycle and growth occurs primarily by hypertrophy. The molecular mechanisms that control cell cycle withdrawal in neonatal CM are not well known. Our previous data shows that FOXO transcription factors act as negative regulators of embryonic CM proliferation by directly promoting the expression of p21^{cip} and p27^{kip}, cyclin-dependant kinase inhibitors (CKIs). Here, we investigate the role of FOXO transcription factors in regulating CM proliferation in neonatal cardiomyocytes. CM-specific combined deficiency of FOXO1 and FOXO3 results in increased CM proliferation in hearts of mice at 1 and 3 days after birth. However, by postnatal day 7 no significant differences in proliferation were observed with CM-specific FOXO-deficiency as compared to control hearts. 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. Activity of AMPK is also induced during the neonatal period relative to the embryonic hearts. This suggests that AMPK-mediated activation of FOXO is required for promotion of 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, IGF1 and NFATc3, all of which are associated with increased CM proliferation. CM-specific loss of both FOXO1 and FOXO3 results in increased expression of FOXM1, IGF1 and NFATc3, in addition to decreased p27^{kip} gene expression. Furthermore, FOXO1 and FOXO3 directly bind to regulatory regions of FOXM1, and FOXO1 inhibits FOXM1 reporter gene expression in cotransfection assays. Interestingly, we also identified IGF1 as a direct transcriptional target of FOXM1. Our results suggest that FOXO activity inhibits FOXM1 and this FOXO/FOXM1 controls IGF1 levels to promote cell cycle withdrawal in neonatal CM. Altogether, our results provide evidence for the importance of an AMPK/FOXO/FOXM1/IGF1 signaling pathway in the promotion of cell cycle withdrawal in neonatal CM. Therefore, understanding the molecular mechanisms by which FOXO regulates CM proliferation could be harnessed in adults for the treatment of cardiac injury.

39. Comparison of noninvasive measurement of cardiac output, Electrical Velocimetry, with Thermodilution measurement of cardiac output in children

David S. Spar MD, Julie A. Vincent MD, Alejandro Torres MD, William E. Hellenbrand MD, Marc Richmond MD, Ganga Krishnamurthy MD

Department of Pediatrics, College of Physicians and Surgeons, Columbia University

Background: The primary function of the cardiovascular system is to meet the metabolic demands of the body and is largely dependent upon cardiac output. Thermodilution (TD), a traditional measure of cardiac output, is an invasive technique requiring catheterization of the pulmonary artery. The use of TD in children is not uniformly favored as it offers uncertain risk-benefit ratio. Electrical Velocimetry, a novel method of noninvasive cardiac output assessment, measures the maximum change in thoracic electrical bioimpedance as the ohmic equivalent of the mean aortic blood flow acceleration and further transforms it into an equivalent of mean aortic blood flow velocity. Stroke volume and cardiac output are then calculated. This novel method has been minimally studied in children. The objective of the study was to compare cardiac output measurements by the noninvasively measured Electrical Velocimetry (EV-CO) with pulmonary artery thermodilution (TD-CO) in children with normal cardiac anatomy and function.

Methods: Forty-four children with a median age of 10 years (Range 0.8 – 17) who previously underwent cardiac transplantation and were undergoing routine cardiac catheterization were prospectively enrolled in the study. Subjects with intra- or extracardiac shunts, moderate or severe tricuspid regurgitation, or hematocrit less than 30% were excluded. Paired measurements of TD-CO by pulmonary artery catheter thermodilution and EV-CO by Electrical Velocimetry were recorded. TD-CO and EV-CO measurements were analyzed for correlation, as well as bias and precision by the Bland-Altman plot. Bias represents the mean difference between the actual TD-CO measured by pulmonary artery TD and the EV-CO measured by Electrical Velocimetry. Precision was represented by two standard deviations of the bias. An *a priori* definition of “acceptable” limits of agreement was set at $\pm 30\%$ given the inherent errors in measurement of the “gold standard” technique TD. The percentage error between the two measurements was calculated as twice the standard deviation of the bias divided by the mean CO.

Results: 26 males and 18 females were enrolled. The mean values of TD-CO (3.66 ± 1.71 liter min^{-1}) and EV-CO (3.44 ± 1.71 liter min^{-1}) did not differ significantly ($p=\text{NS}$). There was a statistically significant correlation between TD-CO and EV-CO ($r=0.89$, $p<0.001$). Bland-Altman analysis of TD-CO and EV-CO demonstrated a bias of 0.22

liter min⁻¹ with a precision of ± 1.55 liter min⁻¹. The mean percentage error was $7.7 \pm 18.7\%$ (± 1 SD).

Conclusions: Noninvasive EV-CO differs from TD-CO measurements by an average of $7.7 \pm 18.7\%$ and exceeds the *a priori* set acceptable limits of agreement in children with normal intracardiac anatomy and function. There was a significant correlation between TD-CO and EV-CO ($r=0.89$). The application of EV-CO monitoring as a trend monitor in outpatient or critical care settings requires further investigation.

40. The Relationship between Cardiovascular Profile Score and Cardiac Output in the Fetus with High Cardiac Output Lesions

Statile C, Cnota J, Gomien S, Divanovic A, Crombleholme T, Michelfelder E

Background: High cardiac output lesions are associated with an increased risk of fetal death due to cardiac failure and hydrops fetalis. Appropriate prenatal management and fetal intervention have been shown to improve survival. The cardiovascular profile score (CVPS) has been related to fetal outcome in intrauterine growth restriction, twin-twin transfusion syndrome, and fetal congenital heart disease, but has not been used to characterize and risk stratify fetuses with high cardiac output lesions. We hypothesized that elevated fetal combined cardiac output (CCO) will be associated with lower CVPS.

Methods: We performed a retrospective case series of fetuses undergoing echocardiography with high cardiac output lesions, including sacrococcygeal teratoma, cervical teratoma, placental chorioangioma, and vein of Galen aneurysm between 7/06 and 11/10. The most recent echo prior to delivery, demise, or intervention was used for analysis. Fetal echocardiographic evaluation included assessment of CVPS, as well as Doppler/2D estimation of CCO, indexed to estimated fetal weight. CCO was log transformed to normalize distribution. CVPS levels were compared by ANOVA. The relationship between logCCO and CVPS was assessed by linear regression. Statistical significance was assigned to $p < 0.05$.

Results: A total of 46 fetuses were studied: 27 sacrococcygeal teratomas, 11 cervical teratomas, 7 chorioangiomas, and 1 vein of Galen aneurysm. The mean gestational age at the time of study was 27.0 ± 5.3 weeks. LogCCO differed across CVPS values ($p < 0.001$) while demonstrating a significant inverse relationship ($r^2 = 0.20$, $p < .002$) (Figure). Ten of 40 subjects with outcome data experienced either *in utero* demise or intervention; the proportion of these fetuses with CVPS < 8 was significantly higher than in those who survived to birth without intervention (80% vs. 16%, $\chi^2 = 13.7$, $p < 0.001$). All fetuses with outcome data available who had a CCO > 625 ml/min/kg underwent fetal intervention or died in utero.

Conclusions: These data demonstrate an inverse relationship between CCO and CVPS in the fetus with high cardiac output lesions. As a measure of fetal cardiovascular well being, the CVPS may be useful as an indication for intervention in this population.

41. Left Ventricular Non-Compaction in Duchenne Muscular Dystrophy

Christopher J. Statile MD, Michael D. Taylor MD, Linda H. Cripe MD, Wojciech Mazur MD, Eileen King PhD, Jesse Pratt, D. Woodrow Benson MD, PhD, Kan N. Hor MD

Background: Left ventricular non-compaction (LVNC) is characterized by deep trabeculations in the left ventricular (LV) endocardium and a thinned epicardial layer. LVNC is often associated with other manifestations of cardiac disease, e.g. LV hypertrophy, LV dilation or a combination of hypertrophy and dilation. Although generally associated with systolic dysfunction, LVNC has also been described in patients with normal global ventricular systolic function. Recent anecdotal data from case reports and clinical MR images suggest an increased prevalence of LVNC in neuromuscular diseases including Duchenne Muscular Dystrophy (DMD). This study aims to define the prevalence of LVNC in the DMD population and characterize its relationship to global LV function as measured by ejection fraction (EF).

Methods: Cardiac MRI (CMR) was used to analyze global LV function and the presence of LVNC in 4 patient groups: DMD boys with EF > 55%, DMD boys with EF < 55%, normal subjects and patients with a primary diagnosis of LVNC. Standard imaging data included steady-state free precession (SSFP) short-axis cine stack images sequences. Global LV function was characterized by EF, stroke volume, and myocardial mass as measured by standard planimetry. To evaluate for the presence of LV non-compaction, the non-compacted to compacted (NC/C) ratio was measured in each of 16 standard myocardial segments in short axis. LVNC was defined as a diastolic NC/C ratio > 2.3 for any segment. Results were described using measures of central tendency, including median and interquartile range (IQR).

Results: 151 CMR were analyzed: 66 DMD boys with EF > 55%, 30 DMD boys with EF < 55%, 40 normal subjects and 15 patients with primary LVNC. The median ages of the groups were 13.7, 15.1, 16.0, and 17.6 years, respectively ($p = 0.02$). Among the 96 DMD boys, there were 27 cases meeting LVNC criteria, with an overall prevalence of 28% (95% CI = 19.1% - 37.1%). Of the 27 LVNC cases in DMD boys, 11 had an EF > 55%, and 16 with an EF < 55%. The prevalence of LVNC in DMD boys with EF > 55% was 16.7 % (95% = 6.8% - 26.6%) compared to 53.3 % (95% = 35.4% - 71.2%) for DMD boys with EF < 55%. The median highest NC/C ratio was 1.8 (IQR = 1.57, 2.02) for DMD boys with EF > 55% compared to 1.54 (IQR = 1.29, 1.78) for the normal group. For DMD boys with EF < 55% median highest NC/C ratio was 2.46 (IQR = 1.84, 3.04) compared to 3.69 (IQR = 2.81, 4.23) for the LVNC group (figure 1). Although the presence of LV trabeculations are common, segment 16 (apical lateral) in both DMD and LVNC most commonly fulfilled criteria for LVNC as described above. No controls subjects were noted to have LVNC.

Conclusion: The data from this DMD population show a high prevalence of LVNC that is dependent on LV systolic function. DMD boys with normal global function have a much lower prevalence of LVNC than those with decreased systolic function in whom over half are positive for LVNC. While, the etiology of this finding is unknown, we speculate that in the dysfunctional DMD LV, LVNC is a compensatory mechanism rather than an embryologic defect. In this case, the presence of LVNC may be part of the DMD progression with fibrofatty replacement of myocardium causing thinning of the compacted myocardium and contributing to the appearance of increased trabeculations which reach detection threshold by CMR, resulting in an increase in NC/C ratio.

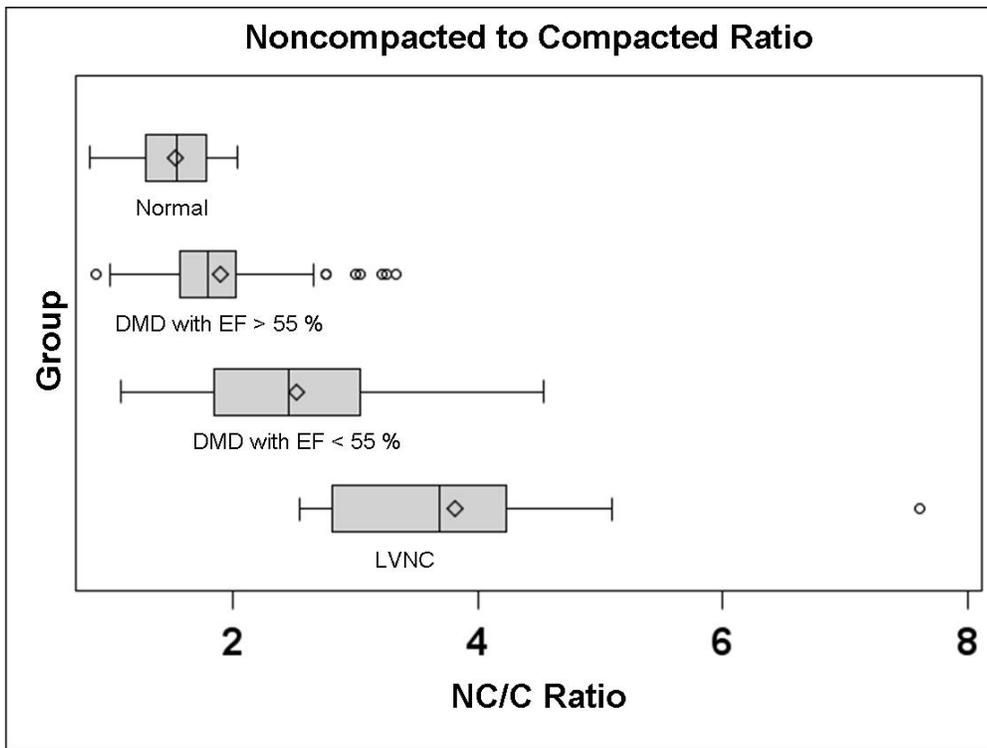


Figure 1

42. Defining the temporal and spatial role of Zic3 in the establishment of the left-right axis

Mardi J. Sutherland, BS, Shuyun Wang, PhD, Malgorzata E. Quinn, PhD and Stephanie M. Ware, MD, PhD

Division of Molecular Cardiovascular Biology, Department of Pediatrics, Cincinnati Children's Hospital Medical Center

Mutations in ZIC3 cause X-linked heterotaxy, a disorder characterized by abnormal left-right asymmetry of organs and frequent cardiovascular malformations thought to result from abnormal heart looping. The molecular mechanism(s) by which ZIC3 mutations cause heterotaxy are unknown. Zic3 is expressed ubiquitously during critical stages for left-right patterning; later expression in the developing heart is controversial. 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. Central node cells, containing motile cilia that generate leftward flow of extracellular fluid, and peripheral crown cells, required to properly initiate asymmetric gene expression, are both critical in the establishment of the left-right axis. We hypothesized that Zic3 expression in node cells is required for proper left-right asymmetry. To address this question, Zic3 was deleted in both cell types of the node using a conditional loss of function approach. Surprisingly, Zic3 deletion in the node results in viable, phenotypically normal mice. However, analysis of node morphology in Zic3 null mice reveals node dysplasia characterized by abnormal clusters of node cells intermingled with enlarged crown cells. In contrast to the accepted paradigm that left-right asymmetry is initiated at the node in mice, these results implicate Zic3 in earlier signaling events affecting node formation and subsequent function.

43. Genetic screening revealed digenic heterozygote variants in junctophilin and troponin T in patient with restrictive cardiomyopathy

Ken Takagi MD, Jeffrey A. Towbin MD, Enkhsaikhan Purevjav MD, PhD

Department of Cardiology, Heart Institute, Cincinnati Children's Hospital

Background: Restrictive Cardiomyopathy (RCM) is the least common of the cardiomyopathies and is characterized by impaired ventricular filling during diastole with preserved systolic function. RCM is characterized by stiffness of the heart muscle, usually due to accumulation of amyloid and scar tissue. RCM in children is a rapidly progressive disorder causing severe heart failure with a high risk of sudden cardiac death; therefore, heart transplantation is the only treatment available to preserve the long term survival. Familial inheritance has been reported, however, identification of gene abnormalities in RCM cases compared to other cardiomyopathies has been difficult due to low prevalence. Less than 20 mutations in the genes encoding sarcomeric and/or structural proteins such as desmin and cardiac troponins have been reported to date.

Rationale: To elucidate genetic basis and discover new candidate genes for inherited RCM in order to invent the mechanisms-oriented clinical management.

Methods: Direct sequencing of exons 11 targeted genes was performed in 67 patients who were diagnosed with RCM. Polymorphisms were excluded using dSNP database, predictions studies were obtained using Polphen-2, SIFT, Panther, SNPs3D and P Mut software.

Results: We identified digenic heterozygote mutations in one patient with RCM who demonstrated ventricular fibrillation: a heterozygous missense mutation p.G505S (c.1513G>A) in junctophilin and a heterozygous missense mutation p.A94C (c.310C>T) in troponin-T. Both mutations have been reported as a single missense mutation in patients with hypertrophic cardiomyopathy (HCM).

Conclusion: Most of mutations in sarcomeric genes identified in RCM patients are associated with HCM. We identified digenic inheritance in RCM patient and propose further studies whether digenic inheritance may cause the RCM phenotype in humans.

44. ***SHROOM3*** is a novel candidate for heterotaxy identified by whole exome sequencing

Muhammad Tariq¹, Stephanie M. Ware^{1,2}

Divisions of ¹Molecular Cardiovascular Biology, ²Human Genetics, Cincinnati Children's Hospital Medical Center, Cincinnati, OH

Background: Heterotaxy-spectrum cardiovascular disorders are challenging for traditional genetic analyses because of clinical and genetic heterogeneity, variable expressivity, and non-penetrance. In this study high-resolution SNP genotyping and exon-targeted array comparative genomic hybridization platforms were coupled to whole-exome sequencing to identify a novel disease candidate gene.

Results: SNP genotyping identified absence-of-heterozygosity regions on chromosomes 1, 4, 7, 13, 15, 18, consistent with parental consanguinity. Subsequently, whole-exome sequencing of the proband identified 26065 coding variants, including 18 non-synonymous homozygous changes not present in dbSNP132 or 1000 genomes project. Of these 18, only 4 -one each in *CXCL2*, *SHROOM3*, *CTSO*, *RXFP1*- were mapped to the absence-of-heterozygosity regions, each of which was flanked by more than 50 homozygous SNPs confirming recessive segregation of mutant alleles. Sanger sequencing confirmed the *SHROOM3* homozygous missense mutation (p.G60V) and it was predicted as pathogenic by four bioinformatic tools. *SHROOM3* has been identified as a central regulator of morphogenetic cell shape changes necessary for organogenesis and can physically bind ROCK2, a rho kinase protein required for left-right (LR) patterning. Screening 96 sporadic heterotaxy patients identified 4 additional patients with rare variants in *SHROOM3*.

Conclusions: Using whole exome sequencing, we identify a recessive missense mutation in *SHROOM3* associated with heterotaxy syndrome and identify rare variants in subsequent screening of a heterotaxy cohort, suggesting *SHROOM3* as a novel target for the control of LR patterning. This study reveals the value of SNP genotyping coupled with high-throughput sequencing for identification of high yield candidates for rare disorders with genetic and phenotypic heterogeneity.

45. Loss of Heart Rate Variability in DMD represents Decreased Autonomic Function which Correlates with Decrease in Myocardial Strain Magnitude.

Tamara Thomas, MD, Elaine Urbina, MS, MD, Lynn Jefferies, MS, MD, D. Woodrow Benson, MD, PhD, Kan Hor, MD, Jeff Anderson, MD, Linda Cripe, MD

Duchenne Muscular Dystrophy (DMD) is an X-linked recessive disorder affecting approximately 1 in 3500 males. DMD results from a mutation in dystrophin, a sarcolemmal protein abundant in skeletal and cardiac muscle; the principal clinical features include skeletal myopathy and cardiac dysfunction usually resulting in death by the third decade of life. In addition to the cardiac dysfunction, an elevated resting heart rate is frequently observed during the natural history of DMD associated cardiac disease. The purpose of this investigation is to study the elevated heart rate, which we believe to be a form of abnormal heart rate variability (HRV) implicating autonomic dysfunction. We plan to enroll 30 control patients and 75 DMD patients with heart rate and HRV assessment via autoregressive algorithms from ambulatory electrocardiographic (EKG) recordings. Cardiac manifestations are characterized by an insidious decline in ventricular function that contributes to premature death. Recent studies from our center and others have shown that indices of global left ventricular (LV) function, e.g. mass, volume and ejection fraction (EF) are not adequate to detect early cardiac dysfunction in DMD patients. However, myocardial strain (ϵ), an indicator of regional LV function, i.e. myocardial deformation normalized to its original dimension, can detect occult cardiac disease early in the course of DMD despite normal global LV function. Therefore, in this study, we hypothesize that DMD boys with elevated resting heart rates will have both decreased HRV and a greater loss of regional LV function, i.e. reduction in strain magnitude, than DMD boys with normal HRV and both groups will be decreased when compared to age-matched controls. Cardiac MRI data from routine clinical visits within 6 months of enrollment will be used. Characteristics of the study subjects will be summarized as frequencies for categorical variables and as mean, standard deviation, median, and inter-quartile range for continuous variables by groups using Statistical Analysis Software. HRV analysis using standardized temporal and spectral power will be assessed from Holter monitor data by MARS V7 program prior to statistical analysis. We will also examine whether there is an association between specific dystrophin mutations and either decreased HRV or decreased strain magnitude.

46. GATA4 and GATA6 display divergent functions after pressure overload

Jop H. van Berlo and Jeffery D. Molkentin

INTRODUCTION The adult heart expresses both GATA4 and GATA6, zinc finger motif containing transcription factors. Our previous work has shown that GATA4 as well as GATA6 are both sufficient and necessary for the development of cardiac hypertrophy. We determined whether GATA4 and GATA6 act redundantly in the pressure overloaded heart.

METHODS We used loxP targeted GATA4 and GATA6 mice crossed with β -MHC promoter driven Cre mice to specifically delete GATA4 and/or GATA6 from the heart. In conjunction, we used cardiomyocyte specific inducible overexpression of GATA4 and/or GATA6.

RESULTS We initially hypothesized that GATA4 and GATA6 would function redundantly. We first assessed a gene dosage model of interaction between GATA4 and 6. We deleted GATA4 and GATA6 alleles in a dosage-dependent manner. Stepwise deletion of GATA4 and GATA6 alleles resulted in a stepwise-blunted hypertrophy response to pressure overload, suggesting that indeed gene dosage is important for cardiac hypertrophy. Since it could be that it was not merely gene dosage, but also synergy between GATA4 and GATA6 that determined the hypertrophic response, we next tested a synergy model of interaction. We overexpressed low levels of GATA4 and GATA6 alone or together. If synergy between GATA4 and 6 were important, we expected to find raging hypertrophy when both are overexpressed. However, we only found slight hypertrophy, which again was more likely due to gene dosage than anything else.

To finally prove that gene dosage was the most important determinant for GATA function in the heart, we performed a rescue experiment. We deleted GATA6 specifically from the heart and overexpressed either GATA6 or GATA4. Surprisingly, overexpression of GATA4 in the absence of GATA6 was unable to rescue, while overexpression of GATA6 did rescue cardiac function in response to pressure overload. A potential mechanism for the divergence in function could be regulation of angiogenesis. We found a decreased angiogenic response to pressure overload in absence of GATA4, but conversely an upregulated response in absence of GATA6.

CONCLUSION Overexpression of GATA4 is insufficient to rescue deletion of GATA6 in the pressure-overloaded heart, suggesting that GATA4 and GATA6 are not functionally redundant.

47. MAGNETIC RESONANCE IMAGING (MRI) ASSESSMENT OF CARDIAC TRANSPLANT REJECTION AND ALLOGRAFT VASCULOPATHY

Chet R. Villa MD, Kan Hor MD, John Lynn Jefferies MD and Michael Taylor MD

Background: Pediatric heart transplantation is a life-saving option for children with congenital heart disease and cardiomyopathy not amenable to traditional surgical or medical therapies. Despite improvements in the field, rejection, both cellular and humoral, is an important cause of acute and sub-acute morbidity and mortality. Meanwhile, the long term success of heart transplantation is limited by cardiac allograft vasculopathy (CAV), which is the leading cause of graft loss among pediatric patients > 1 year following transplant. Transplant rejection and CAV may present with limited clinical symptoms secondary to cardiac denervation, thus screening endomyocardial biopsy and angiography are employed to aid in diagnosis. Despite the fact that invasive, catheter based diagnostic testing is considered the “gold standard” in assessing both rejection and CAV, it has significant limitations. Cardiac magnetic resonance imaging (MRI) is able to provide a non-interventional myocardial tissue content and global function, which may provide ample information to assess rejection and CAV.

Hypothesis: We hypothesize that noninvasive, multiparametric cardiac MRI based myocardial characterization and stress imaging will provide a sensitive surrogate for transplant rejection and CAV.

Specific aim 1: We will assess the ability of cardiac MRI including myocardial strain, T2 quantification (myocardial edema) and gadolinium associated delayed enhancement in conjunction with biochemical markers of rejection to predict cellular and antibody mediated rejection (AMR), which define transplant rejection.

Specific aim 2: We will assess the ability of cardiac MRI derived regional wall motion abnormalities, strain and strain rate imaging and delayed enhancement to detect subclinical ischemic changes associated with angiographically diagnosed CAV.

Study design: The proposed study will be a prospective trial to assess the ability of non-invasive cardiac MRI imaging to diagnose acute rejection and CAV. To accomplish this goal a cardiac MRI will be performed in conjunction with traditional invasive imaging modalities when treating a patient for possible rejection/CAV. All patients presenting for scheduled endomyocardial biopsy and/or angiography within the Heart Institute, at Cincinnati Children’s Hospital Medical Center (CCHMC), will be recruited for this study. Patients will undergo a cardiac MRI within a day of previously scheduled cardiac catheterization. These patients will also undergo a yearly cardiac MRI coincident with further scheduled catheterization.

48. In vitro fertilization does not predict fetal diagnosis of congenital heart disease

Jodie K. Votava-Smith, MD¹, Julie S. Glickstein, MD^{1,2}, Lynn L. Simpson, MD^{2,3}, Ismee A. Williams, MD, MS^{1,2}

¹Department of Pediatrics, Division of Pediatric Cardiology, Columbia University Medical Center, Morgan Stanley Children's Hospital, ²The Center for Prenatal Pediatrics, Morgan Stanley Children's Hospital, ³Department of Obstetrics and Gynecology, Division of Maternal Fetal Medicine, Columbia University Medical Center

OBJECTIVES: Whether in vitro fertilization (IVF) is associated with congenital heart disease (CHD) is unclear. IVF has been named an indication for fetal echocardiography, though this has not been universally implemented. We aimed to compare the rate of IVF conception in fetuses diagnosed with CHD to that of fetuses with normal cardiac structure.

STUDY DESIGN: This is a case-control study consisting of a retrospective review of all patients who underwent fetal echocardiography by pediatric cardiology at Columbia University Medical Center from 2007-2010 to identify the mode of conception.

RESULTS: Echocardiography was performed on 2828 fetuses, of which 2761 (97.6%) had method of conception documented. Of these, 263 (9.5%) were the product of IVF conception. Reasons for referral included suspected CHD (36%), other fetal anomaly (28%), family history (13%), maternal indication (12%), suspected twin-twin transfusion syndrome (TTTS) (9%), IVF (0.3%), and other (2%). CHD was diagnosed in 617/2761 (22.3%) consisting of 497 complex cardiac lesions, 11 isolated arch anomalies, 81 isolated VSDs, and 28 single valve lesions excluding acquired anomalies due to TTTS. Rate of IVF was lower in fetuses with CHD (7% CHD vs 10.3% no CHD, OR=0.66 [95% CI 0.47-0.92], p=0.014). IVF fetuses had higher mean maternal age (35.4 ± 5.2 IVF vs 30.3 ± 6.3 no IVF, p<0.001), and were more likely to be products of a multiple gestation (68.4% IVF vs 15.9% no IVF, OR=11.5 [95% CI 8.7-15.3], p<0.001). Fetuses with CHD were found to have no difference in mean maternal age from those with normal cardiac structure (30.7 ± 6.4 CHD vs 30.9 ± 6.4 no CHD, p=0.95), and were less likely to be products of a multiple gestation (9.1% CHD vs 24.3% no CHD, OR = 0.31 [95% CI 0.23-0.42], p<0.001). In a multivariable model controlling for maternal age and multiple gestation, IVF was no longer associated with fetal CHD diagnosis (OR=1.2 [95% CI 0.78-1.7], p=0.48) but multiple gestation was (OR=0.3 [95% CI 0.22-0.41], p<0.001).

CONCLUSIONS: Among fetuses undergoing echocardiography at a tertiary care referral center, IVF conception was not associated with fetal CHD diagnosis after controlling for maternal age and multiple gestation. These results are in contrast to

previous reports and may be related to the nature of our study population and the inclusion of pregnancies that did not carry to term.

49. Elevated placental vascular resistance without evidence of brain-sparing persists after treatment of twin-reversed arterial perfusion sequence

Jodie K. Votava-Smith, MD¹, James F. Cnota, MD¹, Susan Gomien, RDMS¹, Mounira Habli, MD², William Polzin, MD², James Van Hook, MD², Timothy M. Crombleholme, MD², Erik C. Michelfelder, MD¹.

¹Fetal Heart Program, The Heart Institute, and ²Fetal Care Center of Cincinnati, Cincinnati Children's Hospital Medical Center, Cincinnati, OH

OBJECTIVE: Pump twins of twin-reversed arterial perfusion (TRAP) sequence may have altered cerebrovascular tone to preserve brain oxygenation in response to highly desaturated umbilical venous return from the acardiac twin. We sought to characterize the cerebroplacental Doppler patterns in pump twins before and after therapeutic radiofrequency ablation (RFA) of the acardiac twin.

STUDY DESIGN: We retrospectively reviewed pre and post-RFA echocardiograms of TRAP fetuses performed between 2/04 and 2/11. Doppler evaluation included estimation of pump twin combined cardiac output indexed for weight (CCO), and pulsatility index (PI) of the middle cerebral artery (MCA) and umbilical artery (UA). PI z-scores were calculated. Paired t-tests were used to compare pre and post-RFA Doppler data.

RESULTS: 35 fetuses had complete pre-RFA Doppler data sets. Mean gestational age (GA) was 20.6 ± 2.5 weeks. Mean z-score for UA-PI was 2.8 ± 1.1 , with 80% of fetuses having UA-PI z-score > 2 . Mean z-score for MCA-PI was 1.0 ± 1.0 , including 17% of fetuses with MCA-PI z-score > 2 and none with MCA-PI z-score < -2 . Cerebroplacental resistance ratio (CPR = $MCA-PI \div UA-PI$) was low (< 1.08) in 16/35 (46%), and 14/16 (88%) of those had UA-PI z-score > 2 . Estimated fetal weight was > 5 th percentile for GA in 34/35 (97%). Post-RFA Doppler data was available in 12 pump twins, with follow-up study done a median of 2 days (interquartile range 1,5) post-RFA. Following intervention, pump twin CCO decreased but there was no significant change in MCA-PI, UA-PI, or CPR (Table 1).

CONCLUSION: Prior to RFA therapy, the majority of the pump twins demonstrate low CPR secondary to abnormally high UA-PI rather than low MCA-PI. These findings persist in short term follow-up after RFA, despite a significant decline in CCO indicating interventional success. These results suggest elevated placental vascular resistance, which may be due to abnormal placental vascular connections which persist after cord ablation of the acardius, versus placental vascular remodeling secondary to chronic hypoxia. Further studies to characterize the placental pathology of this condition are warranted.

Table 1: Doppler parameters for the 12 pump twins seen both pre and post-RFA, given as mean \pm SD with p-value assessed by paired sample t-tests.

	Pre-RFA	Post-RFA	p
CCO (ml/min/kg)	733 \pm 210	567 \pm 121	0.0061
MCA-PI z-score	0.99 \pm 1.14	0.84 \pm 0.99	0.45
UA-PI z-score	2.44 \pm 1.45	3.21 \pm 1.72	0.059
CPR	1.18 \pm 0.26	1.14 \pm 0.21	0.57

50. Increased COX2 expression is associated with human calcific aortic valve disease (CAVD) and is observed in the *klotho*-mouse model of CAVD

Elaine E. Wirrig, Jonathan D. Cheek, Robert B. Hinton, and Katherine E. Yutzey

Aortic valve disease affects 2% of the United States population. The process of aortic valve (AoV) calcification has been compared to bone formation and many factors involved in osteogenesis are expressed in CAVD. In this study the *klotho*-deficient mouse, which ages prematurely, is used as a model of CAVD. *Klotho*-deficient mice exhibit nodular calcification in the AoV annulus with a distinct lack of immune infiltration in the calcified lesions. Increased expression of osteogenic genes, such as Runx2 and osteopontin, is observed in AoV tissues from *klotho*-deficient mice, similar to expression previously described in human CAVD. A microarray analysis was performed to identify alterations in gene expression in *klotho*-deficient mice relative to age-matched littermates. COX2 is among the genes with the greatest increase in expression in the *klotho*-deficient animals. COX2, while commonly associated with a role in inflammation, also plays a critical role in osteoblast differentiation and bone repair. We hypothesize that increased expression of COX2 contributes to osteogenic-like AoV calcification. COX2 expression is increased in the valve annulus near areas of calcification in *klotho*-deficient mice, in contrast to primarily endocardial expression in control littermates. COX2 expression colocalizes with regions of calcification and is not associated with macrophage infiltration, an indication of inflammation, in *klotho*-deficient mice. Likewise, in human diseased aortic valves COX2 protein expression is also increased in the valvular interstitial cells (VIC) of adult CAVD specimens compared to control AoVs. Furthermore, preliminary studies in adult mouse VIC cultures treated with osteogenic-inducing media indicate that COX2 inhibition impedes osteogenic differentiation of VICs. These results demonstrate that increased COX2 expression is associated with both human and mouse CAVD and that COX2 activity may contribute to osteogenic differentiation of mouse VICs.

51. Inhibition of p38 α reduces pathology in multiple mouse models of muscular dystrophy

Erin R. Wissing,^{1,2} Kinya Otsu,³ Jeffery D. Molkentin¹

¹Department of Molecular Cardiovascular Biology, Cincinnati Children's Hospital, Cincinnati, OH.

²Department of Molecular Genetics, Biochemistry and Microbiology, University of Cincinnati, Cincinnati, OH. ³Department of Cardiovascular Medicine, Osaka University, Osaka, Japan

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. p38 α 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 p38 α in the skeletal muscle of δ -sarcoglycan (*sgcd*) null mice using a floxed p38 α 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 p38 α / β pharmacologic inhibitor over 9 weeks. A/J *dysferlin* null mice present with a large influx of inflammatory cells and fibrosis in muscle with aging. To determine if this disease pathway was also functioning in A/J mice we treated 4 month old mice with p38 α / β inhibitor, and these data will be presented. Finally, in order to determine if over activation of p38 is sufficient to cause a dystrophy-like pathology in skeletal muscle, we used two genetic approaches 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. Consistent with this observation, mice lacking the p38 inactivating phosphatases DUSP1,4,10 showed a subtle, albeit significant induction of dystrophic-like pathology in skeletal muscle with aging. Taken together, these data suggest a detrimental role of p38 α in the progression of muscular dystrophy and suggest a novel therapeutic approach that could be employed quickly in humans.

52. Cardiac-specific very long chain acyl-CoA dehydrogenase deficiency induces dilated cardiomyopathy and cold intolerance in mice

Dingding Xiong, Zaza Khuchua, Chonan Tokunaga, Kathryn Rafferty, Sarkies Ruben Martherus, Anne-Cecile Huby, Hanna Osinska, Enkhsaikhan Purevjav, Jeanne James, Jeffrey A. Towbin, Arnold W. Strauss

BACKGROUND: Very long chain acyl-CoA dehydrogenase (VLCAD) catalyzes the initial step of mitochondrial beta-oxidation of the fatty acids with chain lengths of 14 to 20 carbons. Patients with VLCAD deficiency present with hypoketotic hypoglycemia, cardiomyopathy and sudden death that can be exacerbated by the stresses of fasting and cold. Global VLCAD knockout (KO) mice recapitulate these phenotypes. However, the contribution of cardiac VLCAD in the development of cardiomyopathy and the response to the environmental stressors is unclear. In this study, we assessed the hypothesis that cardiac specific VLCAD deficiency is sufficient to induce cardiomyopathy and cold intolerance.

METHODS AND RESULTS: A cardiac-specific homozygous VLCAD KO (csVLCAD KO) mouse was generated by crossing VLCAD floxed-allele mouse with transgenic mouse expressing Cre recombinase under the control of the α -myosin heavy chain promoter. Echocardiographic analysis at 6 months of age demonstrated left ventricular dilation and decreased fractional shortening (FS) in csVLCAD KO mice (for FS, $21 \pm 1\%$ vs $29 \pm 4\%$ in wild-type mice, $p < 0.05$). Ultrastructural analysis of ventricular myocardium showed widening of the sarcomeric I band, increased sarcomere length and substantial lipid deposits in csVLCAD KO mice. To test if cardiac VLCAD deficiency leads to susceptibility to death caused by cold stress, csVLCAD-KO mice and controls were placed at 4°C . All csVLCAD-KO mice ($n=9$) became moribund within 4-10 hours, whereas only 1/9 hemizygous and 1/9 wild-type mice died. Unlike global VLCAD KO mice, csVLCAD KO mice did not develop hypoglycemia. To determine if VLCAD deficiency alters mitochondrial fatty acid oxidation in cardiomyocytes, oxygen consumption rates in response to palmitate and glutamate administration were measured. The results indicated that while mitochondrial function was largely preserved, the fatty acid oxidation rate was markedly reduced in csVLCAD-KO mice, measuring 10-15% of WT.

CONCLUSIONS: Cardiac-specific VLCAD deficiency is sufficient to induce ventricular dilation and cardiac dysfunction as well as cold intolerant, which may be related to reduced fatty acid oxidation and increased lipid deposition in cardiomyocytes.

53. Hybrid-Hybrid Cardiac Procedures in Children: A One Stop Approach to Using the Hybrid Catheterization Facility to Combine Non-Cardiac Procedures with Cardiac Catheterization

Matthew Zussman, Robert Beekman, Ryan Leahy, James Spaeth, Russel Hirsch

The Heart Institute, Cincinnati Children's Hospital Medical Center, Cincinnati, OH

Background: Hybrid catheterization procedures traditionally combine cardiac catheterization (CC) and cardiac surgical procedures. During the past 3 years, we have also used the hybrid catheterization facility (HCF) to combine CC and non-cardiac invasive procedures (a “hybrid-hybrid” (HH) approach). This report documents the feasibility of combining such procedures on delivery of care to these high risk patients.

Methods: A prospective database of all CC procedures performed in our HCF since it opened in January 2007 was reviewed. Procedures were defined as HH if a CC and a non-cardiac invasive procedure were performed in tandem in the HCF during the same general anesthesia (GA) session. Hybrid cardiac catheterizations (i.e. those combining CC with cardiac surgery procedures) were excluded from this analysis.

Results: During the review period, 41 patients underwent 51 HH procedures as defined. Airway evaluations (bronchoscopy) were the most common of the interventions (n=34; 64%), followed by gastro-intestinal endoscopies (n=4; 7.5%), tracheal stenting or dilation (n=4 7.5%), Broviac line placement or removal (n=4; 7.5%), myringotomy tube placement (n=3; 5.6%), dental procedures (n=2; 3.7%), ophthalmologic (n=1; 1.8%) and orthopedic procedures (n=1; 1.8%) making up the remainder. The majority of the patients had only 1 non-cardiac procedure, 3 patients had 2 non-cardiac procedures, and 2 patients had 3 non-cardiac procedures by two different subspecialty services. There were no anesthesia complications. In each case, the additional procedures would have required additional separate GA sessions if not performed in an HH fashion. Further, without an HCF design, the majority of the non-cardiac procedures would have been performed in non-cardiac locations, with some patients requiring transport under GA or rescheduling at a different time.

Conclusion: Commitment to arranging HH procedures with non-cardiac services has improved patient safety by decreasing the total number of anesthesia sessions and / or the risk of transportation to other locations while under GA. Combining non-cardiac procedures with CC in high risk cardiac patients improves care efficiency by avoiding delays in non-cardiac testing or treatment. The benefits of HH utilization of HCF's should be actively promoted.