Over the last several years, the pace of research in our field has accelerated rapidly, exploding with new ideas and novel approaches. Additional Ventures is proud to host the **Single Ventricle Investigator Meeting**, which connects the fields of basic science, engineering, computation, and medicine all in one place - creating a true center of gravity for discovery in single ventricle heart research.

When: October 6-9, 2022

Where: Baltimore Marriott Waterfront

SVIM 2022 Key Topic Areas

Understanding Single Ventricle Etiology (e.g., Genetics, Model Organisms, Non-Genetic & Modifiable Risk, Normal Cardiac Development)

Addressing Complications & Comorbidities (e.g., End Organ Mapping & Biomarkers, Personalized Medicine, Alternative Interventions, Learning Networks)

Defining Biological Mechanisms of Outcomes (e.g., Genetic & Non-Genetic Basis, Substrate-Outcome Relationship, Predictive Models, Early Detection, Early Biomarkers)

Developing Functional Cures (e.g., Enabling Technologies, Bionic Approaches, Regenerative Approaches, Standardization and Scalability)

SVIM 2022 will also highlight the important scientific work from Enduring Hearts in a scientific spotlight on **Optimizing Cardiac Transplantation.**

Wifi: MarriottBonvoy_Conference

Password: SVIM2022

SVIM 2022 Extended Agenda

THURSDAY, OCT 6		LOCATION
4:00 – 5:30 PM	**Welcome Session & Keynotes** **Opening Remarks** Erin Hoffmann | Additional Ventures **Spotlight on the Patient Journey** Jameson Rich **Overview of the Single Ventricle Research Roadmap** Kirstie Keller, PhD | Additional Ventures **New tools and concepts in cardiac developmental biology** Michel Pucéat, PhD | Inserm **Role of patient-centric medicine in single ventricle care** Kiona Allen, MD | Lurie Children's Hospital	Harborside Ballroom A-C
6:00 – 8:00 PM	**Evening Reception with Fireside Chat:** Funding Landscape in Single Ventricle Research (Dinner and Drinks Provided) **Panelists:** **Kristin Burns, MD** | Branch Chief, Heart Development and Structural Diseases, National Heart, Blood, and Lung Institute, NIH **Erin Hoffmann** | President & Co-Founder, Additional Ventures **Glenn Dillon, PhD** | Vice President of Research Operations, American Heart Association **David Tancredi, MD, PhD** | Director, Leducq Foundation for Cardiovascular Research **Panel Moderator:** **Ekemini U. A. Riley, PhD** | Founder and President of the Coalition for Aligning Science	Harborside Ballroom A-C

FRIDAY, OCT 7		
7:00 – 8:00 AM	**Breakfast** (Provided)	4th floor foyer
8:00 – 9:30 AM	**Etiology I: Cardiac Development and Morphogenesis** Moderated by: Kimara Targoff, MD **Transcriptional and genomic regulation of chamber formation** **Plenary: Benoit Bruneau, PhD** | Gladstone Institutes **Single cell multi-omics reveal novel cardiac subpopulation critical to valve morphogenesis** Alexander Merriman*, Mauro Costa, Chun Ye, Deepak Srivastava	Harborside Ballroom A-C

(FRIDAY, OCT 7)

		LOCATION
	Dissecting Mechanisms of Chamber-Specific Cardiac Differentiation and its Perturbation Following Retinoic Acid Exposure David M. Gonzalez*, Nadine Schrode, Tasneem A.M. Ebrahim, Nicolas Broguiere, Giuliana Rossi, Lika Drakhlis, Robert Zweigerdt, Matthias P. Lutolf, Kristin G. Beaumont, Robert Sebra, Nicole C. Dubois **Persistent Ventricle Partitioning in the Adult Zebrafish Heart** Hannah R. Moran*, Christian Mosimann **Novel genetic analyses identify roles for proteosome factors in heart development and as candidate genes for congenital heart defects** Lisa Maves*, Kylie Kerker, Gist H. Farr III, Isabelle Young, Alex Goldstein, Juan Pablo Espinosa, Eva Hasegawa, David Beier	(Harborside Ballroom A-C)
9:45 – 10:45 AM	**Concurrent Breakout Sessions**	
	Minimally Invasive Monitoring to Ensure an Enduring Heart Transplant (led by Enduring Hearts) Featured Speakers: Palak Shah, MD, Margaret Samyn, MD, Brian Feingold, MD, and Marius George Lingararu, DPhil, MA, MSc Moderated by: Shelley Miyamoto, MD and Palak Shah, MD	Laurel A-B
	Brain Health Outcomes in Single Ventricle Heart Disease Patients Across Lifespan Featured Speaker: Kathryn Leigh Humphreys, PhD Moderated by: Nadine Kasparian, PhD, Ashok Panigrahy, MD, and Caitlin Rollins, MD	Laurel C-D
	New Frontiers in Single Ventricle Gene Regulation Featured Speakers: Deepak Srivastava, MD, Muge Kuyumcu-Martinez, PhD, and Rolf Bodmer, PhD Moderated by: Susan Liao, PhD	Essex A-B
	Leading Self, Team, and Institution Led by: Jennifer Askey, PhD, PCC | Academic Coach	Essex C
	The Obstacle is the Way: Translating Clinical Challenges into Therapeutic Opportunities Featured Panelists: Chris Breuer, MD, Danielle Gottlieb Sen, MD, Bret Mettler, MD, and George Nicholson, MD Moderated by: Danielle Gottlieb Sen, MD	Kent A-B
	Patient and Family Perspectives on Research Directions Featured Panelists: B. Arman Aksoy, PhD, Taylor Houlihan, Jameson Rich, and Tawanna Williams, CPC Moderated by: Diane Pickles	Kent C
11:00AM – 12:30 PM	**Outcomes I: Outcome Origins and Model Systems** Moderated by: Tasha Garcia, PhD	Harborside Ballroom A-C
	Challenging assumptions: Unique attributes of the failing single ventricle **Plenary: Shelley Miyamoto, MD** | Children's Hospital Colorado	

(FRIDAY, OCT 7)

		LOCATION
	Interim Report on Metabolic Derangements and Biomarker Signatures in Patients with Single Ventricle Heart Disease R. Mark Payne*, Thomas M. O'Connell, Lilian Golzarri-Arroyo, Jean P. Molleston **CFL1-Mediated Actin Remodeling: A Potential Right Ventricular Failure-Specific Therapeutic Target** Jonathan Edwards*, Spencer Williams, Jeffrey Brandimarto, Joshua Rhoades, Kenneth Bedi, Kenneth Margulies, Zoltan Arany **Evaluation of Ectopic Calcification in PTFE and Tissue Engineered Vascular Grafts in a Long Term Large Animal IVC Interposition Graft Model** Kevin Blum*, Mahboubeh Nabavinia, Ting-Heng Chou, John Kelly, Mitch Stacy, Chris Breuer **Pulmonary arteries undergo extensive changes in mechanical properties during hypoxia in both development and maturity** Abhay B. Ramachandra*, Edward Manning, Jay D. Humphrey	(Harborside Ballroom A-C)
12:30 – 2:00 PM	**Lunch** (Provided)	4th Floor Foyer
	Optional Lunch Programming: Building a Culture of Curiosity: Experiments as a Leader Led by: Jennifer Askey, PhD, PCC | Academic Coach	Laurel A-D
2:00 – 3:30 PM	**Care I: End Organ Mapping and Biomarkers** Moderated by: Nadine Kasparian, PhD **Using the FORCE: Development of a CMR Imaging Biomarker Research Platform** **Plenary: Rahul Rathod, MD, MBA** | Boston Children's Hospital **Random Forest Analysis Identifies Important Clinical and Imaging Predictors of Impaired Neurocognitive Development in Children with Congenital heart Disease** Rafael Ceschin*, Dean B. Andropoulos, Ashok Panigrahy **Blood barrier permeability correlates with cognitive function in Fontan patients** John Wood*, Sharon O'Neil, Silvie Siriany, Clio Gonzalez-Zacarias, Jian Shen, Botian Xu, Jon Detterich, Bradley Peterson, Ashok Panigraphy **Endothelial dependent vascular function is decreased in patients with Fontan circulation and is associated with worse Fontan hemodynamics** Jon Detterich*, Julian Cameron, Silvie Suriany, Scott Leopold, Honglei Liu, Christopher Denton, Sarah Badran, Neil Patel, Thomas D. Coates, John C. Wood **Material properties are important for patch sizing in aortic arch reconstruction for single ventricle patients** Shannen B. Kizilski*, Martha D. Chaillo Lizarraga, Nicholas E. Kneier, Emily R. Eickhoff, Noah E. Schulz, Peter E. Hammer, David M. Hoganson	Harborside Ballroom A-C

(FRIDAY, OCT 7)		LOCATION
3:45 – 5:15 PM	**Cures I: Bionic and Regenerative Approaches** Moderated by: Nicole Dubois, PhD **Scaling 3D Bioprinting: From the Petri Dish to a Human-Scale Biopump** **Plenary: Mark Skylar-Scott, PhD** | Stanford University **Human Embryoid Body Bioinks for FRESH 3D Bioprinting of Contractile Cardiac Tissue** Brian Coffin*, Adam Feinberg **Towards Building a Contractile Conduit with FRESH 3D Bioprinting** Jacqueline Bliley*, Maria Stang, Annie Behre, Brian Coffin, Erica Comber, Dan Shiwarski, Adam Feinberg **Analysis of Injection-Jet Configurations for a Self-Powered Fontan Circulation** Ray Prather*, Arka Das, Michael Farias, Eduardo Divo, Alain Kassab, William DeCampli **Development of a fetal valve prototype designed for implantation in utero** Sanchita S. Bhat*, Hieu T. Bui, Anna Farnan, Christopher K. Breuer, Aimee K. Armstrong, Lakshmi Prasad Dasi	(Harborside Ballroom A-C)
5:30 – 7:00 PM	**Poster Session** (with Happy Hour & Hors d'oeuvres)	Harborside Ballroom D-E

SATURDAY, OCT 8		
7:00 – 8:00 AM	**Breakfast** (Provided)	4th Floor Foyer
8:00 – 9:00 AM	**Care II: Personalized Medicine Approaches and Alternative Interventions** Moderated by: Stephanie Nakano, MD **Growing and Evolving: Updates from ACTION on Ventricular Assist Device Therapy in the Fontan Circulation** Kathleen E. Simpson*, Chet Villa, Muhammad F. Shezad, Ari Cedars, Kurt Schumacher, Sharon Chen, David N. Rosenthal, Angela Lorts **Outcomes After Initial Heart Failure Consultation in Fontan Patients** Sharon Chen*, Muhammad F. Shezad, Angela Lorts, Kurt Schumacher; on behalf of the Advanced Cardiac Therapies Improving Outcomes Network (ACTION) **Initial Validation of a Predictive Personalized Computational Model for Patients with Borderline Left Ventricles** Vijay Vedula*, Yurui Chen, Isao Anzai, Justin Tran, David Kalfa **Ambulatory Monitoring of Fontan Pressures Using a Novel Implantable Sensor** Martin Bocks*, Nader Najafi	Harborside Ballroom A-C

(SATURDAY, OCT 8)		LOCATION
9:15 – 10:15 AM	**Concurrent Breakout Sessions**	
	Innovations for Enduring Heart Transplantation (led by Enduring Hearts) Featured Speakers: Jennifer Conway, MD, Anne Halpin, PhD, Stephanie Nakano, MD, Jane O, MD Moderated by: Anne Halpin, PhD and Stephanie Nakano, MD	Laurel A-B
	Computational Fluid Dynamics: Making it a reality in everyday practice Featured Speakers: Mark Fogel, MD, David Hoganson, MD, Vijay Govindarajan, PhD, and Alejandro Roldán-Alzate, PhD Moderated by: Mark Fogel, MD, David Hoganson, MD, and Alison Marsden, PhD	Laurel C-D
	Model Organisms in Single Ventricle: What's the Best Approach? Featured Panelists: Benoit Bruneau, PhD, Anthony Firulli, PhD, Mengmeng Huang, PhD, Luis Hernandez Nunez, PhD, Stephanie Lindsey, PhD, Georg Vogler, PhD Moderated by: Luis Hernandez Nunez, PhD	Essex A-B
	Developing Vision for Your Career Led by: Jennifer Askey, PhD, PCC | Academic Coach	Essex C
	Registries and Data in Single Ventricle: What's out there? What's in there? Featured Panelists: Kristin Burns, MD, Yves D'Udekem, MD, PhD, Nadine Kasparian, PhD, Alexander Opotowsky, MD, Shelby Kutty, MD, PhD, MHCM, Diane Pickles, Rahul Rathod, MD, MBA, Jack Rychik, MD, and Kurt Schumacher, MD, MS Moderated by: Alexander Opotowsky, MD and Jack Rychik, MD	Kent A-B
10:30AM – 12:00 PM	**Scientific Spotlight by Enduring Hearts** **History of single ventricle transplantation** Janet Scheel, MD **Pretransplant morbidities in single ventricle** Kurt Schumacher, MD **Donor availability, new listing criteria and challenges in adults with single ventricle** Ari Cedars, MD **Single ventricle and ventricular assist device support** Shriprasad Deshpande, MD **Panel Discussion:** Not too early not too late: When to Consider Transplantation for a Child with Single Ventricle	Harborside Ballroom A-C
12:00 – 2:00 PM	**Lunch** (Provided)	4th Floor Foyer
2:00 – 2:30 PM	**SVRF 2021 Top Scorer** *Speaker Will Be Announced at the Conference!	Harborside Ballroom A-C

(SATURDAY, OCT 8)

2:30 – 3:45 PM

LOCATION
Harborside Ballroom A-C

Etiology II: Mechanisms of Congenital Heart Disease
Moderated by: Mingtao Zhao, DVM, PhD

Identification of pathogenic genomic structural variation in single ventricle congenital heart disease by short read whole genome sequencing
Daniel Quiat*, Xuefang Zhao, Sarah U Morton, Alexandre Pereira, Sophie McAllister, Steven R DePalma, the Pediatric Cardiac Genomics Consortium, Michael E Talkowski, Harrison Brand, Jonathan G Seidman, Christine E Seidman

Multi-chamber cardioids unravel human heart development and defects
Alison Deyett*, Clara Schmidt, Sasha Mendjan

A CRISPR-Activation CROP-seq Screen to Identify Dosage Sensitive Genes in Congenital Heart Disease
Sanjeev Ranade*, Sean Whalen, Angelo Pelonero, Lin Ye, Rahul Mital, Langley Grace Wallace, Casey Gifford, Katherine Pollard, Deepak Srivastava

Myocardial-intrinsic defects underlie an Rbfox-mediated zebrafish model of hypoplastic left heart syndrome
Mengmeng Huang*, Alexander Akerberg, Xiaoran Zhang, Haejin Yoon, Shakchhi Joshi; Celia Harding, Christopher Nguyen, William T Pu, Marcia C Haigis, C Geoffrey Burns, Caroline E Burns

Evaluation of endocardial HAND2 gene regulatory networks that lead to tricuspid atresia
Anthony B Firulli*, Rajani M George, Beth A Firulli, Ram Podicheti, Douglas B Rusch, Len Pennachio, Marco Osterwalder

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4:00 – 5:00 PM

Harborside Ballroom A-C

Outcomes II: Non-Genetic Factors in Disease Development
Moderated by: Stephanie Lindsey, PhD

Homeostasis & the Developing Vasculature in Single Ventricle Physiology
Jay Humphrey*, Linda Irons, Sae-Il Murtada, Abhay Ramachandra, Bruno Rego

A Computational Framework for Simulating Patient-Specific Vascular Growth and Remodeling
Erica L. Schwarz*, Martin R. Pfaller, Jason M. Szafron, Stephanie E. Lindsey, Marcos Latorre, Christopher K. Breuer, Jay D. Humphrey, Alison L. Marsden

A multimodal approach to investigate the effects of respiration on Fontan flow
Markus Horvath*, Caglar Ozturk, Bryce Starr, Mulan Jiang, Ellen Roche

In utero mitral valve inflow obstruction stunts left ventricular chamber growth and maturation in a fetal lamb model
Daisuke Onohara*, Matthew W. Hagen, Sammantha Louey, George Giraud, Sonnet S. Jonker, Muralidhar Padala

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(SATURDAY, OCT 8)		LOCATION
5:15 – 7:00 PM	**Great Debate: What is the Solution to the Fontan Circulation?** (with Happy Hour & Hors d'oeuvres) Moderated by: Kirstie Keller, PhD **Debate Partners:** **Christopher Breuer, MD** | Nationwide Children's Hospital **T-Y Hsia, MD** | Orlando Health Arnold Palmer Hospital for Children	Harborside Ballroom A-C

SUNDAY, OCT 9		
7:00 – 8:00 AM	**Breakfast** (Provided)	4th Floor Foyer
8:00 – 9:00 AM	**Cures II: Enabling Technologies** Moderated by: Irfan Kathiriya, MD, PhD **Two-Year Follow-Up after Autologous Stem Cell Therapy for Hypoplastic Left Heart Syndrome at the time of Bidirectional Glenn Surgery** Somya Shankar*, Apurva B. Challa, Drew K. Seisler, Clinton Hagen, M. Yasir Qureshi, Timothy J. Nelson **Electrophysiological characterization of engineered micro-heart tissue derived from human pluripotent stem cells using 3D in-vivo like protocols** Lavanya Aryan*, Kuo-Chan Weng, Lauren Huebner, Lauren Boggs, Nathaniel Huebsch, Stacey Rentschler **Simulated Performance Of A Bioprinted Pulsatile Fontan Conduit** Zinan Hu*, Erica L. Schwarz, Jessica Herrmann, Mark Skylar-Scott, Alison L. Marsden **A Computational Model for Cardiovascular Fluid–Solid-Growth Interaction** Martin R. Pfaller*, Marcos Latorre, Erica L. Schwarz, Jason Szafron, Jay D. Humphrey, Alison L. Marsden	Harborside Ballroom A-C
9:15 – 10:15 AM	**Concurrent Breakout Sessions**	
	Surgically Palliated Single Ventricle: Pre- and Post-transplant Patient Care (led by Enduring Hearts) Featured Speakers: Pranava Sinha, MD, MBA, Kathleen Simpson, PhD, Kurt Schumacher, MD, and Sharon Chen, MD Moderated by: Kurt Schumacher, MD and George Nicholson, MD	Laurel A-B
	Artificial Intelligence for Single Ventricle Disease: What are the Challenges and Opportunities? Featured Speaker: Markus Rottmann, PhD	Laurel C-D
	Defining the Mechanistic Basis for theAnatomic Subtypes of Single Ventricle Featured Speaker: Paul Grossfeld, MD	Essex A-B

(SUNDAY, OCT 9)		LOCATION
9:15 – 10:15 AM	**An Open-Source Pipeline for Bioprinting Perfusable Tissues** Led by: Jessica Herrmann, Alison Marsden, PhD, Zachary Sexton, and Mark Skylar-Scott, PhD	Essex C
	Bolstering Your Communication Toolkit: Strategies for Success in Science & Engineering Led by: Jay D. Humphrey, PhD	Kent A-B
10:30 AM – 12:00 PM	**Closing Session: Future of Single Ventricle Science & Medicine** Moderated by: Kaitlin Davis, PhD **Biological cardiac pacing by gene therapy** Hee Cheol Cho, PhD | Johns Hopkins Medicine **Panel Discussion:** Future of Single Ventricle Science and Medicine **Panelists:** **Benoit Bruneau, PhD** | Director and Senior Investigator, Gladstone Institute of Cardiovascular Disease; Professor, Pediatrics, University of California San Francisco **Shelley Miyamoto, MD** | Director, Cardiomyopathy Program and Jack Cooper Millisor Chair in Pediatric Heart Disease, Children's Hospital Colorado; Professor, Pediatrics-Cardiology, University of Colorado Anschutz Medical Campus **Kurt Schumacher, MD** |Associate Professor, Congenital Heart Center, University of Michigan; Director, Pediatric Heart Transplant Program; Director, PC4 Data Coordinating Center; Associate Director, M-CHORD; Associate Director, Pediatric Cardiology Fellowship **Mark Skylar-Scott, PhD** | Assistant Professor of Bioengineering, Stanford University; Basic Science and Engineering (BASE) Faculty, Betty Irene Moore Children's Heart Center **Closing Remarks** Kirstie Keller, PhD | Additional Ventures	Harborside Ballroom A-C

Appendix – Abstracts

SVIM 2022 Scientific Sessions

Keynotes

Thursday, October 6 at 4:00-5:30 PM

Transcriptional and genomic regulation of chamber formation

Michel Pucéat, PhD | Inserm

The origins of Congenital Heart Diseases (CHD) including the Hypoplastic Left Heart Syndrome (HLHS) are far from being understood. Genetics is part of them but does not account for the broad diversity or severity of cardiac malformations. Using genetically modified mice, our understanding of both physiological and pathological heart formation has significantly progressed for the last 2 decades. However, we need new biological tools to study human heart development. We also call for new concepts to understand specific and still unexplained steps of heart morphogenesis that are deficient or dysregulated in CHD. Here, I will discuss the recent engineering of 3D cardiac organoids from human pluripotent stem cells which provide a new tool to study in-vitro physiological and pathological heart formation. I will further introduce two novel biological concepts that apply to major steps of heart morphogenesis as well as to heart regeneration in the context of CHD, namely, the embryonic cell senescence and the in-vivo cell reprogramming process. Both concepts will be discussed in the more specific context of HLHS.

Better Together: Putting Patients and Families at the Center of Single Ventricle Care

Kiona Allen, MD | Lurie Children's Hospital

The talk will focus on the ways that patient, caregiver, and care team partnerships can enhance clinical care, advance research, and ultimately promote innovation by drawing from the different and equally important perspectives, holding the field accountable, and instilling passion and energy into the critical work we do.

SVIM 2022 Scientific Sessions

Etiology I: Cardiac Development and Morphogenesis

Friday, October 7 at 8:00-9:30 AM

Moderated by Kimara Targoff, MD | Columbia University

Transcriptional and genomic regulation of chamber formation

Plenary: Benoit Bruneau, PhD | Gladstone Institutes

Understanding the molecular and cellular underpinnings of chamber formation are key to understanding congenital heart defects, and in particular single ventricle disease. We have examined the role of transcription factors in chamber formation. We find that reduced dosage of TBX5 compromises a compartment boundary that is essential for septation and chamber formation. Further reduction in TBX5 results in single ventricle and single atrium morphology. In parallel, Katherine Pollard's lab is using computational approaches to predict alterations in 3D chromatin structure due to chromosomal deletions. We have found that one deletion in a patient with hypoplastic left heart syndrome is predicted to alter chromatin structure at an important transcription factor locus. With genome engineering to recapitulate this deletion in iPS cells we confirm the predicted alterations in 3D chromatin.

Single cell multi-omics reveal novel cardiac subpopulation critical to valve morphogenesis

Alexander Merriman*[1], Mauro Costa[1], Chun Ye[1], Deepak Srivastava[1]

[1] Gladstone Institutes

Congenital and acquired valvular heart disease are major sources of morbidity and mortality with an anticipated increase in prevalence secondary to an aging population and increase in survivorship for patients with congenital disease. Further developmental studies are required to advance novel therapeutic development including living tissue engineered heart valves and pharmacologic interventions. In this study, we have interrogated the later stages of valvulogenesis, following formation of the endocardial cushions, to understand the molecular mechanisms of valve formation and how these mechanisms are disrupted in the context of disease. Leveraging a combination of single cell RNA/Chromatin Accessibility sequencing in the developing mouse heart, we identified a novel, rare cell population in the developing valve with a unique transcriptional profile comprised of highly specific developmental signaling pathway genes. These cells are first detectable after valve primordia formation at embryonic day (E) 12.5 and are spatially localized at the leading edge of the developing leaflets. Ablation of this rare subpopulation during development results in highly dysplastic valves, characterized by hyperplastic, redundant, immature leaflets associated with valvular stenosis and regurgitation. These dysplastic features are consistent with the features of several congenital valvulopathies including Ebstein's Anomaly, and pulmonary or aortic valve stenosis. Single cell RNA sequencing analysis of a human fetal heart with hypoplastic left heart syndrome and critical aortic stenosis demonstrated a depletion of this cell population in the diseased aortic valve, suggesting these cells may be required for normal human valvular development as well. This study establishes the existence of a novel, rare subpopulation of cardiac cells that are critical to valve development and may contribute to the pathogenesis of congenital valvulopathies.

SVIM 2022 Scientific Sessions

Dissecting Mechanisms of Chamber-Specific Cardiac Differentiation and its Perturbation Following Retinoic Acid Exposure

David M. Gonzalez*[1], Nadine Schrode, Tasneem A.M. Ebrahim, Nicolas Broguiere, Giuliana Rossi, Lika Drakhlis, Robert Zweigerdt, Matthias P. Lutolf, Kristin G. Beaumont, Robert Sebra, Nicole C. Dubois[1]

[1]Mount Sinai School of Medicine

Proper heart development requires specification and differentiation of multiple progenitor populations, and dysregulation of these processes can lead to congenital heart defects (CHDs). Furthermore, different forms of CHDs may be driven by defects in distinct progenitor subtypes, who's heterogeneity remains incompletely understood. To understand the transcriptomic landscape of the developing heart we performed single-cell RNA sequencing (scRNASeq) at the cardiac crescent (E8.25), primitive heart tube (E8.75) and late heart tube (E9.25) stages using Foxa2-Cre;mTmG embryos, allowing us to label atrial/ventricular fated cells prior to and during chamber morphogenesis. Through RNA velocity and lineage trajectory tools we identify heart field progenitors in multiple differentiation states, and uncover the top dynamically regulated genes for each cell type, which represent putative drivers of cell-state transitions during differentiation. We find that clustering of myocardial cell types occurs primarily based on heart field progenitor origin, and that different progenitor populations contribute to ventricular or atrial identity through separate differentiation mechanisms. Furthermore, we find that differentiation of anterior or posterior second heart field (SHF) cells occurs through deployment of separate components of the cardiac gene-regulatory network. Lastly, we show that in utero exposure to exogenous retinoic acid (RA), which plays a role in atrial chamber specification and acts as a teratogen during development, causes defects in ventricular chamber size. scRNASeq of RA-exposed embryos demonstrated dysregulation in FGF signaling in anterior SHF cells and a shunt in differentiation towards formation of head mesenchyme, and defects in cell-cycle exit in myocardial progenitors. These data demonstrate the utility of comparative scRNAseq studies for understanding lineage relationships during development and revealing cell-specific sensitivity to perturbations.

Persistent Ventricle Partitioning in the Adult Zebrafish Heart

Hannah R. Moran*[1], Christian Mosimann[1]

[1]University of Colorado Anschutz Medical Campus

The heart is the first functional organ to form in the developing vertebrate embryo. The zebrafish provides an accessible vertebrate system to study early heart morphogenesis and to gain new insights into the mechanisms of congenital disease. Although composed of only two chambers compared to the four-chambered mammalian heart, the zebrafish heart integrates the core processes and cellular lineages that are central to cardiac development across vertebrates. The vertebrate heart integrates cells from the early-differentiating first heart field (FHF) and the later-differentiating second heart field (SHF), both emerging from the lateral plate mesoderm. In mammals, this process forms the basis for the development of the left and right ventricle chambers and subsequent chamber septation. The single ventricle-forming zebrafish heart also integrates FHF and SHF lineages during embryogenesis, yet the contributions of these two myocardial lineages to the adult zebrafish heart remain incompletely understood. Here, we characterize the myocardial labeling of FHF descendants in both the developing and adult zebrafish ventricle. Expanding previous findings, late gastrulation-stage labeling using drl-driven CreERT2 recombinase with a myocardium-specific, myl7-controlled, loxP reporter results in the predominant labeling of FHF-derived outer curvature and the right side of the embryonic ventricle. Raised to adulthood, such lineage-labeled hearts retain broad areas of FHF cardiomyocytes in a region of the ventricle that is positioned at the opposite side to the atrium and encompasses the apex. Our findings are consistent with the hypothesis that integration of distinct cardiomyocyte lineages is an evolutionarily

SVIM 2022 Scientific Sessions

ancient trait that predates the formation of multi-chambered ventricles.

Novel genetic analyses identify roles for proteosome factors in heart development and as candidate genes for congenital heart defects

Lisa Maves*[1, 2], Kylie Kerker[2], Gist H. Farr III[1], Whitaker Reid[2], Isabelle Young[2], Alex Goldstein[2], Juan Pablo Espinosa[2], Eva Hasegawa[1], David Beier[1, 2]

[1] Seattle Children's Research Institute
[2] University of Washington

Congenital heart defects (CHDs), which include single ventricle disorders, occur in about 1% of live births and are the leading cause of infant death due to birth defects. In spite of the exceptional efforts of large-scale whole-exome sequencing studies on CHD patients, it is estimated that these studies have thus far accounted for only about 30% of the genetic contribution to CHDs. Our long-term goal is to provide increased understanding of the genetic and molecular causes of CHDs. This insight would make it more feasible to use genomic screening for improved risk assessment and more accurate diagnoses for severe CHDs such as single ventricle defects. We have taken a novel approach to identify new CHD-candidate genes. Using the ExAC human genetic database, we identified a set of over 200 new candidate genes for CHDs. This gene set includes a group of five genes encoding proteasome factors. We utilized protein-protein interaction network analysis to find that these proteasome factors are predicted to interact with known CHD genes involved in single-ventricle defects. The proteasome system, one of the main cellular protein degradation pathways, has been shown to be involved in human cardiac disease, but specific roles for proteasome factors in CHDs or heart development have not yet been determined. We used CRISPR screening in zebrafish embryos to preliminarily identify functions of these proteosome factors in heart development. We have recently used CRISPR to create an allelic series of new proteasome gene mutant zebrafish strains. Thus far our analyses show that these proteasome genes share critical roles in zebrafish heart development. Furthermore, we find that proteasome gene mutant embryos show an increase in ubiquitinated proteins, similar to embryos that are treated with the small molecule proteasome inhibitor bortezomib. These results show that there is indeed a block to proteasome function in proteasome gene mutant embryos. Through our characterization of these proteasome genes, a new gene class in heart development and CHDs, our study increases our fundamental knowledge of the genetic mechanisms of heart development. Our results promise to further our understanding of the genetic causes of human CHDs.

Outcomes I: Outcome Origins and Model Systems

Friday, October 7 at 11:00-12:30 PM
Moderated by Anastacia (Tasha) Garcia, PhD | University of Colorado Anschutz Medical Campus

Challenging assumptions: Unique attributes of the failing single ventricle

Plenary: Shelley Miyamoto, MD | Colorado Children's Hospital

The mechanisms underlying the outcome for single ventricle heart disease remain unknown. Why do some patients with single ventricle heart disease survive well into adulthood and others develop heart failure, valve dysfunction, or arrhythmias in infancy or early childhood? While the outcome of patients with single ventricle heart disease is multifactorial, including the impact of variations in anatomy, genetics, and environmental factors, there are also adaptations at the level of the myocardium that are worth considering. In this talk I will review some of the unique features of the single ventricle myocardium that may contribute to development of single ventricle heart failure and provide opportunities for the identification of novel therapeutic targets.

SVIM 2022 Scientific Sessions

Interim report on Metabolic Derangements and Biomarker Signatures in Patients with Single Ventricle Heart Disease

R. Mark Payne*[1], Thomas M. O'Connell[1], Lilian Golzarri-Arroyo[2], Jean P. Molleston[1]

[1] Indiana University School of Medicine
[2] Indiana University

Background - Patients with single ventricle (SV) heart disease have a high rate of progression to heart failure (HF) and death after palliation. Preventative medical therapies are lacking. A biological understanding of the mechanisms contributing to HF in SV is needed. This study explores biomarkers to predict outcome and stratify response to therapies in SV.
Goal - To prospectively evaluate metabolomic derangements in patients with SV heart disease. We will correlate these findings with established clinical metrics, as well as new clinical tools, to determine if metabolomic profiling can identify biomarkers that predict outcome and identify mechanism(s) of HF.
Methods: We are using a multi-platform, nuclear magnetic resonance (NMR) and mass spectrometry (MS)-based metabolomics approach on plasma and fecal samples from a cross-sectional set of 150 SV subjects, ages 1 – 25 years. 75 Control (Con) subjects include healthy and dilated cardiomyopathy (DCM) subjects. Clinical assessments include prospective clinical laboratory evaluations, history and physical examination, echocardiography, and vibration controlled transient elastography (VCTE). Statistical analysis includes ANOVA and Kruskal-Wallis.
Results - 71 subjects have been enrolled with 7 DCM, 19 healthy Con, and 45 SV. Mean age is not different between the 3 groups. There is a 2:1 male predominance in the SV cohort vs 1:1 in the Con and DCM cohorts. Hemoglobin in the SV subjects is statistically higher (14.6 ± 2.42 g/dL, $p=0.042$) than Con and DCM. Brain natriuretic peptide (BNP) is significantly higher in DCM (243 ± 291pg/mL) than Con 24.4 ± 14.7pg/mL) or SV (62.9 ± 56.3pg/mL) ($p<0.001$). Direct and total bilirubin were higher in SV subjects ($p\leq0.005$), while measures of lipid metabolism (cholesterol, triglycerides, HDL, and LDL) were significantly lower in the SV cohort. ALT was higher in SV ($p=0.002$ by Kruskal-Wallis). C-reactive protein was higher in the DCM and SV cohorts than Con by Kruskal-Wallis ($p=0.006$). Liver stiffness measurement (LSM) by VCTE was strikingly higher in SV (17 ± 13, range 2.9 - 75) than either DCM (5.54 ± 3.15, range 3.1 – 12.5) or Con (4.63 ± 1.1, range 3.0 – 7.0) ($p<0.001$).
Conclusion - SV subjects show significant changes to liver early in life with resultant abnormalities in hepatic function. LSM, a measure of fibrosis, is markedly elevated in SV but not DCM, indicating hepatic fibrosis; elevated LSM can also indicate expected hepatic congestion. BNP is elevated in DCM but not SV subjects. These data suggest underlying hepatic fibrosis and dysfunction in subjects with SV anatomy early in life. Our pilot metabolomics study found significant differences in liver associated metabolites in the SV and SVHF groups including primary and secondary bile acids. Upcoming metabolomic analysis of the next set of blood and fecal samples will focus on hepatic and gut biology as an initial hypothesis for HF in the SV subjects as compared to DCM.

CFL1-Mediated Actin Remodeling: A potential Right Ventricular Failure-Specific Therapeutic Target

Jonathan Edwards*[1,2], Spencer Williams[1,2], Jeffrey Brandimarto[2], Joshua Rhoades[2], Kenneth Bedi[2], Kenneth Margulies[2], Zoltan Arany[2]

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Background - Right ventricular failure (RVF) is a significant health problem in patients with single ventricular congenital heart disease (SV-CHD) that is a strong predictor of death, and for which there are no proven therapies. A major barrier to such therapeutic development is a limited understanding for the molecular mechanisms that drive RVF in humans and clinically relevant disease models.

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Objective - To identify RV-specific heart failure mechanisms in humans and test their applicability in an animal model of RVF.
Methods - RNAseq was performed separately on matched nonfailing (n=16) and dilated cardiomyopathy (n=33) RVs and left ventricles (LVs). Fisher's Meta-Analysis was performed using MetaboAnalyst to compare RV and LV dilated cardiomyopathy vs nonfailing differential gene expression (DGE). Peptidomics was performed on arterial and coronary sinus blood (SomaLogic) in a separate cohort of individuals with reduced LV function with (n=7) or without (n=14) concomitant RV dysfunction. Pulmonary artery banding (PAB, n=6) or sham (n=4) was performed on 9-week old C57/BL6 (50% female for each) and sacrificed after 10 days.
Results - RNAseq revealed 303 genes with DGE common to RV and LV, 1008 unique to RV, and 132 unique to LV (Bonferroni adjusted $p<0.05$). PantherDB gene set enrichment analysis revealed broad downregulation for Actin remodeling among RV-specific DGE, including CFL1, PFN1, CAP2, and WASF3 (all, adj $p<5x10\text{-}4$). In parallel, peptidomics of arterial and coronary sinus blood revealed that CFL1 was consistently excreted by the heart in the context of RV dysfunction but not LV dysfunction alone (adj $p<0.05$). CFL1/PFN1 maintain sarcomeric thin filament equilibrium by actin depolymerization and polymerization, respectively. To understand if altered actin dynamics play a role in mouse RVF pathogenesis, PAB or sham was performed on wild type mice. PAB led to RV hypertrophy (RV/tibia length 1.94 vs 1.28 mg/cm, $p=0.006$) and an increase in total CFL1 (2.4 fold) in the RV of PAB mice. CFL1 phosphorylation—a marker of CFL inhibition—was also reduced 3.5 fold ($p=0.0007$) and correlated to an increase in triton-soluble actin ($R2=0.73$, $p=0.03$) in PAB RV.
Discussion - Actin remodeling, and therefore maintenance of sarcomeric thin filament, may be uniquely disrupted in RVF and serve as an RVF-specific therapeutic target. Further investigation is necessary to reconcile the CFL1 transcriptional and translational/posttranslational changes in human and mouse RVF.

Evaluation of Ectopic Calcification in PTFE and Tissue Engineered Vascular Grafts in a Long Term Large Animal IVC Interposition Graft Model

Kevin Blum*[1], Mahboubeh Nabavinia[1], Ting-Heng Chou[1], John Kelly[1], Mitch Stacy[1], Chris Breuer[1]

[1] Nationwide Children's Hospital

A significant source of postoperative morbidity and mortality the congenital heart disease patient population arises from the use of prosthetic biomaterials utilized in the surgical treatments of these conditions. Recent data has demonstrated that polytetrafluoroethylene (PTFE), the standard material used for vascular grafts, can develop ectopic calcifications over long implant times. These calcifications can lead to vascular wall stiffening as well as conduit stenosis. Tissue engineered vascular grafts (TEVGs) hold promise in these patients through their ability to serve as a scaffold for the development of functional neovessels with native form and function. In order to evaluate the development of ectopic calcifications, six month old Dorset lambs were implanted with either PTFE (N=3) or TEVG (N=3) grafts as thoracic IVC interposition grafts. At three years post-implantation, sheep from both cohorts were imaged using non-contrast computed tomography scans to evaluate for the development of ectopic calcification. Ectopic calcification was evident in 100% of the PTFE cohort, and 0% of the TEVG cohort (calcium mass 284.4±60.9 HU*mL PTFE vs 0.8±0.7 HU*mL TEVG, $p=0.0013$ unpaired t-test). These results demonstrate the ability of the sheep model to recapitulate the calcification complications seen in humans, as well as supporting TEVGs as a beneficial conduit for congenital heart disease surgical repairs.

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Pulmonary arteries undergo extensive changes in mechanical properties during hypoxia in both development and maturity

Abhay B. Ramachandra*[1], Edward Manning[2], Jay D. Humphrey[1]

[1] Yale University
[2] Yale University School of Medicine

The mechanical properties of large pulmonary arteries play important roles in both lung and cardiac function. Altered hemodynamics and associated hypoxia in single ventricle physiology alters these pulmonary artery properties, triggering a series of remodeling processes in the ventricle, vasculature, and lungs. Longitudinal changes in pulmonary arteries during and after periods of hypoxia, central to understanding the sequelae, are not well quantified or understood. We addressed this gap in knowledge by biomechanically phenotyping pulmonary arteries as a function of duration of exposure in juvenile (hypoxic exposure starting at 3 weeks of age) and adult (hypoxic exposure at 8 weeks of age) mice of both sexes. Mechanical properties were quantified using a custom computer-controlled biaxial testing device. We observed thickening and a reduction in stored energy of the pulmonary arteries under hypoxic exposure. Further, the circumferential stiffness and stress increased after 5 weeks of hypoxic exposure in both juvenile and adult mice. Surprisingly, despite differences in initial stress state, both juvenile and adult pulmonary arteries remodeled to the same final mechanical state (i.e., stress and stiffness) at the end of hypoxic exposure. Detailed time-course data in the adult male mice also revealed that the mechanical properties evolved rapidly during the first couple of weeks, reaching an asymptote by about 3 weeks. Smooth muscle responses to potassium chloride (membrane depolarization) or phenylephrine (adrenoreceptor agonist) and endothelial response to acetylcholine were compromised in hypoxic conditions, compared to normoxic controls in the adult male mice. Overall, both active and passive vascular mechanics are compromised upon hypoxic exposure and so too the lung parenchyma, similar to that in emphysema. Our current efforts are directed towards quantifying associated microstructural changes using histology and quantifying mechanics in normoxic recovery to probe reverse remodeling.

Care I: End Organ Mapping and Biomarkers

Friday, October 7 at 2:00-3:30 PM
Moderated by Nadine Kasparian, PhD | Cincinnati Children's Hospital

Using the FORCE: Development of a CMR Imaging Biomarker Research Platform

Plenary: Rahul Rathod, MD, MBA | Boston Children's Hospital

Cardiac MR imaging (CMR) has improved our understanding of Fontan physiology and outcome prediction. Unfortunately, most of these reports are small, single center studies. To address these limitations, we have created the Fontan Outcomes Registry using CMR Examinations (FORCE) platform. We will describe our journey during its development and our goal to become a CMR imaging biomarker research platform to accelerate our advancement and care for patients with single ventricle heart disease.

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Random Forest Analysis Identifies Important Clinical and Imaging Predictors of Impaired Neurocognitive Development in Children with CHD

Rafael Ceschin*[1], Dean B. Andropoulos[2], Ashok Panigrahy[1]

[1] University of Pittsburgh School of Medicine, Department of Radiology
[2] Texas Children's Hospital, Department of Anesthesiology

A majority of term born infants with congenital heart disease (CHD) are now surviving to adulthood due to effective medical and surgical management;1 however, they are more likely to present with neurocognitive deficits commonly associated with preterm birth2 and show delayed cortical maturation in utero3. These deficits persist through adolescent development4,5. With improvements to surgical intervention and palliative care, there are now more adults living with CHD than ever before – a population growing an estimated 5% per year.6 While the causal mechanisms of these neurocognitive and neurobehavioral defects are not fully understood, it is imperative to identify clinical and imaging risk factors to enable early intervention. Here, we implement Random Forest to predict longitudinal neurocognitive outcomes in neonates and adolescents with CHD. Random Forest allows for the interpretability of feature importance directly via the Gini score as a measure of separable features which most contribute to the output prediction. This has the direct benefit of providing potential mechanistic insight into aberrant brain development in this population.

We trained random forest algorithms to predict developmental delay using the 1-, 3-, and 5-year Bayley's composite scores. A separate RF model was created for each outcome measure. We included patient clinical variables, perioperative clinical measures, structural white matter (DTI), and structural volumes (volumetric MRI) as predictors. Structural white matter at pre- and first post-op timepoints was measured using along-tract diffusivity measures of 13 inter-hemispheric and cortico-association fibers. Structural volumes were measured using an automated segmentation pipeline using the Albert neonatal template, resulting in 50 total segmented structures. Volumes were corrected for PMA using a ridge regression (alpha=0.4) including both age and scan timepoint as regressors. In total, 265 predictors were included in the model. The 100 subjects were split into non-overlapping train (n=70)/test (n=30) sets by random stratified sampling to ensure a similar proportion of normal/abnormal cases in each dataset. We estimated the best parameters for the random forest algorithm via a randomized grid search of parameters using 2-fold cross-validation on the training set only. The best parameters were then used to fit the model on the full training set and validated on the test set. Algorithm performance was measured using Precision, Recall, and Area Under the ROC Curve (AUROC). Variable importance was measured by the average Gini-impurity of each feature across all decision trees in which that feature is present in the model.

In the neonatal cohort, the best model performance was achieved on the Bayley's 1 year social composite score with an AUC of 0.79 +/- 0.15. Of the top 5 important features, 4 were intra-operative clinical features (circulatory arrest time, opiate time, low temp intra-op, and epinephrine dose post-op), with the remaining feature being intra-ventricular CSF volume. Future work is currently under way to include importance socio-demographic factor (including maternal IQ) and also replicating this methodology in older subjects include two datasets of adolescents with CHD and Single Ventricle HLHS Disease (SVRIII Brain Connectome) to identify more granular sets of predictive features on multiple domains of neurocognitive and neurobehavioral outcomes.

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Blood barrier permeability correlates with cognitive function in Fontan patients

John Wood*[1], Sharon O'Neil[1], Silvie Siriany[1], Clio Gonzalez-Zacarias[2], Jian Shen[2], Botian Xu[2], Jon Detterich[1], Bradley Peterson[1], Ashok Panigrahy[3]

[1] Children's Hospital Los Angeles
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[3] Children's Hospital Pittsburgh

Introduction - Survivors of the Fontan palliation for single ventricular heart disease suffer from neurocognitive dysfunction. The etiology is likely multifactorial and includes genetic predisposition, ischemic insults during surgical and catheter palliations, as well as persistent circulatory insufficiency. Fontan physiology mimics right heart failure with resultant high central venous pressures and low cardiac output. Cyanosis impairs oxygen delivery, and the resultant polycythemia increases blood viscosity. We postulated that abnormal blood brain barrier (BBB) function might represent a logical mediator linking abnormal cardiovascular dynamics to neurocognitive dysfunction.
Methods - To test this hypothesis, we evaluated neurocognitive function, brain blood flow and blood brain barrier permeability in a cohort of 27 Fontan subjects (age 20.7 ± 2.7 years, 16 male) and 17 control subjects (22.9 ± 4.9, 9 male). All subjects provided informed consent under an IRB approved protocol. MRI was performed on a Philips 3T Achieva using a 32 element head coil and a digital receiver chain. Total brain blood flow was measured using phase contrast assessment of carotid and vertebral artery flow and normalized to brain weight nomograms based on age and sex. The permeability surface area product of water was assessed using the WEPCAST arterial spin labelling technique. This technique uses a four second labelling duration and three second labelling delay to ensure a stable passage of labelled water signal into the sagittal sinus. Labelled spins were normalized to a proton density image to calculate the fractional water extraction and resultant permeability surface (PS) area product. Neurocognitive testing was performed by a licensed neuropsychologist and investigator (SO) in a soundproof room. The screening neurocognitive exam consisted of subtests derived from the NIH Toolbox, Weschler Adult Intelligence Scale, Beery Visual-Motor Integration, Weschler Abbreviated Scale of Intelligence, Delis Kaplin Executive Function System, Perdue Pegboard, and the Conners Continuous Performance test. Total testing time was approximately two hours.
Results - Cerebral blood flow was lower in Fontan patients (40.7 ± 8.5 ml/100g/min) compared with control subjects (49.4 ± 10.5 ml/100g/min, p = 0.005). PS was not different on univariate analysis (137.7 ± 24.1 vs 131.7 ± 15.8, p=0.36). In Fontan patients, PS was positively associated with cerebral blood flow and diastolic pressure, with a combined r2 of 0.58, but not age or sex. PS was correlated with matrix reasoning in Fontan patients (r2 = 0.50, p=0.0001), but not control subjects (r2=0.004,p=0.84). PS was not correlated with the other neurocognitive metrics.
Discussion - BBB water transport is tightly regulated through aquaporin channels. In some neurodegenerative diseases, dysregulation of water transport precedes BBB disruption to large molecules such as gadolinium. The WEPCAST arterial spin labeling technique is a contrast-free method for quantifying BBB water permeability, making it potentially an attractive marker of neurocognitive risk. In Fontan patients, PS measured by WEPCAST was associated with logical predictors of cerebral perfusion and highly correlated with a specific neurocognitive task. This observation will be validated in a larger cohort and coupled with regional assessments of vascular perfusion, brain morphometry, and functional connectivity to probe for mechanistic insights.

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Endothelial dependent vascular function is decreased in patients with Fontan circulation and is associated with worse Fontan hemodynamics

Jon Detterich*[1], Julian Cameron[1], Silvie Suriany[1], Scott Leopold[2], Honglei Liu[1], Christopher Denton[1], Sarah Badran[3], Neil Patel[1], Thomas D. Coates[1], John C. Wood[1]

[1] Children's Hospital Los Angeles
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[3] Helen Devos Children's Hospital

Introduction - Fontan palliation results in passive blood flow to the lungs, decreasing shear stress on the pulmonary endothelium that results in endothelial dysfunction and vascular remodeling. Despite pulsatile blood flow in the systemic circulation, systemic endothelial dysfunction is also present. Prior work has demonstrated that endothelial dysfunction in the systemic vascular bed is predictive of severity of disease and cardiac output in pediatric idiopathic pulmonary hypertension, but the relationship between systemic and pulmonary endothelial function has not been explored in the Fontan population. Endothelial control of vascular tone is critical to the regulation of blood flow and pressure but the degree of dysfunction and its relationship to cardiac output in the Fontan circulation is also unknown. We aimed to determine the magnitude of macro- and microvascular endothelial dysfunction as well as the impact of endothelial dysfunction on Fontan hemodynamics.
Methods - We enrolled patients presenting to our heart institute clinic with Fontan circulation. We collected data from surveillance cardiac catheterization done within 1-2 months of vascular testing. The protocol was reviewed and approved by the Children's Hospital Los Angeles institutional review board. Endothelial function (FMD) was measured using a forearm cuff occlusion method with ultrasound visualization of the brachial arterial within 2 to 5cm of the antecubital fossa. Real time video screen grab was used to monitor baseline and post-occlusion dilation. The brachial artery measurement was made proximal to the cuff to avoid ischemia of the brachial artery. Laser doppler flowmetry was used to simultaneously assess post-occlusive microcirculatory hyperemia (PORH) in the skin as a response to tissue ischemia.
Results - We enrolled 29 Fontan (48% female, 13.3±4.6 years) and 15 healthy controls (73% female, 11.8±3.9 years); there was no difference in age but the healthy group was predominantly female. FMD, which is dependent on shear-mediated nitric oxide release, is decreased in 1V compared to healthy participants (p=0.0035). Microcirculatory resting flow was not different between the 2 groups, however, PORH trended lower in 1V vs. healthy (p=0.0765). There was no association between endothelial function and microcirculatory function in healthy participants (R=+0.01, p=0.79) but there was a trend toward PORH association with FMD (R=+0.2, p=0.086). Improved FMD in the 1V group is associated with lower IVC pressure (R=-0.65, p=0.03) and lower pulmonary artery wedge pressure (R=-0.59, p=0.027) on cardiac catheterization. Improved FMD was also associated with improved pulmonary (R=+0.68, p=0.03) and systemic blood flow (R=+0.87, p=0.001) post inhaled NO. Similarly, higher PORH was associated with lower IVC pressure (R=-0.84, p=0.009) and lower pulmonary artery wedge pressure (R=-0.63, p=0.03) as well as improved pulmonary (R=+0.88, p=0.01) and systemic (R=+0.87, p=0.01) blood flow.
Conclusion - Conduit artery and microvascular dysfunction, FMD and PORH respectively, are prevalent in patients with Fontan circulation. Improved endothelial function in conduit arteries and microcirculation is associated with improved Fontan hemodynamics, including higher systemic and pulmonary blood flow, and lower central venous and pulmonary artery wedge pressure. These non-invasive biomarkers of vascular function may act as surrogates for hemodynamic changes in the Fontan population and provide insight into mechanisms of cardiovascular dysregulation.

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Material properties are important for patch sizing in aortic arch reconstruction for single ventricle patients

Shannen B. Kizilski*[1], Martha D. Chaillo Lizarraga[1], Nicholas E. Kneier[1], Emily R. Eickhoff[1], Noah E. Schulz[1], Peter E. Hammer[1], David M. Hoganson[1]

[1] Boston Children's Hospital

Patch augmentation of the hypoplastic aortic arch is often a challenging part of Stage 1 single ventricle palliation. During this procedure, a biologic patch is manually shaped and sewn into the open aorta to increase its diameter to a normal size with smooth tapering to the unpatched sections. This procedure, however, is conducted when the aorta is unpressurized, so the reconstructed diameter at physiologic blood pressures is unknown until blood flow is restored after the procedure. Depending on stiffness of the patch material relative to the highly compliant aorta, the reconstructed segment might be significantly undersized or oversized compared to the surrounding vessel when pressurized. Diameter mismatch in the aorta is associated with abnormal flow and early ventricular failure. Prospective patch design to achieve the correct pressurized dimensions is achievable through computational modeling with accurate estimates of the mechanical properties of the vessel and patch. We have been developing this patch planning workflow through extensive tissue characterization and application of fundamental mechanical principles. Our growing database of aortic and biologic patch mechanical properties enables estimation of the zero-pressure configuration of patient-specific hypoplastic aortas. With this information, and with a target pressurized diameter for the reconstructed vessel, we calculate dimensions of the patch to be sewn in at the unpressurized state. Calculated patch sizes for pulmonary homograft versus pericardium are compared to dimensions obtained through the current technique of matching the unpressurized vessel diameter. Our results demonstrate the importance of considering both aortic and patch properties for proper patch sizing.

Cures I: Bionic and Regenerative Approaches

Friday, October 7 at 3:45-5:15 PM
Moderated by Nicole Dubois, PhD | Icahn School of Medicine at Mount Sinai

Scaling 3D Bioprinting: From the Petri Dish to a Human-Scale Biopump

Plenary: Mark Skylar-Scott, PhD | Stanford University

The convergence of human induced pluripotent stem cell and 3D bioprinting technologies are enabling rapid advancement in organ-scale biomanufacturing. For patients with single ventricle physiology, the availability of therapeutic quantities of contractile autologous tissue could provide a curative solution to overcome Fontan failure. In this talk, we highlight a few recent advances in high throughput bioprinting and the scaling of cell manufacturing, and we provide a roadmap ahead for assembling functional and vascularized cardiac tissues at human scale.

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Human Embryoid Body Bioinks for FRESH 3D Bioprinting of Contractile Cardiac Tissue

Brian Coffin*[1], Adam Feinberg[1]

[1] Carnegie Mellon University

End-stage heart failure affects more than 64 million persons worldwide, with the vast majority ineligible for transplant due to lack of donor supply, comorbidities, or other risk factors. While whole organ biofabrication has the potential to bridge this gap, current tissue engineering approaches cannot build 3D cardiac muscle with sufficient cell density, cell volume and precise anatomical structure to repair or replace the heart. As a step towards addressing this challenge, we have developed a human embryoid body (EB) based bioink that supports cell proliferation, EB fusion, and subsequent differentiation into cardiomyocytes and other cell types to form contractile 3D cardiac tissues. To create the EB bioink, human embryonic stem cell derived EBs of controlled dimensions were combined with a high concentration fibrinogen solution. We then the used Freeform Reversible Embedding of Suspended Hydrogels (FRESH) 3D bioprinting to fabricate ring, tube, and ventricle shaped cardiac constructs. Thrombin added to the FRESH support bath was used to rapidly gel the fibrinogen in the EB bioink into fibrin to maintain the printed structure. The constructs were cultured for a period of 2 days to allow the EBs to grow and fuse, after which they were differentiated into cardiomyocytes in chemically defined media with CHIR and WNT-C59. Electrophysiology and contractility were examined during spontaneous contraction and under paced field stimulation, showing successful cardiomyocyte differentiation, regions of synchronized contraction, and calcium transients with electrical pacing. Finally, scaffolds were imaged by confocal microscopy for residual structural fibrin, cell generated matrix, cardiac troponin T and myofibril alignment. In total, these results demonstrate that FRESH 3D bioprinted cardiac tissues from EB bioinks followed by in situ, 3D differentiation is a viable biofabrication strategy. Future work will focus on identifying the optimal day of EB differentiation for forming and printing the bioink and the role of specific reagents on cell type specification and differentiation.

Towards Building a Contractile Conduit with FRESH 3D Bioprinting

Jacqueline Bliley*[1], Maria Stang[1], Annie Behre[1], Brian Coffin[1], Erica Comber[1], Dan Shiwarski[1], Adam Feinberg[1]

[1] Carnegie Mellon University

Though long-term outcomes for patients with single ventricle disease have dramatically improved, many suffer from complications associated with having a univentricular circulatory support. One potential solution to this problem is building a contractile conduit that is able to assist with the pumping of the single ventricle and reduce systemic venous pressures (Biermann et al. Plos One, 2016). The ideal contractile conduit should produce unidirectional pumping of fluid in order to reduce systemic venous pressures and other Fontan circulation-associated complications. Freeform reversible embedding of suspended hydrogels (FRESH) 3D bioprinting is one potential approach to build a contractile conduit where we have previously demonstrated the precise printing of heart muscle structures, including cardiomyocytes and ventricular chambers made out of type I collagen (Lee, Hudson et al., Science, 2019). Here, we engineer a proof of concept contractile conduit composed of (i) collagen and (ii) embryonic stem cell derived cardiomyocytes and primary fibroblasts. Bioprinted conduits were fabricated first on a small scale (1.4 mm I.D x 13.5 mm length) to validate design requirements with lower cell and material usage. Contractile conduits displayed isotropically aligned cardiomyocytes on the tissue surface that began synchronized contraction ~2-4 days following fabrication. Linear action potential propagation of ~5 cm/s was observed across the tissue surface, which is below the level observed in the adult human heart but on par with measurements obtained in engineered heart muscle (Bliley et al. Science Translational Medicine, 2021) and embryonic heart tissue (Gu et al., Biomedical Optics Express, 2015). Contractile conduits were able to displace fluorescent beads with tissue contraction; however, due to the absence of incorporated valve structures, only a minimal unidirectional pumping through the lumen at 400 μm/s was observed. In an effort to improve unidirectional pumping, bileaflet heart valves were bioprinted and incorporated at either end of the conduit. Finite element modeling was utilized to optimize valve leaflet design to allow for higher leaflet opening, as well as approximation

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of valve leaflets during tissue relaxation. Successful printing of heart valve leaflet structures was verified with Optical Coherence Tomography. Valve leaflets could also be cyclically opened and closed via increased pressure within the tube lumen with minimal regurgitation based on fluorescent bead tracking. In conclusion, we demonstrate the creation of a small, proof-of-concept contractile conduit, which in the future could potentially be utilized as an accessory tissue pump for patients with single ventricle disease.

Analysis of Injection-Jet Configurations for a Self-Powered Fontan Circulation

Ray Prather*[1], Arka Das[2], Michael Farias[1], Eduardo Divo[2], Alain Kassab[3], William DeCampli[1]

[1] The Heart Center, Arnold Palmer Hospital for Children
[2] Embry-Riddle Aeronautical University, Department of Mechanical Engineering
[3] University of Central Florida, Department of Mechanical and Aerospace Engineering

Introduction - The Fontan circulation is a fragile system in which imperfections at any one of multiple levels may compromise quality of life, produce secondary pathophysiology, and shorten life span. One mode of Fontan failure and the main target of this study is increased inferior vena caval (IVC) pressure. We propose to augment the passive Fontan circulation with an injection jet shunt (IJS) drawing flow from the aortic arch, balanced by a fenestration the cost of a small increase in cardiac load. The IJS working principle involves momentum transfer from high-velocity jet to a low-velocity co-flow by means of entrainment. The combination of entrainment with the fenestration leads to a significant reduction of upstream (i.e., IVC) pressure. We describe a tightly coupled multi-scale lumped parameter/computational fluids dynamics (LPM-CFD) model to further investigate this paradigm.
Methods - A synthetic 3D CAD model of the fenestrated total cavopulmonary connection (TCPC) was generated (including pulmonararteries, IVC and SVC), with average dimensions matching those of a 2-4 yo patient (BSA 0.675m^2) and a cardiac output of about 2.3L/min. The detailed 3D pulsatile hemodynamics in the fluid domain are modeled as unsteady, turbulent and incompressible (constant density). Blood is assumed to be non-Newtonian. Turbulence is approximated using a large eddy simulation (LES) approach, well suited for complex flow interactions allowing for two-way laminar-to-turbulent transitions encountered in the pulsatile confined co-flowing jet. Further parametric exploration of several geometric design parameters such as TCPC morphology (2Y=double Y graft, skew and torus configurations), shunt diameter, shape and location, and fenestration diameter and location. The effects of the IJS-fenestration implementation on IVC pressure and systemic oxygen saturation were calculated.
Results and Discussion - A set of baseline simulations representing a failing Fontan has an elevated IVC pressure (+17.8mmHg), following a sequential enlargement of the fenestration to 7.5 mm leads to a 5.0mmHg IVC pressure drop but also significant reduction in systemic oxygen saturation (<85%). Addition of a 2mm IJS with an elliptical nozzle to this model preserves the IVC pressure drop between 3.5mmHg-4.2mmHg while improving systemic oxygen saturation (>85%) with only a small additional volume load to the ventricle (CO/Qs = 1.2). Offset of the SVC anastomosis along the left pulmonary artery by as much as 100% of the SVC diameter further enhances IVC pressure drop and systemic saturations up to 88%.
Conclusions - The data for the ongoing analysis illustrate the potential salutary effect of the proposed IJS-fenestration configuration on the Fontan circulation. Further optimization of these configurations is ongoing. In-vitro simulations are currently being generated to cross-validate the optimal outcome from the in-silico model.

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Development of a fetal valve prototype designed for implantation in utero

Sanchita S. Bhat*[1], Hieu T. Bui[1], Anna Farnan[1], Christopher K. Breuer[2], Aimee K. Armstrong[2], Lakshmi Prasad Dasi[1]

[1] Georgia Institute of Technology
[2] Nationwide Children's Hospital

Introduction - Single ventricle physiology (SVP) treatment consists of interventions that are palliative and not curative. In the fetal population, balloon valvuloplasty is a common procedure to open stenotic valves in utero and restore biventricular anatomy. Recently, prenatal intervention rates to prevent SVP have improved, but even patients who avoid SVP require or eventual valve replacement, limited by poor durability and somatic outgrowth. To combat this issue, tissue engineering has become an emerging technology to develop scaffold like structures, where the scaffold allows for neotissue growth overtime leaving the patient's own cells in place of an implant. The goal here is to expand these techniques to develop a scaffold that will allow for "neovalve" formation. Thus, this project aims to develop a fully biodegradable tissue-engineered heart valve (TEHV) that will normalize the fetal hemodynamics to prevent SVP and allow the valve to repair, remodel and grow with the patient.
Methods - We manufactured valve prototypes as follows - cobalt chromium metal stent with polycaprolactone (PCL) leaflets. The stents were cut using a femtosecond laser and two leaflet attachment techniques were incorporated. PCL was attached to the stent using a sutureless spray-on technique since we have observed gradual commissural failure in sutured valves in the past. We tested these prototype in our fetal pulse duplicator using physiological conditions of 140bpm and 70/45 right ventricle-pulmonary artery pressures using a 60/40 water/glycerin mixture. We also conducted preliminary animal implants to establish the deployment system for the prototypes.
Results - Our Co-Cr with PCL prototype fetal valve was tested for 100 consecutive cardiac cycles and had a regurgitant fraction of 3.9% with an effective orifice area of 0.18 cm2 and a mean transvalvular pressure gradient of 3.84 mmHg. We had moderate success with the valves in animal models with minimal paravalvular leakage and mild to no stenosis as measured from echocardiography. There was trivial flow acceleration through the stent with peak velocity ~1.6 m/s. We are experimenting with other leaflet material and stent designs to generate more data for presentation. We are also developing a degradation protocol to characterize leaflet degradation in a physiological setting. This project is the first step to design to develop and manufacture a fully biodegradable tissue-engineered valve for use in utero. The current drawback in the lack of interventional techniques for congenital anomalies can be overcome with the progress of such a replacement device.

Care II: Personalized Medicine Approaches and Alternative Interventions

Saturday, October 8 at 8:00-9:00 AM
Moderated by Stephanie Nakano, MD | Children's Hospital Colorado

Growing and Evolving: Updates from ACTION on Ventricular Assist Device Therapy in the Fontan Circulation

Kathleen E. Simpson*[5], Chet Villa[1], Muhammad F. Shezad[1], Ari Cedars[2], Kurt Schumacher[3], Sharon Chen[4], David N. Rosenthal[4], Angela Lorts[1]

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Background - The initial experience with ventricular assist device (VAD) therapy in Fontan patients was positive. This study sought to investigate updated outcomes in an era of evolving anticoagulation practices and more widespread application of VAD therapy in Fontan patients.
Methods: We conducted a retrospective study of Fontan patients in the Advanced Cardiac Therapies Improving Outcomes Network (ACTION) implanted with a VAD between 01/2012 and 5/2022. Descriptive statistics are presented as count (percentage) and median (interquartile range) for categorical and continuous variables respectively.
Results - Eight-nine Fontan patients were implanted with a total of 101 devices. The median age was 10.8 years (4.7 – 17.5) with a median weight and BSA of 31.5 kg (15.3-61.9) and 1.11 m2 (0.64-1.65), respectively. Twenty-five (28%) were female. The majority of implants were implantable continuous flow (57 [56%]), including 21 Heartmate 3s. Eighteen patients (18%) were implanted with a Berlin Heart EXCOR. The majority of patients were implanted as a bridge to transplantation (60 [67%]). The remaining implants were placed as bridge to candidacy (24 [27%]), bridge to recovery (3 [3%]), and chronic therapy (2 [2%]). Most patients were INTERMACS profile 2 (48 [54%]) or profile 1 (25 [28%]) at the time of implant. Profile 3 (8 [9%]) and profile 4-6 (7 [8%]) were less common. Twenty (23%) were supported on ECMO at time of implant. The median duration of support was 82 days (15-170). Sixty eight patients (75%) had a positive outcome at time of last follow-up: 57 (64%) were transplanted, 9 (9%) were alive on device, and 2 (2%) were weaned from VAD support. Sixteen patients (18%) suffered a stroke (8 ischemic, 2 ischemic + hemorrhagic, and 6 hemorrhagic). Forty seven patients (32%) experienced major bleeding. Forty-two patients (47%) were anticoagulated with bivalirudin at some point during VAD support.
Conclusions - VAD remains an effective approach to support patients with Fontan circulation with cardiac failure in an era of evolving anticoagulation practices and device options. Hemocompatibility related complications remain an important source of morbidity and mortality and should be a focus of quality improvement projects.

Outcomes After Initial Heart Failure Consultation in Fontan Patients

Sharon Chen*[1], Muhammad F. Shezad[2], Angela Lorts[3], Kurt Schumacher[4]; on behalf of the Advanced Cardiac Therapies Improving Outcomes Network (ACTION)

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Purpose - Many individuals with a Fontan palliation require advanced heart failure (HF) care during their lifetime. There are no standardized guidelines dictating the threshold for referral to a formal HF or heart transplant (HTx) service. Delays in referral can result in irreversible end-organ damage or clinical instability such that transplant or ventricular assist device (VAD) implant is too risky. The specific aims of this study were to characterize Fontan patients referred for HF care, describe outcomes after initial HF consultation, and identify risk factors for poor outcome after HF consultation. The overall goal is to improve timely referrals for HF care, which in turn may lead to better VAD and transplant outcomes in Fontan patients.
Methods - Centers participating in the Advanced Cardiac Therapies Improving Outcomes Network (ACTION) were invited to complete a survey within 5 days of seeing a Fontan patient (any age) at the time of an initial consultation with a HF/HTx team. A follow-up survey was sent to the provider 30 days later to determine outcome after initial consultation. The primary outcome was "late referral", defined as death or declined for HTx and/or VAD due to too sick; a secondary outcome of "care escalation" was defined as VAD implant, inotrope initiation or HTx listing. Characteristics at time of initial consultation were examined.
Results - From 7/2020 to 10/2021, 13 ACTION centers contributed data on 70 Fontan patients seen for an initial HF/HTx consultation. At the time of consultation, median age was 13.5 (7, 17) years, patients were 8 (2, 12) years from Fontan surgery, 49% had normal systolic function and 34% had protein-losing enteropathy. 54% were already hospitalized by the time of consult, and of those hospitalized, 34% were already on inotropic support. At 30 days, 11% were found to be late referrals (too sick for HTx/VAD=5, died=3), and 39% had care escalation (initiated inotrope = 6 and/or VAD implant = 4 and/or HTx

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list = 20). Conversely, at 30 days, 31% were seen with recommendation for continued co-management with the referring cardiologist, and 11% were seen with no further scheduled HF visits. Initial consult < 1 year post-Fontan was strongly associated with late referral (OR 8.8, 95% CI 1.6-47.7, p=0.012); there were no other significant distinguishing variable for those referred too late.
Conclusion - Over 10% of Fontan patients die or are declined for HTx, and almost 40% had care escalation, within 30 days of an initial HF consultation. This suggests that earlier HF referral should be considered, especially for those within 1 year of initial Fontan surgery.

Initial Validation of a Predictive Personalized Computational Model for Patients with Borderline Left Ventricles

Vijay Vedula*[1], Yurui Chen[1], Isao Anzai[2], Justin Tran[3], David Kalfa[2]

[1] Columbia University
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Background and Motivation - Borderline left ventricle (BLV) is a complex congenital heart disease in which neonates are born with an undersized left ventricle. The spectrum of this heterogeneous condition includes patients with concomitant mitral and aortic valve stenoses, aortic coarctation/arch hypoplasia, hypoplastic left heart complex, septal defects, and endocardial fibroelastosis. Decision-making between biventricular repair (BiVR) and single ventricle palliation procedure (SVPP) for BLV patients remains quite difficult and subjective. While BiVR is aimed at creating two functioning ventricles in a single surgery, SVPP involves multiple staged open-heart surgeries resulting in a single functional ventricle with high inter-stage mortality and poor long-term outcomes. On the other hand, patients undergoing BiVR may exhibit reduced heart function post-surgery and may have to be subsequently switched to SVPP. We aim to create a personalized computational model that may assist decision-making in these critical patients.
Methods - We developed a closed-loop lumped parameter network (LPN) model of the circulatory system for BLV patients. The model accounts for potential septal defects, arch hypoplasia, and valvular stenoses. We developed an automatic tuning framework that personalizes the LPN model parameters to match patient characteristics and available echocardiographic and cath data. The preoperative LPN circuit layout was then modified to virtually perform BiVR and Norwood procedures for each patient. For instance, an aortic valve repair and arch repair were performed virtually by lowering the hemodynamic resistance to the 4 th power of the change in the diameter. The virtual Norwood included the addition of a resistive element mimicking the surgical implantation of a 3.5mm BT-shunt. Computer simulations were performed on 10 BLV patients, retrospectively selected, for each surgical procedure to compare the hemodynamic outcomes and assess if the clinically performed procedure was the optimal choice.
Results - The preoperative LPN models were tuned to match the patients' hemodynamic data derived from cath and echocardiographic measurements within the standard deviation. In the patient subset who clinically underwent Norwood, performing a virtual BiVR resulted in increased mean pulmonary artery pressure (mPAP, 32±4.7 vs. 16±3.4 mmHg), mean left atrial pressure (mLAP, 18±1.2 vs. 5±1.0 mmHg), and LV end-diastolic pressure (LVEDP, 16±3 vs. 5±2 mmHg). On the other hand, performing a virtual Norwood on the patient subset who underwent BiVR led to nominal changes in pressures but a high pulmonary to systemic flow ratio (Q p /Q s) due to abnormally high systemic vascular resistance (SVR). All patients in this analysis were still alive at the last follow-up, except for one patient who expired at 8 months of age due to pulmonary vein atresia and respiratory failure.
Discussion - We developed a predictive computer model that enables surgeons to perform virtual surgeries and predict hemodynamic quantities for BLV patients using clinical, echocardiographic, and cath data as input. On a small initial cohort, our model predictions corroborate the validity of the clinical performed procedure. In particular, the model correctly identifies candidates unsuitable for BiVR by showing high PAP, LAP, and LVEDP. The results need to be validated on a larger and prospective cohort.

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Ambulatory Monitoring of Fontan Pressures Using a Novel Implantable Sensor

Martin Bocks*[1], Nader Najafi[2]

[1] UH Rainbow Babies & Children's
[2] Integrated Sensing Systems

Congenital heart disease patients with functional single ventricle (FSV) anatomy ultimately require a Fontan operation for long term survival. The goal of the Fontan operation is to re-route systemic venous blood to the pulmonary circulation without passing through an intervening ventricular chamber. As a result, blood flow to the lungs is a passive, non-pulsatile process. Although morbidity and mortality associated with the Fontan procedure has improved considerably over the last two decades, there are still many patients who develop complications and eventual Fontan failure for reasons we do not yet entirely understand. The pressure in the Fontan pathway is arguably the single measurement that most closely predicts the overall health of the palliated circulation. This measurement reflects the general condition of all the cardiac and vascular structures that lie between the proximal branch pulmonary arteries and the systemic single ventricle. Unfortunately, Fontan pressure measurements obtained in the catheterization laboratory are variably, and often erroneously, influenced by elements involved in performing the procedure itself. The unique physiology of the Fontan allows outside factors to have a more significant influence on the pressure measurements compared to patients with biventricular anatomy and physiology. Furthermore, the invasive cath lab measurement provides only a snapshot of what is occurring within the unique circulation and does not represent what is taking place during normal activities of daily living or times of exertion. The ability to measure chronic, serial Fontan pathway pressures in an ambulatory setting will result in a better understanding of the Fontan physiology and should ultimately improve morbidity and mortality associated with this high risk patient population. We believe commercial development of such a device would represent a significant technological advancement in providing care to this high risk patient population. Herein, we report on the development of a novel implantable, wireless pressure sensor that is being designed for surgical placement in the central Fontan pathway at the time of the Fontan operation. We have demonstrated the device to be safe for intracardiac implantation in a GLP preclinical study and results will be reviewed. We will also reveal data on MRI compatibility and long term functionality. Finally, the issue of sensor drift will be discussed as the final step needing to be addressed before seeking FDA approval for first-in-human Investigational Device Exemption study.

Etiology II: Mechanisms of Congenital Heart Disease

Saturday, October 8 at 2:00-3:45 PM
Moderated by Mingtao Zhao, DVM, PhD | Nationwide Children's Hospital

Congenital heart disease at single cell resolution

SVRF 2021 Top Scorer: James Martin, MD, PhD | Baylor College of Medicine

With current therapies, more than 90% of CHD patients survive into adulthood but often suffer from heart failure. We performed single nuclear RNA sequencing (snRNA-seq) and analyzed 157,273 nuclei from donors and CHD patients, including hypoplastic left heart syndrome (HLHS) and Tetralogy of Fallot (TOF). We observed CHD specific cell states in cardiomyocytes (CMs) and Cardiac fibroblasts (CFs). Moreover, Imaging Mass Cytometry (IMC) uncovered the spatially resolved perivascular microenvironment to validate the transcriptomics data. Peripheral immune cell profiling revealed defective monocytic immunity in CHD and may be useful for both diagnostic and prognostic purposes. Our comprehensive CHD phenotyping provides a first effort to develop pipelines and datasets for personalized medicine in CHD.

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Identification of pathogenic genomic structural variation in single ventricle congenital heart disease by short read whole genome sequencing

Daniel Quiat*[1, 2], Xuefang Zhao[3, 4], Sarah U Morton[1, 2], Alexandre Pereira[2], Sophie McAllister[2], Steven R DePalma[2], the Pediatric Cardiac Genomics Consortium, Michael E Talkowski[3, 4], Harrison Brand[3, 4], Jonathan G Seidman[2], Christine E Seidman[2, 5, 6]

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[2] Harvard Medical School
[3] Massachusetts General Hospital
[4] Broad Institute of Harvard and MIT
[5] Brigham and Women's Hospital
[6] Howard Hughes Medical Institute

Genomic analyses identify aneuploidy, copy-number variants (CNVs), and damaging single nucleotide variants as an underlying genetic cause in ~45% of congenital heart disease (CHD) cases but fail to detect a causal variant in many cases despite clinical evidence supporting a genetic etiology. Recent advances in high-throughput sequencing technology and computational approaches have identified genomic structural variants (SVs) as an important class of pathological genomic variation that has been largely excluded from prior analyses. Here, we perform comprehensive SV genotyping in over 1,800 previously unsolved CHD cases with trio (proband-parent) short read whole genome sequencing (srWGS) enrolled in the Pediatric Cardiac Genomics Consortium, including 318 probands with single ventricle CHD (hypoplastic left heart syndrome, n=221; double inlet left ventricle, n=43; tricuspid atresia, n=56). The number of rare SVs resulting in loss-of-function (LoF) of protein coding genes per genome were similar in CHD cases and controls (median 4, p=0.84). Furthermore, the de novo SV (dnSV) mutation rate did not differ between cases and controls (0.12 vs 0.12 per genome, p=0.56), however dnSVs in CHD probands trended towards altering genes more intolerant to LoF (median gnomAD LoF observed/expected upper bound fraction or LOEUF, 0.12 CHD vs 0.53 controls, p=0.06). Overall, ~2% of CHD probands, including those with single ventricle CHD, harbored a LoF SV affecting a known CHD gene, with 30% of these SVs measuring less than 100 kilobases in size. Using matched srWGS and bulk RNA sequencing of discarded surgical tissue from 238 CHD probands, we confirmed the effects of a subset of rare LoF SVs on gene dosage and found that the magnitude of transcriptional effects on genes within LoF and copy-gain SVs related to SV allele frequency. These results suggest that LoF SV identified by srWGS are a rare genetic cause of single ventricle CHD and highlight the need for further investigation of additional genetic mechanisms that contribute to the missing heritability in single ventricle CHD.

Multi-chamber cardioids unravel human heart development and defects

Alison Deyett*[1], Clara Schmidt[1], Sasha Mendjan[1]

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The number one cause of fetal death are defects in heart development. Determining the underlying causes faces many challenges, including the complexity and inaccessibility of the embryonic heart, the unclear impact of drugs and environmental factors during pregnancy, and the lack of in vitro models representing all the compartments of the human heart. Here, we established a cardioid organoid platform recapitulating the development of the major compartments of the human embryonic heart, including the right and left ventricles, the atria, the outflow tract, and the atrioventricular canal. These cardioids have the compartment-specific in vivo-like gene expression profile, morphology, and functionality. We use this platform to unravel the developmental ontogeny of signal propagation between interacting heart chambers and dissect how genetic and environmental factors cause specific defects in different regions of the developing human heart.

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A CRISPR-Activation CROP-seq Screen to Identify Dosage Sensitive Genes in Congenital Heart Disease

Sanjeev Ranade*[1], Sean Whalen[2], Angelo Pelonero[1], Lin Ye[1], Rahul Mital[1], Langley Grace Wallace[1], Casey Gifford[3], Katherine Pollard[1], Deepak Srivastava[1]

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[2] Gladstone Institute of Data Science and Biotechnology
[3] Stanford University Medical School

Congenital heart defects can arise due to aberrant dosage of genes essential for heart development. Down Syndrome, or Trisomy 21, affects numerous physiological organ systems in development and is caused by an extra copy of chromosome 21. Between 40-60% of children with Down Syndrome are born with congenital heart defects, with a high prevalence of septal defects. Children with Down Syndrome are 1000-fold more susceptible than the general population to have complete atrioventricular septal defects (AVSD). We hypothesize that the presence of an extra copy of specific gene(s) on chromosome 21 disrupts transcriptional specification within subpopulations of cells relevant to atrioventricular canal (AVC) formation. A critical challenge in identifying dosage sensitive chromosome 21 genes is the ability to modulate gene expression at physiologically relevant levels within the appropriate cells. To address this, we first performed single cell transcriptomics (scRNA-seq) on human induced pluripotent stem cells (hIPSCs) derived from a mosaic DS patient wherein we compared trisomic and isogenic control cells in an in vitro cardiomyocyte differentiation model (hIPSC-CM). We identified a subpopulation of myocardial cells with a signature of atrioventricular myocardium that displayed dysregulation in genes essential for AVC myocardial specification. Having established a relevant in vitro model system, we then engineered a CRISPR-Activation allele (dCas9-VPR) into the disomic isogenic control cells through homologous recombination to the CLYBL safe harbor locus and confirmed functionality at hIPSC stage through cardiomyocyte differentiation. Using a filtering strategy based on previously published mouse models and our own scRNA-seq atlas of the embryonic mouse heart, we identified 66 genes on chromosome 21 that may contribute to heart development in a dosage sensitive manner. We designed guide RNAs (sgRNAs) for these 66 genes and generated a pooled lentivirus based CRISPR droplet sequencing (CROP-seq) library to increase expression of target genes and provide a single cell RNA-seq based readout. We then developed a machine learning algorithm to predict a minimal set of features reflective of trisomic or disomic states and identified a narrow set of candidate dosage sensitive chromosome 21 genes that shifted the transcriptional state of disomic cells to a more trisomic-like state. These results highlight the power of CRISPR based scRNA-seq screens to identify dosage sensitive genes relevant to congenital heart defects and multigenic disorders such as single ventricle disease.

Myocardial-intrinsic defects underlie an Rbfox-mediated zebrafish model of hypoplastic left heart syndrome

Mengmeng Huang*[1, 2], Alexander Akerberg[1, 2], Xiaoran Zhang[1, 2], Haejin Yoon[2], Shakchhi Joshi[2]; Celia Harding[1, 2], Christopher Nguyen[1, 3], William T Pu[1, 2, 4], Marcia C Haigis[2], C Geoffrey Burns[1, 2], Caroline E Burns[1, 2, 4]

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Hypoplastic left heart syndrome (HLHS) is characterized by underdevelopment of left sided structures including the ventricle, valves, and aorta. Although the mechanisms of disease pathogenesis remain elusive due to a paucity of candidate genes and animal models, prevailing paradigm suggests that HLHS is a multigenic disease of co-occurring phenotypes. Here, we report that zebrafish lacking two orthologs of the RNA binding protein RBFOX2, a gene previously linked to HLHS in humans, display cardiovascular defects overlapping those in HLHS patients. In contrast to current paradigm, we demonstrate that co-ex-

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isting ventricular, valve, and aortic deficiencies in rbfox mutant zebrafish arise secondary to impaired myocardial function as all three phenotypes are rescued when Rbfox is expressed specifically in the myocardium. On a molecular and cellular level, we find diminished expression and alternative splicing of sarcomere and mitochondrial components in rbfox-deficient hearts that compromise sarcomere assembly and mitochondrial respiration, respectively. Injection of human RBFOX2 mRNA restores ventricular structure and function in rbfox mutant zebrafish, while HLHS-linked RBFOX2 variants fail to rescue. Taken together, our data suggest that mutations in RBFOX2 are causal for HLHS pathogenesis and provide a complimentary paradigm for HLHS emergence where co-existing ventricular, valve, and aortic deficiencies have a monogenic etiology caused by myocardial dysfunction.

Evaluation of endocardial HAND2 gene regulatory networks that lead to tricuspid atresia

Anthony B Firulli*[1], Rajani M George[1], Beth A Firulli[1], Ram Podicheti[2], Douglas B Rusch[2], Len Pennachio[3], Marco Osterwalder[4]

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[4]Department for BioMedical Research, University of Bern, Switzerland

Congenital heart defects represent nearly 1% of all births and those that result in a single functional ventricle (hypoplastic left heart syndrome, HLHS; Tricuspid Atresia, TA; or double inlet left ventricle, DILV) are amongst the most severe threats to survival. The bHLH transcription factor HAND2 lies within the NOTCH signaling pathway functioning within the developing endocardium. HAND2 endocardial loss-of-function is causative of TA or DILV in addition to severe septum, trabeculation, and vascular abnormalities. These findings reveal the critical nature of the cell-cell communications between endocardium and myocardium that that coordinate ventricle morphogenesis. To gain an understanding of the molecular mechanisms downstream of HAND2 within the endocardium, we performed single cell RNA-Seq from E11.5 control (Hand2f/f) and HAND2 endocardium loss of function mutants (NfactC1IresCRE;Hand2f/f, H2CKO). Bioinformatic analysis of the datasets reveal a number of canonical pathways that are disrupted including wound healing, cardiac hypertrophy, and Apelin Endothelial signaling. The Apelin pathway regulates endothelial cell polarity which is necessary for normal ventricular morphogenesis. Defects in Apelin signaling result in trabeculation defects and regulatory pathways that detect shear stress are compromised. Shear stress response is dependent on the actions of the transcription factor Krüpple-like factor 2 (KLF2). Our results show that Klf2 expression is specifically downregulated within the endocardium where Klf2 AV cushion and systemic vascular expression appears unaffected. Using HAND2 DNA-occupancy data, we identified a putative HAND2-dependent enhancer within the Klf2 upstream sequences. Using transgenic b-galactosidase reporter analysis to test this 1.7KB enhancer sequence we determined that this enhancer sequence is sufficient to drive all Klf2 endothelial/endocardial expression. Crossing this reporter mouse line onto the H2CKO background reveals a specific endocardial downregulation of b-galactosidase reporter activity while cushion and vascular expression is unaffected. Together, these data show that HAND2 functions within a number of important canonical endocardial pathways and that shear stress response, a necessary step in endothelial cell polarization, is compromised in H2CKO mice through direct regulation of Klf2, suggesting that a breakdown within the Apelin gene regulatory network can lead to single ventricle disease via an endothelial cell polarization etiology.

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Outcomes II: Non-Genetic Factors in Disease Development

Saturday, October 8 at 4:00-5:00 PM
Moderated by Stephanie Lindsey, PhD | University of California San Diego

Homeostasis & the Developing Vasculature in Single Ventricle Physiology

Jay Humphrey*[1], Linda Irons[1], Sae-Il Murtada[1], Abhay Ramachandra[1], Bruno Rego[1]

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Many palliative surgeries designed to treat single ventricle patients target associated segments of the vasculature, including introduction of a Blalock-Thomas-Taussig shunt, the Norwood procedure, a bidirectional Glenn, and Fontan completion. Appropriately, computational models of the complex hemodynamics continue to provide critical insight into both the pre- and post-surgical single ventricle circulation. There is, however, a pressing need to understand better how these many different vessels – systemic arteries, pulmonary arteries, and veins – respond to changing hemodynamics, first to the single ventricle circulation and second to the surgical correction. Such understanding can only be achieved against the backdrop of a better understanding of the processes by which these vessels normally develop from the perinatal period to maturity. In this talk, we will present initial experimental findings and novel computational results aimed toward understanding the developing vasculature from transcript to tissue. To increase throughput, we use mouse models and employ transcriptional profiling and biomechanical phenotyping to inform multiscale computational models that synthesize information from cell signaling, extracellular matrix turnover, and tissue level responses to the hemodynamics. This synthesis of data is achieved within a framework that is built upon the ubiquitous concept of homeostasis, which represents an inherently stable negative feedback system. Vascular maladaptations arise, in part, from compromised homeostasis, often resulting in insidious positive feedback loops. Initial results demonstrate the fundamental homeostatic role of cellular mechano-sensing and mechano-regulation of the extracellular matrix, which endows the different types of blood vessels with site-specific properties that normally contribute to circulatory efficiency. We submit that our multiscale computational models have promise to aid in understanding the single ventricle physiology and in surgical planning for its treatment.

A Computational Framework for Simulating Patient-Specific Vascular Growth and Remodeling

Erica L. Schwarz*[1], Martin R. Pfaller[1], Jason M. Szafron[1], Stephanie E. Lindsey[2], Marcos Latorre[3], Christopher K. Breuer[4], Jay D. Humphrey[5], Alison L. Marsden[1]

[1] Stanford University
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[5] Yale University

Introduction - Predicting vascular growth and remodeling (G&R) remains an area of significant clinical interest with relevance to pediatric cardiology and single ventricle physiology. The constrained mixture model (CMM) of vascular G&R, in particular, has been successful at modeling vessel evolution in response to a wide variety of hemodynamic and pathological conditions by capturing the turnover of constituent families constrained to deform with the whole tissue. However, coupling CMM with high-fidelity fluid-solid interaction (FSI) simulations in patient-specific geometries has been limited by simplifying 2D spatial and temporal assumptions and by high computational cost which requires tracking individual constituent history and creates an exponentially increasing time complexity. However, in congenital heart disease and tissue engineering applications, capturing the 3D evolution of tissue morphology is critically important for quantifying and predicting disease progression such

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as stenosis formation. Thus, there remains a need for a fully 3D fluid-solid-growth (FSG) framework. In this work we present a novel fluid-solid-growth handshake (FSGh) framework for CMM simulations. In the FSGh framework the time-evolving material stress response is approximated as an updating linearized hyperelastic material around the current vessel state. The resulting reduction in computational cost allows for the simulation of time-resolved FSG on patient-specific geometries.
Methods - In the FSGh framework, the evolution of the structural configuration of the tissue is found by using the arbitrary Lagrangian-Eularian FSI framework to find the geometry that satisfies mechanical equilibrium using the linearized response of the full, constituently-derived stress and enforces predicted volume change using a swelling penalty formulation. Using this framework, the solid tissue is modeled as a time-independent hyperelastic material within each FSI iteration. Therefore, FSI iterations are computationally cheaper than evaluating the full constitutively-defined material history. FSI solutions are iterated at given G&R timesteps until the model configuration converges to a prescribed tolerance.
Results - The FSGh framework was implemented in the SimVascular open-source multiphysics solver svFSI. Its performance was validated against hypertension and flow perturbation models with known time-evolving behavior. We then simulated the G&R of a tissue-engineered vascular graft used in single ventricle physiology and an asymmetric abdominal aortic aneurysm. These examples demonstrate the value of the FSGh framework to in predicting complex evolving vascular pathologies.
Discussion - The FSGh framework represents a novel implementation of CMM that allows for evaluation of time-resolved FSG in a fully 3D FSI framework while avoiding the computationally expensive evaluation of the full constituent stress response during the FSI solution. Initial results indicate agreement of the FSGh framework with full CMM method. Additional validation is underway to verify the behavior of the FSGh framework across a wide variety of hemodynamic perturbations and patient-specific geometries.

A multimodal approach to investigate the effects of respiration on Fontan flow

Markus Horvath*[1, 2], Caglar Ozturk[1], Bryce Starr[3], Mulan Jiang[1], Ellen Roche[1]

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The current preferred treatment for single ventricle physiology culminates in the Fontan circulation which connects the systemic and pulmonic vasculature in series. While it allows patients to survive with a single ventricle, the relentless hemodynamic burden triggers severe pathophysiological consequences. Despite great interest, understanding of the physiological interactions and development of support strategies remain limited which results in continuously high mid- to long term mortality rates over the past 20 years. One important example of limitation is highlighted in the current Fontan models; animal, benchtop, and computational. Recently, respiratory mechanics have been identified as a governing contributor to Fontan flow patterns and resulting reverse flow in the systemic venous return, yet currently available animal models fail to recreate the impact of respiration on the hemodynamics. Similarly, sophisticated models have been developed in vitro and in silico, but physiologically relevant interaction of respiratory and vascular pressures is limited. We present the development of quantitative tools that recreate the impact of respiration on the hemodynamics and serve as test platforms for interventions. In this work we introduce a platform of physical and computational models of the Fontan physiology including a clinical validation study which mimic our natural breathing and flow mechanics to recreate the venous blood flow in the Fontan circulation. This will allow to study the effects of different breathing patterns and particular physiologies on the flow characteristics. Finally, it will serve as a platform to test different support strategies to improve the Fontan circulation.

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In utero mitral valve inflow obstruction stunts left ventricular chamber growth and maturation in a fetal lamb model

Daisuke Onohara*[1], Matthew W. Hagen[2], Sammantha Louey[2], George Giraud[2], Sonnet S. Jonker[2], Muralidhar Padala[1]

[1]Emory University
[2]Oregon Health & Science University

Introduction - Mitral valve lesions that obstruct left ventricular (LV) inflow and chamber filling are associated with LV hypoplasia, in babies born with hypoplastic left heart syndrome (HLHS). This no-flow/no-grow hypothesis has long been hypothesized and confirmed in chick embryos, but is yet to be confirmed in larger animal models relevant to humans. In this study, we report an in-utero fetal lamb model of mitral valve inflow obstruction with an implantable balloon, to test the no-flow/no-grow hypothesis.
Methods - Pregnant ewes (n=12) with twin fetuses (gestation day 120) were used in this study. One fetus was instrumented to achieve left ventricular inflow obstruction (LVIO), and the other fetus was considered a control (Control). Surgery was performed in all ewes under anesthesia, gaining access to the fetuses through a laparotomy and a hysterotomy. LVIO was achieved by surgically implanting an inflatable latex balloon into the left atrium of the beating heart of one fetus, via a left thoracotomy. Inflating the balloon with 30% glycerol, increased its volume, filled the left atrium and obstructed left atrial filling from the foramen ovale, and also obstructed the mitral valve. An ultrasound flow probe implanted on the ascending aorta was used to monitor reduced LV output from reduced LV filling, and fetal health was monitored with catheters in the carotid artery, jugular vein, and the amniotic fluid. Upon closing the incisions, the ewe was recovered from anesthesia, and stanchioned for 2 weeks with continuous fetal health monitoring. LVIO was achieved by gradual balloon inflation, to avoid fatal arrhythmias. 2 weeks after the surgery (gestation day 134), the ewes were anesthetized, and the fetuses were recovered to compare the hearts between the LVIO and control. Fetal ultrasound was performed before surgery, after surgery, and at termination.
Results - 7 of the fetuses completed the 2-week experiment (58.3% survival). At explant, the fetal body weight was not different (LVIO: 4.6±0.9kg vs Control: 4.5±0.6kg, p=0.8597). Heart weight differed between groups, with a higher weight in LVIO: 31.4±5.6g than control:26.4±2.4g (p=0.0471). LV weight was 7.4±1.8g in LVIO, vs 7.9±1.1g in control (p=0.5652), RV weight was 8.35g (7.73 to 11.25g) in LVIO vs. 7.59g (6.92 to 7.95g) in control (p=0.0670). The LV/RV weight ratio was 0.83±0.17 in LVIO, compared to 1.08±0.19g in Control (p=0.0209). LV end-diastolic volume was 2.3±0.7ml in LVIO group vs. 7.1±0.8ml in control (p<0.0001), and end-systolic volume was 1.01ml (0.95 to 1.95ml) in LVIO group vs. 3.38ml (3.28 to 3.57ml) in control (p=0.0025). LV/RV cavity ratio in the mid-level of the heart was significantly smaller in the LVIO group (0.21±0.06), vs. Control (0.56±0.05) in diastole (p=0.0005), 0.15±0.04 and 0.53±0.16 in systole, respectively (p=0.0054).
Conclusion - Left ventricular inflow obstruction at late gestation in a twin fetal lamb model stunted left ventricular growth, and induced right ventricular remodeling. This preliminary work indicates the feasibility of a large animal model to investigate the no-flow/no-grow hypothesis underlying HLHS.

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Cures II: Enabling Technologies

Sunday, October 9 at 8:00-9:00 AM
Moderated by Irfan Kathiriya, MD, PhD | University of California San Francisco

Two-Year Follow-Up after Autologous Stem Cell Therapy for Hypoplastic Left Heart Syndrome at the time of Bidirectional Glenn Surgery

Somya Shankar*[1], Apurva B. Challa[2], Drew K. Seisler[2], Clinton Hagen[2], M. Yasir Qureshi[2], Timothy J. Nelson[2]

[1]Sidney Kimmel Medical College at Thomas Jefferson University
[2]The Mayo Clinic

Purpose - This phase I clinical trial examined the safety at two years after Autologous Umbilical Cord Blood-derived Mononuclear Cells (UCB-MNCs) intramyocardial injections during the Stage II Surgery for Hypoplastic Left Heart Syndrome.
Methods - 23 subjects with HLHS were enrolled across six different hospitals. Prenatal diagnosis allowed umbilical cord blood banking and processing into cryopreserved UCB-MNCs by ReGen Theranostics Inc. UCB-MNCs were administered via intramyocardial injections in the right ventricle at the time of Stage II surgery. Patients were followed for two years. The primary endpoint was to assess safety and feasibility of cell therapy. The secondary endpoint was to assess right ventricle function and somatic growth evolution six months after the cell injections.
Results - 21 out of 23 subjects completed the two-year follow-up. None required cardiac transplantation and two died during the study duration. Both deaths were determined to not be related to UCB-MNC cell-based product or delivery. A total of 187 adverse events were reported. Ten adverse events were possibly, probably or certainly related to cell delivery or therapy: post procedure fever (4), transient decrease of ejection fraction (3), tricuspid regurgitation (1), intermittent sinus tachycardia (2), and pericardial bleeding (1). All were rated to be events of mild or moderate severity. No serious adverse event related to cell therapy was reported. There was no significant decline in right ventricular ejection fraction noted in the cohort when compared baseline to the 6-month follow-up: 49.9 ± 9.0% vs. 49.6 ± 8.9% , respectively. There was a slight improvement in growth in patients who received cell therapy compared to natural history data, which did not meet statistical significance: 9.1 ± 1.3 kg at six months post-operation (p-value = 0.054).
Conclusion - This study demonstrated the safety and feasibility of intramyocardial autologous UCB-MNC injections during the Stage II surgical palliation of patients with HLHS. No major or serious adverse event related to UCB-MNC therapy or delivery were reported in the two-year follow up period. There was no decline in right ventricular ejection fraction at six months post-surgery in these patients, and there was a small improvement in growth in the patients who received the cell therapy.

Electrophysiological characterization of engineered micro-heart tissue derived from human pluripotent stem cells using 3D in-vivo like protocols

Lavanya Aryan*[1], Kuo-Chan Weng[1], Lauren Huebner[1], Lauren Boggs[1], Nathaniel Huebsch[1], Stacey Rentschler[1]

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The hPSC-derived cardiac tissue had promising potentials for cardiac tissue engineering and regenerative medicine. However, the electrophysiological properties of cardiac tissue derived from in-vitro differentiation were usually showing immature features. Our group developed engineered micro-heart tissue (uEHT) and used an automated and non-invasive image-based measuring system to determine the conduction velocities of these engineered micro-heart tissues. This allows us to assess the electrophysiological properties and functions of cardiac tissues differentiated from multiple protocols in higher throughput and high content concept. We differentiated cardiac tissue with 3D in-vivo like differentiation protocols using both cytokines (Activin and BMP) and small molecules (CHIR and IWP2). We successfully differentiated cardiomyocytes to up to 80% of cTnT positive cardiomyocytes from multiple iPSC lines, including iPSC-GCaMP6 and iPSC-MYL2-GFP line, using

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both cytokines and small molecules. We measured the conduction velocities of uEHT generated from both protocols and showed the CV were 3.6 cm/s and 2.6 cm/s, when differentiated using cytokine and small molecule method, respectively. We further investigated the effect on different components of engineered cardiac tissue such as the percentages of fibroblasts and cardiomyocytes. We found that mixing with 20% of cardiac fibroblasts and 80% of pure cardiomyocytes yielded the best structural and functional outcomes of uEHT. Currently, we optimized the culturing condition for isolating cardiomyocytes using SIRPAa labeling to be used in uEHT to improve conduction velocity. Together, our results show that we could differentiate cardiac tissue with multiple protocols and cell lines and we measured the electrophysiological properties of micro-tissues with automated imaging system.

Simulated Performance Of A Bioprinted Pulsatile Fontan Conduit

Zinan Hu*[1], Erica L. Schwarz[2], Jessica Herrmann[3], Mark Skylar-Scott[3], Alison L. Marsden[3]

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Introduction - Single ventricle patients with Fontan physiology live with a state of circulatory inefficiency, which contributes to various morbidities including heart failure, venous congestion, and arrhythmias. To resolve unfavorable hemodynamics, our interdisciplinary team is developing a 3D bioprinted conduit that could theoretically contract to provide a pulsatile energy source to the lungs. However, bioprinting of large-scale contractile materials is an expensive and complex procedure. Computational modeling provides a means to accelerate conduit design by allowing for efficient exploration of the design space and an assessment of how the hemodynamics of a Fontan patient change in response to conduit design choices. In this study, we constructed a reduced order model and a 3D model of the pulsatile conduit to evaluate design parameters of interest.
Methods - We began by creating a 0D model of the pulsatile conduit by representing it as a time-varying elastance element in a close-loop lumped-parameter network (LPN) model of the Fontan circulation. We evaluated the effect of varying the number of valves in the model, maximum elastance, and pulsatile frequency. We then created a 3D finite element model of the pulsatile conduit by combining electromechanics with fluid-solid interaction and applying a flow waveform at the inlet and using a valve-like boundary condition at the outlet. This was implemented in the open-source software SimVascular and used to evaluate the effect of varying fiber direction and active stress.
Results - In the 0D model of the pulsatile conduit, two valves were needed to optimally reverse the Fontan paradox. This configuration decreased the liver pressure from 16.38 to 15.23 mmHg, while the pressure of the conduit decreased from 11.17 to 9.87 mmHg. Doubling the elastance led the volume amplitude to increase from 29.4% to 48.77%. Elevating the frequency of the conduit to f = 2f0 induced a 15.0% reduction in liver pressure despite having roughly the same volume amplitude of approximately 32.3%. Further, it better transferred pulsatility to the pulmonary junction as well as reduced the IVC pressure. In the 3D model, we found that symmetrically-oriented fiber family directions generated a more circumferential contraction while asymmetrically-oriented fiber family directions led primarily to twist motion. For symmetric contraction, a 25kPa active stress could generate 15% volume amplitude while a 20 mmHg increment in conduit pressure. In twisting contraction, the same stress generated a 7% ejection fraction and 15 mmHg pressure elevated. The volume amplitude for the latter case may be limited due to the pulsatile conduit length being fixed by the prescribed boundary conditions. Future work will consider the effect of longitudinal shortening which may provide a larger volume amplitude. In addition, the 3D model provided characterization of local hemodynamics including wall shear stress and vorticity which have been shown to affect overall vascular performance.
Discussion - Reduced-order models provide convenient ways to optimize the conduit design considering the factors of cylinder geometry, valve placements, and active stress. Such results could provide optimal design guidance prior to manufacturing. In the 3D model, the multi-physics simulation gives comprehensive design guidance from Purkinje fiber to myocardium fiber direction. Moreover, the geometry is no longer limited to cylindrical form and more efficient geometry can be designed and evaluated.

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A Computational Model for Cardiovascular Fluid–Solid-Growth Interaction

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Local hemodynamics play a crucial role in blood vessels adapting to changes in loading conditions, e.g., in pulmonary arteries in single ventricle patients. The microstructural changes in blood vessels are characterized by growth and remodeling, i.e., the continuous deposition and degradation of tissue constituents. This work presents a new open-source framework that enables three-dimensional fluid-solid-growth interaction simulations using a fast mechanobiologically equilibrated model.

The constrained mixture theory describes mechanisms that drive growth and remodeling (G&R), capturing the different rates of turnover and material properties exhibited by individual tissue constituents. However, this approach can be computationally expensive due to the need for tracking constituent configuration and perturbation history. This limitation was overcome by the recent concept of mechanobiologically equilibrated G&R. This rate-independent theory can compute evolving homeostatic states efficiently by enforcing mechanical and mechanobiological equilibrium without the need to track the history of deposition and removal or to integrate evolution equations.

In cardiovascular problems, G&R is commonly triggered mechanically through fluid-structure interaction by pressure-induced intramural stress and flow-induced wall shear stress. Previous implementations of the equilibrated constrained mixture model relied on an assumed form for intramural stress and flow-induced wall shear stress. We propose combining fluid-structure interaction and G&R in a novel fluid-solid-growth (FSG) framework to predict disease progression, using the mechanobiologically equilibrated constrained mixture theory, enabling efficient three-dimensional FSG simulations for patient-specific geometries.

Our FSG model is implemented in our open-source multiphysics finite element solver svFSI (available at github.com/SimVascular/svFSI). In the following, we briefly outline the ingredients of our FSG framework. Blood flow in the fluid domain is modeled by the incompressible Navier-Stokes equations. We pass local fluid pressure and wall shear stress to our solid model at the fluid-solid interface. We use a fast and efficient formulation of the constrained mixture theory that is based on the assumption that each G&R state is mechanobiologically equilibrated. This rate-independent formulation eliminates time-dependency and directly predicts grown and remodeled states for given external loads and boundary conditions. We explore three coupling methods to inform the G&R model with fluid pressure and wall shear stress. First, we utilize a semi-analytical approach, assuming fully-developed laminar Poiseuille flow through a long cylinder. This approximation yields good results in simple tube-like geometries, with pressure assumed uniform axially, but is inaccurate for more complex flow fields. In our second approach, we propose partitioned coupling, where we consecutively solve steady-state fluid flow, solid G&R, and mesh displacement. Finally, we simultaneously solve for fluid velocity, pressure, and solid displacements in a monolithic coupling approach.

We validated our purely solid implementation in svFSI against the implementation in FEBio from Latorre and Humphrey, assuming uniform pressure and the analytical approximation for wall shear stress. We compare all three coupling approaches, semi-analytical, partitioned, and monolithic, in blood vessels during hypertension and aneurysmal enlargement. In the future, we will analyze the influence of fluid-derived wall shear stress stimuli and demonstrate the robustness of our fluid-solid-growth framework in a complex flow example.

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Future of Single Ventricle Science and Medicine

Sunday, October 9 at 8:00-9:00 AM

Moderated by Kaitlin Davis, PhD | Additional Ventures

Biological cardiac pacing by gene therapy
Hee Cheol Cho, PhD | Johns Hopkins University

Single ventricle disease patients are medically fragile and exhibit complex disease manifestations. One of the most significant comorbidities in these patients are heart rhythm abnormalities, including severe bradyarrhythmias. Current pacemaker therapy is largely palliative and far from ideal for pediatric patients with single ventricle disease-related bradyarrhythmias. In this talk, I present biological pacemakers as alternatives to implantable pacemaker devices. Powered by our proof-of-concept studies in small and large animal models of bradyarrhythmia, I will highlight somatic cell reprogramming approaches to regenerate pacemaker tissues in situ by direct myocardial gene transfer and present opportunities for single ventricle patients.

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Poster 01 - A mouse model of Tbx5 dosage-sensitive single ventricle anatomy
Irfan S. Kathiriya*[1], Kavitha S. Rao[2], Kevin M. Hu[2], Bayardo I. Garay[2], Piyush Goyal[2], Sarah Winchester[2], Benoit G. Bruneau[2]

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Single ventricle (SV) anatomy is a consequence of abnormal chamber formation and occurs in combination with cardiac septal defects, such as atrial septal defects (ASDs) or ventricular septal defects (VSDs). Hypoplastic left heart syndrome (HLHS) manifests as left ventricular (LV) hypoplasia, while tricuspid atresia displays right ventricular hypoplasia. Both lesions require ASDs for anatomic shunting of blood for SV physiology. Notably, atrioventricular canal (AVC) defects include VSDs, ASDs and abnormal development of the atrioventricular (AV) valves, with severe cases leading to chamber hypoplasia and functional single ventricle physiology.
TBX5 is essential for chamber formation and cardiac septation. TBX5 haploinsufficiency causes CHDs in both humans and mice. In humans, inherited heterozygous mutations in TBX5 can reduce TBX5 levels and cause CHDs, including VSDs, ASDs, AVC defects, and HLHS. In mice, Tbx5 loss leads to ventricular and atrial hypoplasia with apparent developmental arrest at embryonic day (E) 9.5. Deletion of Tbx5 in the LV causes loss of interventricular septum (IVS) patterning.
Here, we used an allelic series of Tbx5 mutant alleles to study the role of proper Tbx5 dosage for chamber formation and cardiac septation. Stepwise reduction of Tbx5 dosage correlated with severity of CHD phenotypes, including patterning defects of the interventricular septum, interatrial septum and AV cushions. Severely reduced Tbx5 dosage displayed early lethality by E12.5, absent IVS and single ventricle anatomy with ventricular chamber hypoplasia at E10.5. Integrated analysis of gene expression and chromatin accessibility at the single cell level revealed vulnerable, dose-dependent gene regulation for chamber formation and cardiac septation. Therefore, we uncover insights for genetic susceptibility to CHDs and gene regulatory mechanisms underlying single ventricle disease.

Poster 02 - Building Enhancer-Gene Regulatory Maps of the Noncoding Genome for the Developing Human Heart
Stephanie D Conley*[1], Rosa Ma[1], Helen Y Kang[1], Lauren Duan[1], Dulguun Amgalan[1], Chad Munger[1], Oriane Matthys[1], William R Goodyer[1], Jesse M Engreitz[1]

[1]Stanford University

Genetic variation plays a key role in the etiology of single ventricle heart disease. The vast majority of candidate genetic variants are located in non-protein coding regions of the genome, where they may affect the functions of enhancers that control gene expression in specific cell types in the developing heart. Human genetic studies have now identified thousands of candidate genetic variants, each of which could reveal molecular insights into heart development and the causes of single ventricle heart disease. However, several important questions remain unanswered, including: 1) which cell types and genes are critical for human cardiac development, 2) which of the known genetic variants are truly causative for congenital heart disease, and, ultimately, 3) which cellular pathways are impaired by these genetic variants, as these may represent novel therapeutic targets in the prevention and/or treatment of single ventricle heart disease. To address these questions and link variants to functions, a comprehensive regulatory map is required of all genes and enhancers active in human heart development at deep temporal and cell type/state-specific resolution.

To create such a map, we have simultaneously applied single-cell RNA-sequencing and single-cell ATAC-sequencing to generate an initial atlas of >95,000 cells in 11 structurally normal human fetal hearts ranging from 8-22 weeks post conception. To date we have identified at least 21 cell types and states, including 2 endocardial and 5 cardiomyocyte subpopulations.

Furthermore, using these data, we have built cell-type specific enhancer-gene maps using the Activity-by-Contact (ABC) model. We used these maps to predict the regulatory effects of distal enhancers and single-nucleotide variants on gene expression by intersecting these enhancer maps with disease variants associated with congenital heart disease or quantitative

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measurements of heart morphology. These variant-to-function maps highlight numerous cases where noncoding risk variants appear to disrupt the functions of cell-type specific regulatory elements, including enhancers active in endocardial cell subpopulations predicted to regulate PLPPR4 (variant associated with aortic valvular calcification) and GOSR2/WNT9B (variant associated with anomalies of thoracic arteries and veins and 20 other cardiovascular phenotypes).

Overall, this work will advance our fundamental knowledge of heart development by cataloguing cardiovascular cell states throughout development and providing a data-driven, unbiased approach to identify key cell types, genes and genetic variants that influence risk for single ventricle heart disease.

Poster 04 - Toward understanding physiological cardiac embryonic senescence in better deciphering the biological origin of hypoplastic left heart syndrome

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The Hypoplastic Left Heart Syndrome (HLHS) is a complex and multifactorial cardiac congenital disease. More than 30 mutated genes encoding both cardiac and non-cardiac proteins have been uncovered for the last years. However, the function of these mutated proteins is several and not interrelated. Rather, dysregulated biological pathways turn out to likely be at the origin of hypoplastic ventricle and valve defects, a signature of HLHS. One of these pathways that has been highlighted is the cell cycle. However, the trigger for myocyte cycle arrest is unknown. Interestingly a report[1] pointed the presence of cardiomyocytes featuring DNA damage and senescence in 20 weeks old fetal HLHS human hearts.
We report replicative cell senescence in mouse embryonic hearts. We raised the hypothesis that HLH features aberrant or persistent fetal senescence. Accordingly, we aim at understanding the role of physiological embryonic myocyte senescence in formation of the heart.
Senescent myocytes were mostly located in trabeculae and expressed H2AX and p21 but not p16, a gene profile signature of embryonic senescence[2]. Rare senescent myocytes were observed at E9.5 (week 4 in human) and in neonatal mouse left ventricle (LV). The number of p21+ cells were scored in embryonic mouse LV (as % of total cells). We found 2.6±0.3% (n=3), 4±1% (n=7) and 7.8±0.8% (n=3) p21+ cells at E10.5, E13.5 and E16.5, respectively (weeks 6-8 in human).
Next, we used a cell cycle inhibitor that exacerbates senescence (Palbociclib) to look at its impact on cardiac trabeculation and/or compaction. The CDK4/6 inhibitor given to pregnant mice increased the score of H2AX (47±4.4%;n=5 vs 21.6±9.7;n=3 non-treated p≤0.03) and p21+ (4.7±0.7%;n=5 vs 2.2±0.6;n=4 non-treated p≤0.04) myocytes in LV of E13.5 embryos without affecting growth of the whole embryos.
E13.5 embryonic LV from Palbociclib-treated mothers featured hyper-trabeculation and thinner ventricular wall compared to embryos of non-treated mothers (27.7±2.8m ;n=5 vs 57±2.2 m; n=7 p≤0.0001).
In order to visualize whole ventricles and to understand the trabeculation/compaction processes, we combined High Resolution Episcopic Microscopy, 3D cell imaging, and fractal analysis. E16.5 embryonic hearts from Palbociclib-treated mothers still revealed hyper-trabeculation scored by fractal analysis and thin ventricular wall. This suggests that cell senescence might be a major regulator of ventricular compaction.
Analysis of single cell RNA-sequencing of FACS-sorted high tomato+ trabeculae myocytes from E13.5 and E16.5 hearts from embryos generated by breeding SmaCreERT2 with Rosa26tdtomato mice revealed 2 clusters of senescent p21+ myocytes in trabeculae. Gene profiling and trajectory inference of p21+ cells pointed to a role of Tgf1 and sphingosine-dependent pathways as main regulators of myocyte senescence.
A fine tuning of space- and time-dependent physiological embryonic senescence might be the trigger of ventricular compaction and is thus required for normal heart morphogenesis. A dysregulation of both timing and location of the occurrence of this druggable cell process may be at the origin of HLHS. An early diagnostic of the syndrome is warranted to foresee a pharmacological strategy to improve the growth of the LV in HLHS.

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Poster 05 - Case Report: Acute manifestation of endocardial fibroelastosis in an adolescent with mitral valve stenosis and recurring aortic disease – Is it the mix that matters?

Ingeborg Friehs*[1], Daniel Diaz-Gil[1,2], Chrystalle Katte Carreon[3,4], Natalia Silva-Gomez[1,2], Alan Benheim[5], Sitaram M. Emani[1], Pedro J. del Nido[1], Gerald R. Marx[1]

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Background - The most severe congenital heart defect is hypoplastic left heart syndrome/complex (HLHS/HLHC) with underdevelopment of the left-sided structures of the heart. A high number of patients pre- and early postnatally present with endocardial fibroelastosis (EFE), a unique form of fibrosis covering the inner surface of the LV. The presence of EFE is a major putative effector in the maldevelopment of the heart. We advocate for surgical resection of EFE to achieve growth of the underdeveloped LV, allowing for corrective surgery to create a normal heart. Not all patients can be treated successfully due to recurrence of EFE. Our results associate flow disturbances with EFE development through a process called endothelial-to-mesenchymal transition (EndMT), which we have shown as the underlying root cause of EFE formation. Despite EFE being a childhood disease and rarely progressing beyond adolescence, we present the first description of an acute manifestation of EFE and active remodeling of the endocardium via EndMT in an adolescent with HLHC and a genetic heterozygous ABL1 variant.
Case Description - A male, white patient presented at one-month-of-age with mitral valve stenosis, subaortic stenosis, bicuspid aortic valve, and aortic coarctation. After a cortectomy as a neonate, the patient underwent several transcatheter interventions for coarctation and aortic stenosis. At age 6, the patient required his first surgery for a mitral valvuloplasty. At this point no EFE was visualized neither by the surgeon nor on pre- and postoperative imaging. For 7 years, the patient underwent repeated percutaneous valvuloplasties and stent placements for aortic stenosis. Due to the unusual aortic disease, whole-exome sequencing was performed which detected a heterozygous ABL1 variant c.986 C>G; p.P329R. At 13 years, a balloon valvuloplasty reduced an asymptomatic aortic valve gradient from 55 to 30mmHg, however, post-interventional measurements demarked a new trans-mitral gradient, mild regurgitation and increased pulmonary capillary wedge pressures of 21 mmHg. In the following year, the patient reported a steady clinical decline consistent with worsened heart failure symptoms. On follow-up, echocardiographic and MRI imaging revealed acute global LV dysfunction, dilation, hypertrophy, noncompaction, and thickened amorphic tissue encroaching on the endocardial LV surface from the base to the apex, involving both the mitral valve leaflets and subaortic region, and late gadolinium enhancement consistent with EFE, respectively. The patient underwent surgery with removal of EFE. On histological evaluation, tissue from all LV regions were comprised of paucicellular, organized, avascular collagen and elastin matrix, which is consistent with the histological picture of EFE. Immunofluorescence analysis showed a high number of cells expressing both endothelial and mesenchymal markers, indicative of active EndMT.
Conclusion - This case emphasizes the likely multifactorial nature of EFE. Distinct flow alterations in the LV in combination with genetic alterations of intrinsic EndMT pathways (i.e. ABL1), led to acute manifestation of EFE in adolescence. In an attempt to elucidate how genetic and transcriptional factors influence intrinsic and extrinsic stimuli of EndMT, we are currently investigating molecular pathways and gene dysregulation in our EFE patients which will lead to the identification of clinically applicable therapeutic targets for EFE treatment.

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Poster 06 - Interpretable machine learning to decipher RNA splicing regulatory logic
Susan E. Liao*[1], Mukund Sudarshan[1], Oded Regev[1]

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Alternative RNA splicing plays a critical role in the developmentally regulated transfer of information from DNA to functional RNA and protein products. Alternative splicing plays particularly important roles in cardiac development, particularly during embryonic organogenesis and the establishment of cardiac asymmetry. However, our understanding of the regulatory logic underlying alternative splicing decisions remains incomplete. To decipher splicing regulatory logic, we designed a synthetic reporter assay and implemented an "interpretable-by-design" neural network. Our network accurately predicts splicing outcomes and describes how it arrives at its predictions in HeLa cells. We plan to apply these platform technologies to understand regulation of alternative splicing during cardiac development. We expect that additional data generation efforts paired with the interpretable-by-design framework will stimulate advances in understanding other developmentally regulated processes (e.g. transcription, translation, etc.).

Poster 08 - Mechanisms of cardiac de novo sarcomerogenesis
Bhavana Shewale*[1], Andrew Kurland[1], Nan Yang[1], Silvia DeRubeis[1], Joseph Buxbaum[1], Jeffery Johnson[1], Nicole Dubois[1], David Sanders[2], Clifford Brangwynne[2]

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Defective sarcomeric assembly is implicated in the pathophysiology of several inherited cardiomyopathies. While the mature sarcomere structure has been extensively studied, the molecular mechanisms that drive de novo sarcomere assembly are largely unknown. One of the earliest components in de novo sarcomere assembly is alpha-actinin 2 (ACTN2). ACTN2 localizes to the z body which fuses to form the z disc that serves as a linker for adjacent sarcomeric units. Here we used human pluripotent stem cell-derived cardiomyocytes (hPSC-CMs), which closely follow this temporal progression of sarcomere formation, to interrogate the early ACTN2 interactome. We performed RNAseq and mass spectrometry on whole cells and ACTN2 co-IP components at different times during hPSC-CM differentiation. We find that ACTN2 sequentially associates with structural components such as intermediate filament and junctional proteins, and functional regulators such as calcium channel and metabolic proteins reflecting a coordinated program over time. The early cardiomyocyte coordinates translation and processing of sarcomeric proteins with their spatial organization and incorporation into developing myofibrils. ACTN2 in the early z body associates with chaperones and actin binding proteins, suggesting that the z body acts as a nidus for de novo actin filament assembly. In support of this role WNT and ROCK inhibition result in an increase of ordered sarcomeric filaments. We further identified ribosomal subunits and RNA binding proteins in the ACTN2 interactome, suggesting a role of the z body as a dynamic condensate. We confirmed this dynamic state of the z body using ACTN2 FRAP. We further show that epiblast-specific loss of Ddx3x, an early ACTN2 interactor and RNA binding protein, leads to embryonic lethality and cardiac defects in the mouse. Together these findings highlight the importance of the z body in the assembly process and provide a window to study the mechanisms of sarcomere organization.

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Poster 09 - The neural control of cardiac function in larval zebrafish
Luis Hernandez-Nunez*[1], Florian Enger[1], Mark Fishman[1]

[1]Harvard University

Studying the neural control of cardiac function from a systems-level perspective is challenging because it requires measuring neural activity with single-cell resolution in the central, autonomic, and intracardiac nervous systems. We have developed new optic tools to study this question leveraging the translucency and genetic access that larval zebrafish provide. Combining calcium imaging, optogenetics, and microsurgical laser ablations, we are starting to uncover the neural dynamics that underly cardiac homeostasis in this small vertebrate. We would like to also study the neural dynamics generated by a heart with mechanical defects, such as a single ventricle heart, and how they may affect feedback to the brain.

Poster 10 - Duke iSVC: Building an interdisciplinary, translational research team to understand the genetic etiology of single ventricle congenital heart disease
Lauren Parker*[1], Leonie Kurzlechner[1], Jacob Scherba[1], Minu-Tshyeto Bidzimou[1], Leslie Pace[1], Nicholas Andersen[1], Joseph Turek[1], Andrew Landstrom[1]

[1]Duke University

Introduction - Single ventricle (SV) congenital heart diseases represent ~0.05% of congenital heart disease yet carry high morbidity and mortality, requiring early surgical intervention and lifelong cardiovascular care. It is critical to understand the mechanistic basis of SV diseases to improve outcomes. At our institution, we developed a cross-disciplinary research team, the Duke Interdisciplinary Single Ventricle Research Collaborative (Duke iSVC), to synergize SV research across the institution, support trainees' research and career development, and establish a comprehensive biobank and tissue repository for SV research.
Methods - We established a group of faculty and researchers, including liaisons, that linked fetal cardiology, pediatric cardiology, congenital heart surgery, pediatric cardiovascular critical care, and basic scientists. We developed a "best practices" pipeline for the early identification of fetal SV patients for enrollment. After birth, triads consisting of both biological parents and the SV infant were contacted to obtain informed consent. Deep clinical phenotyping was abstracted into a secure REDCap database. Tissue collection was coordinated between labor and delivery, pediatric cardiac ICU, and the operating room to obtain genomic DNA of the triad and whole blood and cardiac tissue of the SV proband. Whole blood was collected in EDTA, PaxDNA, and PaxRNA tubes, and peripheral blood mononuclear cells suitable for iPSC generation were cultured within hours of sample collection. Surgically resected myocardium was collected and flash frozen in <1 minute after excision.
Results - From January 2022 through May 2022, we established regular meetings and direct lines of communication between stakeholders. We recruited 39 probands with SV disease, including 27 with hypoplastic left heart syndrome (HLHS), 7 with tricuspid atresia, and 5 with other SV defects. These included 41% female patients. Individuals identified as White/Non-Hispanic in 72% of the cohort, Black in 15%, White/Hispanic in 5%, unknown in 5%, and multiracial in 3%. The median age of probands enrolled as trios was 1.5 days [Range: 0 – 145 days] while the median age of all other probands was 2.9 years [Range: 0 – 34.9 years]. Peripheral blood mononuclear cells were isolated and successfully expanded in culture for ~70% of the probands. PaxDNA was collected in 73%, PaxRNA in 51%, and surgical tissue in 38%.
Conclusions - Close collaboration between pediatric cardiologists, congenital heart surgeons, basic scientists, and families is necessary to create an intentional pipeline for recruiting neonatal SV patients for research. Moreover, early identification of SV patients and obtaining consent shortly after birth and before surgery improves the yield of blood and tissue collection, given the need for perioperative blood transfusion. These improved features of patient enrollment and tissue collection are expected to substantially improve the ability to study the mechanistic underpinnings of SV disease in order to work towards improving patient outcomes."

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Poster 11 - Human Induced Pluripotent Stem Cell Model of Hypoplastic Left Heart Syndrome
Hananeh Fonoudi*[1], Richard Harvey[2], Paul Burridge[1]

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Hypoplastic left heart syndrome (HLHS), one of the most severe forms of congenital heart defects, is predominantly characterized by underdevelopment of the left side of the heart. Although conventionally HLHS was considered to have hemodynamic origins, recent studies suggest complex genetic etiology. However, our current knowledge of the disease-causing pathways is very limited. To harness the molecular underpinnings of the disease, we have generated an in vitro model of HLHS using human induced pluripotent stem cells (hiPSCs). hiPSCs were generated from 10 unrelated HLHS patients and their parents (trio design; 3 clones per individual; 87 hiPSC lines in total), thus providing controls that are as genetically similar to the patients as possible. To investigate differences during early stages of cardiovascular development, hiPSCs were differentiated into cardiac and vascular smooth muscle cells, and their cellular populations and gene expression were studied. Our gene expression analysis revealed no significant differences between vascular smooth muscle cells derived from HLHS-hiPSCs and their parents. In contrast, flow cytometry analysis performed on hiPSC cultures after directed cardiac differentiation at 5-day intervals (day 0-30) showed that ventricular cardiomyocyte differentiation in HLHS-hiPCSs was perturbed. Time course analysis using RNA sequencing on hiPSCs differentiated into cardiomyocytes from 5 HLHS families revealed that the greatest differences between patients and parents were at day 20 post-differentiation initiation, with down-regulation of cell cycle being the main driver. Moreover, transcriptome analysis suggested maturation defect in cardiac cells derived from HLHS-hiPSCs. These findings were further confirmed using remaining 5 independent HLHS families. Cell phenotyping also indicated that beating cardiomyocytes derived from patients were more immature and their calcium flux properties were significantly different ($n>1000$; $P<0.001$). In summary, our findings thus far suggest that the progression of cardiogenesis in HLHS-hiPSCs is perturbed, which may be due to disruptions in cell cycle control and maturation. Finally, we developed a serum-free chemically defined cardiac organoids, mimicking early chamber development in vitro. Analysis of cardiac organoids form HLHS would shed light on the mechanism of disease formation. In conclusion, our data suggest a common pathogenic pathway underlying the early development of HLHS despite genetic heterogeneity of disease causation.

Poster 13 - Functional analysis across model systems implicates ribosomal protein genes in growth defects associated with hypoplastic left heart syndrome
Alexandre Colas*[1], Karen Ocorr[1], Rolf Bodmer[1], Geo Vogler[1]

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Hypoplastic left heart syndrome (HLHS) is the most lethal congenital heart disease (CHD). The pathogenesis of HLHS is poorly understood and definitive HLHS-causing genes have not been identified yet, in part due to the likely oligogenic complexity of the disease. However, emerging evidence points towards impaired cardiomyocyte proliferation as a contributing mechanism to HLHS pathogenesis. A genome-wide siRNA screen for genes affecting proliferation of human iPSC-derived cardiomyocytes (hPSC-CMs) identified ribosomal protein (RP) genes as a most prominent class of effectors. In parallel, whole genome sequencing (WGS) and rare variant filtering (RVF) of a familial CHD case (HLHS proband and fifth-degree relative with CHD) identified a shared rare, predicted-damaging promoter variant affecting the RP gene RPS15A. Patient-derived iPSC-CM proliferation and RPS15A expression was reduced in the proband compared to the parents. Also, WGS/RVF of a cohort of 25 HLHS proband-parent trios revealed rare variant enrichment of RP genes. Functional testing with an integrated multi-model system approach reinforced the idea that RP genes are major regulators of cardiac growth and proliferation, thus potentially contributing to hypoplastic phenotype observed in HLHS patients. Cardiac knockdown (KD) of RP genes with promoter or coding variants (RPS15A, RPS17, RPL26L1, RPL39, RPS15) reduced proliferation in generic hPSC-CMs and impaired cardiac differentiation in Drosophila resulting in malformed hearts, heart-loss or even lethality. In zebrafish, diminished

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rps15a function caused reduced cardiomyocyte numbers, defective heart looping, or weakened contractility, without overt effects on overall embryonic development, while reduced rps17 or rpl39 function caused reduced ventricular size or systolic dysfunction of the atrium, respectively. Furthermore, We found synergistic interactions between RPS15A and homologs of cardiac transcription factors (Dorsocross, pannier and tinman in flies, and tbx5 and nkx2-7 (nkx2-5 paralog) in fish, thus supporting a specific role for RP genes in heart development. Furthermore, RPS15A KD-induced heart/CM proliferation defects were significantly attenuated by p53 KD in both hPSC-CMs and zebrafish, and by Hippo activation (YAP/yorkie overexpression) in developing fly hearts. Based on these findings, we conclude that RP genes play critical roles in cardiogenesis and constitute an emerging novel class of gene candidates likely involved in HLHS and other CHDs

Poster 14 - Abundance of CCR2+ Macrophages in the Pediatric Single Ventricle Myocardium
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Rationale - Distinct cardiac macrophage populations have been delineated in adult heart failure, with CCR2+ macrophages promoting inflammation and pathologic remodeling. Conversely, the presence and function of cardiac macrophage subtypes have not been well-defined in the setting of single ventricle disease (SV). The overall aim of this study is to immunophenotype cells within the myocardium of pediatric SV subjects to identify immune cell populations that may contribute to heart failure progression in SV patients.
Methods - Paraffin-embedded sections of explanted right ventricular (RV) myocardium from pediatric SV subjects (n=16, median age 1.4 years, 69% male) undergoing heart transplantation or pediatric non-failing (NF, n=2, median age 4.4 years, 50% male) donors were stained (antibodies against DAPI, alpha-actinin, CCR2, CD14, CD64, CD90, vimentin) and imaged using multi-spectral immunohistochemistry (Vectra3). All SV subjects had RV- dominant morphology and included infants undergoing primary heart transplantation (no surgical palliation), those with end-stage systolic failure after stage 1 or 2 palliation, and children with refractory protein losing enteropathy following Fontan completion. Image analysis was preformed using Akoya Inform v2.6 and cell count per tissue area (mm^2) were quantified.
Results - When compared to NF RV myocardium, SV RV tissue contains a higher number of CCR2+ cells (median 0 cells/mm^2 vs. median 6.5 cells/mm^2, p=0.0297), primarily located near vimentin+ cells in the connective tissue surrounding vessels. Additionally, the proportion of CCR2+ macrophages to cardiac macrophages (defined as CD14+) is also higher in SV than in NF myocardium (median 1.00 vs. median 0.07, p=0.0147). Among SV hearts, both CCR2+ frequency and proportion were comparable between those with normal RV systolic function and those with RV systolic failure. The overall number of CD14+ or CD64+ macrophages were similar between NF and SV.
Conclusions - Our preliminary results suggest upregulation of CCR2+ macrophages within SV myocardium, regardless of systolic function or stage of surgical palliation. While there is an increased presence of CCR2+ macrophages in adults with heart failure, CCR2+ macrophage abundance may be similar between SV hearts with preserved systolic function and those with end-stage systolic failure. Nevertheless, future work is needed to determine the functional significance of increased CCR2+ macrophage in the SV myocardium, as well as elucidate the contribution of resident versus recruited CCR2+ macrophages. The spatial resolution provided by multi-spectral immunohistochemistry techniques may also suggest interactions between CCR2+ macrophages and other cardiac cell types. Ultimately, improved understanding of the inherent immune cell alterations associated with SV may lead to novel therapeutic approaches and improved outcomes in this vulnerable population.

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Poster 15 - Intramyocardial administration of human bioengineered cardiac lineage cells in a nonhuman-primate model of right ventricular pressure overload

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Stem cell-derived cardiomyocyte-based therapies have emerged as a potential therapeutic approach to support ventricular function and delay or prevent the need for cardiac transplantation in patients with congenital heart disease. Induced pluripotent cell (iPSC)-derived cell therapy holds promise due to the proliferative capacity and the potential to differentiate into any specific cell type. However, transplantation of such cell products presents a risk of arrhythmia. The purpose of this randomized and blinded study was to evaluate the cardiac safety of an iPSC-derived cardiac lineage (iPSC-CL) product in a nonhuman primate model of right ventricular pressure overload, which can occur with single ventricle congenital heart disease. Rhesus macaques (n=28; 4-9 years of age; male and female) underwent pulmonary artery banding (PAB) surgery to induce right ventricular pressure overload, and cardiac event recorder implantation. Untreated control animals (Group I; n=4) underwent PAB only. Animals in Groups II-IV underwent PAB and two weeks later were administered vehicle (n=8), low dose hiPSC-CL (n=8), or high dose hiPSC-CL (n=8), respectively. Animals were followed for 4 or 12 weeks with periodic analysis of blood measures (hematology, serum chemistry, coagulation, cardiac biomarkers) and an echocardiogram. Cardiac rhythm was continuously monitored by an implanted cardiac event recorder. At study endpoint, animals were euthanized, gross necropsy and histological analysis of all tissues was performed. This included the entire heart (4 mm slices), and the cardiac tissue was stained against human cardiac troponin (hCTnI) to assess engraftment. Engraftment was confirmed in 12 of the 15 cell-injected animals surviving to study end point. Engraftment size and success rate at endpoint was dependent upon dose group; all three animals without engraftment were in the low-dose cohorts. There were no iPSC-CL-related alterations in gross pathology, blood measures, or cardiac function. Of the 16 animals that received iPSC-CL, two animals developed significant clinical events due to ventricular tachycardia: one animal (high dose group) died 3 days after cell delivery, and one animal (low dose group) developed ventricular tachycardia and was successfully treated with amiodarone and completed the study. Three additional animals developed ventricular arrhythmia including episodic or singular ventricular tachycardia events, premature ventricular contractions, and ventricular couplets. These episodes were subclinical and the animals completed the study without incident. Arrhythmia events were more frequent in the high-dose groups, with ventricular arrhythmias occurring 3-19 days post-administration. In conclusion, we have established a nonhuman primate model of right ventricular pressure overload and demonstrated the ability of this iPSC-CL product to engraft into the myocardium. Arrhythmia occurred in both the low-dose and high-dose groups, with the highest frequency occurring in the high-dose group. Future studies will be aimed at evaluating dosing strategies and determining the ability of standard anti-arrhythmic therapy to prevent iPSC-CL-induced rhythm disturbances.

Poster 17 - Endothelial loss of ETS1 leads to coronary vascular defect and ventricular non-compaction

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Jacobsen syndrome (JBS) is a rare chromosomal disorder caused by deletions in the long arm of human chromosome 11, resulting in multiple developmental defects including congenital heart defects (CHDs). Combined studies in humans and genetically engineered mice implicate that loss of ETS1 is the cause of CHDs in JBS, but the underlying molecular and cellular mechanisms are unknown. To determine the role of ETS1 in heart development, specifically its roles in coronary endothelium and endocardium and the mechanisms by which loss of ETS1 causes coronary vascular defects and ventricular non-compac-

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tion, ETS1 global and endothelial-specific knockout mice were used. Phenotypic assessments, RNA sequencing and chromatin immunoprecipitation analysis were performed together with expression analysis, immunofluorescence and RNAscope in situ hybridization to uncover phenotypic and transcriptomic changes in response to loss of ETS1. Loss of ETS1 in endothelial cells causes ventricular non-compaction, reproducing the phenotype arising from global deletion of ETS1. Endothelial-specific deletion of ETS1 decreased the levels of Alk1, Cldn5, Sox18, Robo4, Esm1 and Kdr, six important angiogenesis-relevant genes in endothelial cells, causing a coronary vasculature developmental defect in association with decreased compact zone cardiomyocyte proliferation. Down-regulation of ALK1 expression in endocardium due to the loss of ETS1, along with the up-regulation of TGFβ1 and TGFβ3, occurred with increased TGFBR2/TGFBR1/SMAD2 signaling and increased extracellular matrix (ECM) expression in the trabecular layer, in association with increased trabecular cardiomyocyte proliferation. These results demonstrate the importance of endothelial and endocardial ETS1 in cardiac development. Delineation of the gene regulatory network involving ETS1 in heart development will enhance our understanding of the molecular mechanisms underlying ventricular and coronary vascular developmental defects and will lead to improved approaches for the treatment of patients with congenital heart disease.

Poster 18 - Single-Cell Transcriptomic Analysis of Myocardial Remodeling after Ventricular Unloading in Pediatric Dilated Cardiomyopathy and HLHS

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Heart failure (HF) is a major cause of morbidity and mortality in children and adults. To bridge end-stage HF patients to heart transplantation, mechanical circulatory support devices called Ventricular Assist Devices (VADs) have been developed. Approximately 5-20% of failing adult hearts exhibited recovery of ventricular function after VAD placement. The underlying molecular mechanisms behind such recovery is an area of active investigation. Furthermore, it is unclear whether similar recovery is possible in pediatric patients with heart failure, including patients with Hypoplastic Left Heart Syndrome (HLHS).

Dilated Cardiomyopathy (DCM) and HLHS are two major causes of progressive HF in children. We collected ventricular tissues from patients at the time of placement of a VAD (pre-VAD) and at the time of heart transplantation (post-VAD). Tissue was collected from 5 pediatric patients with DCM and 2 patients with HLHS. The age of the DCM patients ranged from 3 months to 16 years and duration of VAD support ranged from 25 days to 533 days. The two patients with HLHS were of ages 13 mo and 16 years and length of VAD support was 97 days and 209 days respectively. Single-cell RNA sequencing (scRNA-seq) was performed on the DCM and HLHS pre-VAD/post-VAD tissue pairs. A total of >200,000 single-cell transcriptomes were analyzed. We present data on changes in cell composition and gene expression profiles after ventricular unloading in DCM and HLHS tissue pairs.

This study represents the first scRNA-seq analysis of pre- and post- VAD myocardium from pediatric patients with DCM and HLHS. Our analyses will elucidate critical pathways involved in myocardial remodeling after ventricular unloading in failing pediatric hearts.

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Poster 19 - Pathological Remodeling and Metabolic Dysfunction in Peripheral Blood Mononuclear Cells is Associated with Heart Failure in Patients with Single Ventricle Heart Disease

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Rationale - While heart failure (HF) remains a leading cause of death and indication for transplant in single ventricle heart disease (SV) patients, the molecular mechanisms associated with the progression to HF are poorly understood. Additionally, it remains a challenge to predict which patients will develop clinically significant HF, and when. We previously demonstrated that the pre-transplanted myocardium of SV patients displays a profound reduction in immune cell-specific transcripts and pathways. These findings are in stark contrast to what is seen in the failing adult heart. Additionally, our prior data demonstrate the SV heart is typified by decreased myocardial energetic capacity and an altered lipid milieu, including increased levels of a pathogenic lipid species, lactosylceramide (LacCer).
Objective
The purpose of this study was to assess the use of circulating peripheral blood mononuclear cells (PBMCs) from SV patients pre- and post- cardiac transplant to predict clinical sequelae, optimize therapeutic recommendations, and improve outcomes for these patients. Additionally, we aim to elucidate the potential mechanisms by which immune cell metabolism is modified in SV.
Methods - Patients (male and female) undergoing scheduled visits at the Children's Hospital of Colorado were recruited for the study. All subjects gave informed consent and donated their blood to the IRB-approved protocol. SV patients ages 0-18 years were included and categorized as SV nonfailing (NF) or HF according to the presence or absence of systolic HF at the time of collection (n=20, SVNF; n=18, SVHF). Children and adults 0-30 years of age with normal biventricular cardiac function were included as a control group (n=11). Post-transplant SV ventricle patients without rejection were also included (n=6, post-TX). PBMCs were freshly isolated from whole blood using density gradient centrifugation and used immediately. Mitochondrial Bioenergetics of intact PBMCs were measured using the Seahorse Bioanalyzer. Total Reactive Oxygen Species (ROS) were assessed using Amplex Red Reagent. Sphingolipid content in PBMCs were quantified using liquid chromatography mass-spectrometry (LC-MS-MS). To test the direct effect of LacCer accumulation on PBMCs, we acutely treated control PBMCs with the 2uM of exogenous LacCer (n=4) and assessed mitochondrial bioenergetic profiles.
Results - Our data suggest circulating mononuclear cells from SVHF patients display reduced mitochondrial energetics, manifesting as decreased maximal respiratory capacity, decreased energy production, and increased ROS relative to normal controls. Interestingly, in paired data comparison, PBMC metabolic function was recovered following cardiac transplantation. To begin elucidating the potential mechanisms that modify SV immune cell function, we assessed alterations in the glycosphingolipid (GSL) milieu. Similar to what is seen in the SV heart, we identified a significant accumulation of LacCer in PBMCs from SV patients with systolic HF. Further, we determined that acute treatment of exogenous LacCer is sufficient to impair mitochondrial respiration in non-failing control PBMCs, as indicated by decreased maximal respiratory capacity and energy production. Together, these data suggest a clinically relevant spectrum of molecular and metabolic impairment in SV immune cells. These intrinsic SV immune cell defects may predispose patients to life-limiting complications including cardiac failure and post-transplant graft failure. "

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Poster 20 - Differential RNA Signature of HLHS Patients based on Post-Norwood Outcomes
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Introduction - Hypoplastic left heart syndrome (HLHS) is characterized by a missing left ventricle and is surgically treated by the Norwood operation at the time of birth. The Norwood operation is associated with significant changes in cyanosis and volume overload in the right ventricle. In 20-30% patients, myocardial dysfunction occurs after the Norwood operation, and this leads to high patient mortality and increased costs. However, one postulate is that cardiomyocyte dysfunction in response to post-Norwood physiology results in patient mortality. The purpose of this study was to make use of bulk RNA-sequencing to determine the differential gene expression signature of post-Norwood patients based on outcomes.
Methods - Right ventricle biopsies were collected from HLHS patients at the time of the Norwood operation. All biopsies were collected by the CHS Biorepository and stored at -80oC. Once the outcome was known, unfavorable (transplant or mortality within 1-year) or favorable (free of transplant or mortality for 1-year) samples were grouped and used for the proposed study under IRB protocol H-49709. During preliminary studies, we used 4 samples from patients with favorable outcomes and 3 samples from patients with unfavorable outcomes (2 transplants, 1 mortality).
Results - Our first task was to understand changes in RNA expression between HLHS patients with favorable vs unfavorable outcomes at the time of Norwood. A total of 1095 genes were differentially regulated, with FDR<0.05 and fold change exceeding 1.5x. A summary of the differential expression analysis is presented as a volcano plot (Figure 1A), and a hierarchical clustering was generated for differentially expressed genes (Figure 1B). Collectively, this data serves to demonstrate the changes in gene expression between patients with favorable vs unfavorable post-Norwood outcomes.
Discussion - The results of this study suggest a differential RNA-sequencing expression profile between post-Norwood patients with favorable vs unfavorable outcomes. Identifying the specific pathways that are differentially regulated between these two patient groups will provide mechanistic insight into the clinical outcomes. This information will be invaluable in the management of HLHS patients and has the potential to lead to the development of therapies that can block these pathways and reduce the number of patients with unfavorable outcomes.

Poster 21 - Adaptation of Blood Viscoelastic Properties in Fontan and Glenn Circulations Compared to Healthy Individuals: the Single Ventricle Circulation as a Rheologic Disease Model
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Introduction - Fontan palliation results in passive blood flow to the lungs, altering shear stress on the vascular system, which results in vascular remodeling and dysfunction. This exploratory research study aimed to determine whether viscoelastic properties of blood, specifically viscosity, red blood cell deformability and aggregation, are affected by passive flow in Glenn and Fontan circulations as compared to healthy controls.
Methods - 90 Fontan (41% female, 14.2±1.09 years), 34 Glenn (44% female, 3.7±0.42 years), and 40 healthy controls (53% female, 22.5±3.77 years) were included in this study. Whole blood viscosity was measured at native and varying hematocrits using cone-plate viscometry and tube viscometry over various shear rates in healthy controls and patients with Glenn and Fontan circulations. Red blood cell (RBC) deformability and aggregation were measured using couette and cone-plate aggregometers. Aggregation was also measured after RBC-plasma switching between compatible healthy and Glenn/Fontan blood.
Results - Blood viscosity at native hematocrit was not different between Fontan [n=63], Glenn [n=34] and healthy [n=40]

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groups at any shear rate. Hematocrit-to-viscosity ratio (HVR) was higher in Fontan [n=30] and Glenn [n=12] vs. controls [n=40] across all hematocrits and shear rates ($p \leq 0.0002$), suggesting improved microcirculatory oxygen delivery potential. Glenn HVR was significantly higher than Fontan at shear rates of 37.5 s-1 and 75 s-1 for hematocrit 60, as well as shear rate 1500s-1 for hematocrit 20 ($p<0.019$). RBC deformability in Fontan [n=62] was higher than healthy controls at shear stresses of 0.5, 5, 8.89, 15.81, and 50.0 Pa ($p< 0.0491$) and tended to be higher at 0.89 and 1.58 Pa ($p=0.0576, 0.0844$). Glenn [n=33] RBC deformability was also significantly higher than healthy RBC [n=40] at shear stress below 15.81 Pa ($p<0.0116$), while it tended to be higher at 50 Pa ($p=0.059$). Glenn and Fontan RBC aggregation at stasis (7.00±1.64 a.u. and 11.20±1.14 a.u., respectively) and low shear (13.35±1.56 a.u. and 17.18±1.28 a.u., respectively) were significantly lower than healthy controls (stasis=15.29±0.79 a.u, low shear=22.24±1.07 a.u.), $p<0.0037$. Suspended in healthy matched plasma, Glenn and Fontan RBC aggregation increased at stasis (+5.058±1.56 a.u. [$p=0.0317$], +5.87±1.86 a.u. [$p=0.0159$], respectively) and low shear (+4.48±1.9 a.u. [$p=0.0793$], +6.29±2 a.u. [$p=0.0161$], respectively). When suspended in Fontan and Glenn plasma, healthy RBC aggregation decreased at stasis (-6.39±0.98 a.u., $p<0.0001$ and -7.74±1.5 a.u., $p=0.0004$) and low shear (-6.31±0.93 a.u., $p<0.0001$ and -6.37±0.87 a.u., $p<0.0001$).
Conclusion - Fontan and Glenn had higher HVR across multiple hematocrits and shear rates, suggesting improved microcirculatory oxygen delivery potential in the single ventricle circulation. The adaptation in blood viscosity is shear-dependent with increased RBC deformability and decreased RBC aggregation in both Fontan and Glenn circulations compared to healthy controls. The plasma suspending medium is responsible for changes in RBC aggregation as plasma switch experiments increased RBC aggregation from single ventricle participants or decreased RBC aggregation in healthy. Decreasing aggregation, and thus low shear rate viscosity, is a physiological compensation that may be a target for improvement of pulmonary blood flow in patients with passive pulmonary circulation.

Poster 22 - Centrosome reorganization is essential for perinatal cardiomyocyte development
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During cardiomyocyte (CM) maturation, the centrosome, which functions as a microtubule organizing center (MTOC) in CMs, undergoes dramatic structural reorganization where its components, localized to the centrioles embryonically, reorganize to the nuclear envelope perinatally. This programmed developmental process, referred to as centrosome reduction, has been previously associated with cell cycle exit. However, understanding of how this process influences broad CM cell biology, and whether its disruption results in human cardiac disease, remains unknown. Here, we describe an infant with a rare case of congenital dilated cardiomyopathy (cDCM) whose impaired cardiac function, and disrupted sarcomere and mitochondria structures were modeled using induced pluripotent stem cells (iPSCs). Through whole-exome sequencing and CRISPR/Cas9 gene knockout/correction, we identified the centrosomal protein rotatin (RTTN) as the causal gene, representing the first time a centrosome defect has been implicated in a non-syndromic dilated cardiomyopathy (DCM). In vivo genetic knockdowns in zebrafish and Drosophila confirmed an evolutionarily conserved requirement of RTTN for cardiac structure and function. To uncover mechanistic insights, we conducted extensive analysis of patient derived iPSC-derived cardiomyocytes (cDCM-CMs). Single cell RNA-sequencing (scRNA-seq) showed profound impaired maturation of cDCM-CMs, which underlie the observed CM structural and functional deficits. Interestingly, we observed persistent localization of the centrosome at the centriole, contrasting with expected programmed perinuclear reorganization, which led to subsequent global microtubule network defects. Finally, we identified a small molecule that restored centrosome reorganization, and significantly improved the structure and function of cDCM-CMs. In summary, this study demonstrates a role for RTTN in perinatal cardiac development through maturation of the centrosome, and suggests a novel process to investigate as the source of other congenital heart diseases.

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Poster 23 - Perfusion Optimization of Autogenerated Vascular Networks for Engineered Tissues and Bioreactors
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Ensuring adequate vascular perfusion within engineered tissues remains a salient challenge limiting the fabrication of tissues at clinically relevant scales. Although 3D biofabrication techniques are increasingly capable of achieving resolutions necessary for viable tissues, designing vascular networks capable of supporting tissues is a cumbersome and non-trivial process. Recently, methods have been developed to automatically design de novo vascular networks and simulate flow to predict hemodynamic performance within bioreactors. Although hemodynamic quantification provides necessary information for vascular integrity, it does not directly quantify oxygen/nutrient delivery through bulk tissue, thus it is unclear to what degree specified perfusion requirements satisfied. Because of difficulties capturing the heterogeneous perfusion behavior of multiscale circulation, many models approximate vessels within the tissue bulk as boundary conditions, ignoring contributions of vascular architecture. While this approach is acceptable for descriptive modeling of experimental perfusion data, this is a poor approach when designing vascular networks. Leveraging recent advances in multiscale perfusion modeling, we couple single, porous-media compartment models governed by Darcy's law to de novo vasculature generated for bioreactor perfusion spaces. This approach has been shown to demonstrate good agreement in patient-specific myocardial perfusion simulations when compared to [15O]H2O PET scans for coronary circulation (Papamanolis et al., 2021). Using our open-source hemodynamic modeling platform, SimVascular, we couple multiple levels of simulation fidelity within multiscale vascular networks to provide predictions of perfusion efficacy within bioreactor bulk tissue. Perfusion and hemodynamic simulations are used to form a black-box optimization problem in which the physical characteristics of vascular networks represent a vector of design parameters within a design space. This shape optimization problem is well-posed for derivative-free surrogate management framework (SMF) methods in which an expensive deterministic system is evaluated through combinations of surrogate-based infill techniques and patterned-based search procedures. Incorporating recent methods in concurrent SMF shape optimization, we demonstrate a novel method for automatically generating and optimizing de novo vasculature for key perfusion requirement to minimize the number of hypoxic regions within given bioreactor domains (Verma et al., 2020). This work further solidifies the utility of physics-based, rationally-informed design of engineered tissues towards functional constraints. In vitro, minimizing hypoxic regions correlates with decreased necrotic zones and increased tissue viability. The presented work has significant implications for decreasing biofabrication costs and understanding functional performance of engineered tissues.

Poster 24 - Safety and Long-term Survival of Human iPSC Cardiac lineages (hiPSC-CL) in Humanized Mice for Facilitating Patient Safety in the Clinical Application
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Hypoplastic Left Heart Syndrome (HLHS), severe congenital heart disease is a major cause of death and morbidity for which transplantation remains the only viable long-term treatment. There is no viable long-term treatment option for patients with single ventricle defects, other than heart transplantation. For this reason, stem cell-derived cardiomyocyte-based therapies have emerged as a potential therapeutic approach for myocardial repair in heart failure patients. The use of iPSC-derived clinically relevant cell types, such as cardiomyocytes, as cell therapies, poses potential safety concerns. Here, we provide an

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overview of potential safety issues and analyzed the long-term engraftment of the transplanted day 20 hiPSC-CL in a highly immune-deficient (NSG) normal mouse heart. Eight-week-old NSG mice were randomly selected into 5 groups that received either the Day 0 iPSC (n=14), Day 20 iPSC-CL (n=70), Day 20 iPSC-CL with 1 & 10% spike of iPSC (n=52) or vehicle alone (n=42). All test groups of animals received an intramyocardial injection of 3.0x106 cells/animal with an injectable volume of 20 µl. Each subject was assessed for cell engraftment, biodistribution, tumor formation, blood chemistry, and test article-related cardiac adverse events (Telemetry) for up to 9 months. All surviving mice were sacrificed at either 1,4 or 9-months post-treatment. Results demonstrate high-quality 3D culture production of Day 20 iPSC-CL achieved a very high-level long-term cell engraftment and maturation within the myocardium with minimal fibrosis around the graft, induced no teratomas in the heart, and incurred no significant changes in telemetry electrocardiogram. Results from our study suggest that transplanting iPSC-CL into the mouse myocardium in large numbers allows robust engraftment for long-term and did not reveal any cardiac toxicity events.

Poster 25 - Multiscale Investigations of the Hybrid Comprehensive stage II circulation

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Introduction - Hypoplastic Left Heart Syndrome (HLHS) is a Congenital Heart Disease (CHD) that leads to a single ventricle circulation (SV). A multitude of complications can occur with the existing three-stage palliative operation procedure leading to 50% survival rates. To reduce the failure rate and mitigate the trauma associated with the procedure, a novel palliation alternative technique called Hybrid Comprehensive Stage II (HCSII) featuring the inclusion of a stent and baffle in the left and right pulmonary arteries is proposed. This multiscale study aims to experimentally measure the overall coupled effects of the stent and baffle to the flow field and provide better insight into the hemodynamics of the HCSII alternative surgical technique to calculate the path lines of the blood flow passing through the main pulmonary artery (MPA).
Materials and Methods - The mock flow loop (MFL) was modeled to reflect the circulatory system of patients with a body surface area of approximately 0.34 m^2. The bench-top study includes a patient-derived 3D printed phantom of the reconstructed anatomy, incorporating an intra-pulmonary baffle graft and a Palmaz Genesis stent. The malformed aorta in the phantom is completely stenosed from the pulmonary trunk. The four-compartment MFL is based on a reduced lumped-parameter model (LPM) of the HCSII circulation and is tuned to match post-operative cardiac catheter reports of two patients. A digital video otoscope and a high-speed camera allow capturing videos of the deformation of the stented baffle and particle transport from their release at the inlet of the MPA as they travel through the HCSII vasculature. Particle residence time (PRT) was derived by training a Kalman filter-based adaptive tracker using a training algorithm. The cyclic baffle deformation videos were post-processed using a multi-featured tracker algorithm to determine coupled stent-baffle displacement at different locations in the pulmonary artery (PA) across each heart cycle. To cross-validate, the experimental deformation results, in-silico modeling by means of finite element analysis in Abaqus was carried out.
Results and Discussion - The stent and baffle deformation study reveal that for more than 10^3 cardiac cycles, all the tracked in-situ location on the stented baffle surface minimally deform under pulsatile loading conditions. The geometrical modification applied to the models had little effect on the oxygen delivery. Similarly, the PRT study reveals that particles injected in the MPA have successfully ejected within one cardiac cycle and no pathological flows were observed in the HCSII phantom.
Conclusions - This study indicates that the left and right pulmonary flow remain unobstructed given the small cyclic deformation of the stented baffle and no significant vortices are shed from the baffle in the MPA conduit of this reconstructed anatomy. The calculated particle residence time is shorter than a cardiac cycle implying no significant pathological flows present in this alternative surgical technique.

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Poster 26 - Effect Of Hepatic Vein Angle And Offset Anastomoses To Inferior Vena Cava On Augmenting Vascular Resistance And Total Energy Dissipation In Fontan Circulation
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In pediatric congenital heart defects, one ventricle cannot effectively pump the requisite quantity of blood to its intended location, requiring surgery to strategically optimize the existing cardiac infrastructure to re-distribute the blood flow in an energy efficient manner. Although augmenting the inferior vena cava(IVC) anastomosis within the pulmonary arterial circulation of the Fontan procedure is heavily researched, there is lack of investigation regarding the impact of how such circulation places undue stress upon the liver, increasing the overall pressure gradient within venous return, and thus progressively increasing the susceptibility of exacerbating the uni-ventricular function. Recognizing that the hepatic venous inlets are sensitive to such pressure changes, we sought to evaluate whether one could alleviate vascular resistance and energy loss within the hepatic venous circulation, to prolong Fontan circulation. To evaluate how perturbing the hepatic venous circuit could augment hemodynamics within the IVC, we performed cardiovascular fluid dynamic simulations using finite element analysis where an idealized geometric construction was utilized to assess how geometrically altering the angle and offset of attachment of the hepatic vein(HV) to the IVC would augment both vascular resistance and total energy dissipated. In testing angle anastomosis configurations ranging from 15-90 degrees, we found that the greater the anastomosis angle between the HV and IVC, the greater the vascular resistance and overall energy loss. Offset configurations including a single HV displacement, double same-side HV displacement, and bi-directional split offset along the center longitudinal axis, were analyzed and conveyed minimal differences in vascular resistance and energy dissipation in comparison with a geometry of no offset from the center longitudinal axis. However, the split-axis offset profile seemed to generate slightly lower vascular resistance and energy loss in comparison to the other offset profiles, warranting further investigation. Such analyses can optimally guide patient selection for the Fontan operation by using the HV-IVC morphology to identify those whom are more suitable for the Fontan operation and will successfully adapt to the Fontan hemodynamics, in comparison with patients whom may require more intensive care; thus elevating the ability provide patient-specific care to improve Fontan operation outcomes.

Poster 27 - Achieving consensus, severity-graded definitions of Fontan-associated complications
Kurt R. Schumacher*[1], Ari Cedars[2], Kiona Allen[3], David Goldberg[4], Adrianna Batazzi[1], Garrett Reichle[1], David Rosenthal[5], Melissa Cousino[1]; on behalf of the Fontan Circulatory Failure Study Investigators

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Objective - To develop consensus, severity-graded definitions of complications resulting from Fontan physiology.
Rationale - Fontan physiology leads to chronic changes in other organ systems that may affect long-term survival and success of advanced therapies such as heart transplantation. Inadequate assessment and treatment of the extra-cardiac effects of Fontan may contribute to lower post-heart transplant survival in Fontan patients compared to other cardiac diseases. Severity graded/ordinal consensus definitions of Fontan complications have never been developed which has significantly limited characterization of how Fontan-specific morbidity affect patient outcomes. Other disciplines, such as oncology and their Common Terminology for Criteria for Adverse Events, have successfully developed severity graded, disease-specific definitions and applied them to research and clinical care. This study aimed to build on experience in other disciplines to develop consensus definitions for Fontan-associated cardiac and extra-cardiac morbidity which may later be used characterize the full scope of morbidity for use in future studies risks for poor outcomes.

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Methods - A panel of Fontan patient and physiology experts, including pediatric, adult congenital, heart failure, and critical care cardiologists, as well as a pediatric nephrologist, hepatologist, and psychologist convened to develop definitions of Fontan complications. Definitions were created using this severity-graded, ordinal scale: grade 1 – mild; grade 2 moderate; grade 3 – severe; grade 4 – disabling or life-threatening. Following definition creation, a second panel of 21 experts in Fontan circulatory failure used modified Delphi methodology to modify and vote on definitions until consensus (>90% agreement without recommended further modification) was reached on final definitions.
Results - After 3 rounds of modification and voting over 6 weeks, consensus agreement was achieved on all Fontan-specific definitions. The following potential complications and morbidities of Fontan physiology were defined: anatomic Fontan pathway obstruction, cyanosis, systemic venous abnormalities resulting from venous insufficiency, atrial arrhythmia, ventricular arrhythmia, bradycardia, chronic pleural effusions, chronic ascites, protein-losing enteropathy, plastic bronchitis, hemoptysis and pulmonary hemorrhage, sleep apnea, Fontan-associated liver disease, portal and hepatic variceal disease, acute kidney injury affecting clinical treatment, polycythemia, thrombotic disease, recurrent or severe bacterial infection, skin atrophy, adrenal insufficiency, physical impact of previous stroke, mood/behavior disorder and neurodevelopmental disorder.
Conclusion - Consensus, severity-graded definitions of Fontan-specific cardiac and extra-cardiac complications were achieved and are available for use in research. They will allow more robust analyses of Fontan patient outcomes than previously possibly. Both retrospective and prospective studies of risks for pre- and post-heart transplant non-survival using the consensus definitions are underway. "

Poster 28 - Neurodevelopment in infants and toddlers with single ventricle physiology
Alexa Escapita*[1], Kelsey Renard Lambou[1], Julienne Thomas[1], Heather Raiees-Dana[1], Kenneth Knecht[1], Lawrence Greiten[1], Brian Reemtsen[1], Dala Zakaria[1], Shruti Tewar[1], Tara Johnson[1]

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Children with single ventricle physiology are at some of the highest risks for neurodevelopmental disabilities, which present as motor delays in infancy, behavioral problems in toddler years, and learning problems in school-age years. We hypothesize that children with single ventricle physiology have early detectable visual-motor (cognitive) and language delays. To test our hypothesis, we performed an exploratory pilot study to quantify development in a retrospective cohort of infants and toddlers with single ventricle physiology.

With IRB approval, we identified a retrospective cohort of full-term infants and toddlers with single ventricle physiology. These children have frequent neurodevelopmental evaluations in Arkansas Children's Hospital's Cardiac Neurodevelopmental Program, using the American Heart Association categories for high-risk infants and toddlers. Providers perform frequent assessments of these children using the Capute Scales, which results in developmental quotients that quantify development in the cognitive and language domains. The Cognitive Adaptive Test (CAT) measures visual-motor (cognitive) skills, and the Clinical Linguistic and Auditory Milestones Scale (CLAMS) measures language skills. When appropriate, providers refer children to therapy, and they treat them for comorbid or co-occurring conditions. Our goal was to quantify developmental trajectories within the population by tracking changes in developmental quotients over time. Using anecdotal evidence from our clinic, we used the assumption that initial developmental quotients were approximately in the age-appropriate range, and that they would decrease to a clinically significant level by three years of age. To measure the change in development quotients over time, we created a univariate linear regression model. We defined age-appropriate developmental quotients as 85-115, borderline delay as 70-84, and mild delay as 55-69. Statistical significance was defined with alpha < 0.05.

We recruited all 21 infants and children with single ventricle physiology. Using our data from our univariate linear regression model, we determined the relationships between developmental quotients and age at evaluation. Cognitive (CAT) developmental quotients were in the age-appropriate range at 3 months 103 (95% CI:[96-111]), and then they decreased significantly in a linear fashion to 75 (95% CI:[65-84]) by 36 months of age, $R2 = 0.32$, $p < 0.001$. Language (CLAMS) developmen-

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tal quotients were in the age-appropriate range at 3 months 102 (95% CI:[92-112]), and then they decreased significantly in a linear fashion to 66 (95% CI:[54-78]) by 36 months of age, R2 = 0.28, p < 0.001. CAT developmental quotients decreased from age-appropriate to borderline-mild delay, and CLAMS developmental quotients decreased from age-appropriate to mild delay over the first three years of life

Children with single ventricle physiology have declining language and visual-motor development over their first three years of life. The Capute Scales are predictive of future language delays and visual-motor delays in infants and toddlers with single ventricle physiology. By tracking early neurodevelopment in this population, we can recommend and provide early developmental intervention at a younger age, when neuroplasticity is greatest. Through early detection and early intervention, we aim to improve the quality of life of children with single ventricle physiology."

Poster 29 - Parental Impressions and Perceptions of Efficacy in Prenatal Counseling for Single Ventricle Congenital Heart Disease

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Background/Objectives - Fetal echocardiography readily allows for identification of a wide variety of congenital heart disease before birth. Prenatal detection provides the opportunity for proper timely counseling and education of prospective parents. In no condition is there a greater need for detailed comprehensive prenatal counseling than following diagnosis of single ventricle type of congenital heart disease (SVCHD). Treatment requires a series of complex surgical interventions, for which there is no complete cure, in addition to a lifelong burden of health-related challenges. Although commonly performed by experienced practitioners, optimal styles, strategies, and techniques to achieve the most effective prenatal counseling for SVCHD have not been extensively explored. We investigate the perceived efficacy of prenatal counseling via feedback from a survey to parents of children prenatally diagnosed with SVCHD.
Methods - A survey was developed and distributed to parents of children with SVCHD diagnosed and counseled by the Fetal Heart Program (FHP) at Children's Hospital of Philadelphia. Counseling included a structured approach covering multiple domains from a multidisciplinary team including cardiologist, nurse coordinator, surgeon, and social worker. Serial encounters occurred prenatally every 4 weeks from initial diagnosis until birth. The survey utilized Likert scale grades (1 lowest-5 highest) with queries concerning: 1) quantity of counseling, 2) explanation of the heart defect, 3) preparation for surgery, 4) preparation for hospital course, 5) preparation for complications/outcomes of a Fontan circulation, and 6) preparation for neurological/behavioral problems. Queries solicited grades on usefulness of meeting with specific FHP team members including: 1) fetal cardiologist, 2) fetal heart nurse, 3) fetal heart social worker, and 4) cardiac surgeon. A composite score was generated. Data on burden of care and complications was evaluated as modifiers of grades.
Results - 248 families with prenatal diagnosis of SVCHD met study criteria, of which 65 (26%) responded. All completed or were planned to shortly complete Fontan procedure, with median child age of 5 years (range 1-10 years). With regards to preparation for various aspects of care, average scores were highest for description of the surgery and lowest for preparation concerning neurological/behavioral issues. Average level of preparation for complications and outcomes of the Fontan circulation was lower than preparation for surgery ($p<0.01$) and hospital course ($p<0.05$). Average level of preparation for neurological/behavioral issues was lower than surgery ($p<0.0001$) and hospital course ($p<0.001$). Overall, satisfaction with members of the FHP team was high. Cardiac surgeon had the highest average while social worker had the lowest average rating with fetal cardiologist and nurse coordinator ratings in between. Negative correlation ($r=-0.43$; $p<0.001$) was found between the total length of hospitalization prior to Fontan palliation and the composite score for each participant.
Conclusions - Feedback from families inform on strengths and weaknesses in prenatal counseling for SVCHD. Greater emphasis on neurological/behavioral outcomes is necessary. Subsequent hospital course influences impressions of prenatal counseling efficacy. Prenatal engagement with cardiac surgeon is viewed favorably. Our results inform on development of initiatives and protocols for improving efficacy in prenatal counseling in SVCHD.

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Poster 30 - Disruption of Notch1 and Gata5 in mice results in clinically relevant congenital aortic valve stenosis
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Congenital aortic valve stenosis can occur as an isolated defect or in the setting of hypoplastic left heart syndrome (HLHS), a severe form of congenital heart disease (CHD) which encompasses underdevelopment of all left-sided heart structures. Critical congenital aortic valve stenosis and HLHS are associated with significant mortality and long-term morbidity. Despite significant advances in the understanding of the genetic architecture of CHD, the etiology and underlying mechanisms of congenital aortic valve stenosis and other left-sided cardiac malformations including HLHS are largely unknown. Interestingly, heterozygous pathogenic variation in NOTCH1 has been associated with left-sided CHD including aortic valve stenosis and HLHS. While NOTCH1 heterozygote mice do not display CHD, we previously reported that Notch1;Nos3 mutant mice display congenital aortic valve disease along with additional cardiac outflow tract malformations, however, this model has limitations since ~65% of mutant mice suffer neonatal lethality. GATA5 is also implicated in human aortic valve disease, and Gata5-/- mice exhibit bicuspid aortic valve but with only 25% incidence, showing an associated reduction in Notch signaling. Therefore, we aimed to generate novel murine models of congenital aortic valve stenosis with high disease incidence. We hypothesized that the interaction of Notch1 and Gata5 was critical for aortic valve morphogenesis, and generated mouse models by intercrossing Notch1 and Gata5 heterozygote mice. Mice heterozygous for Notch1 and Gata5 (Notch1+/-;Gata5+/-), and heterozygous for Notch1 and null for Gata5 (Notch1+/-;Gata5-/-) demonstrated no neonatal lethality. Echocardiographic analyses demonstrated aortic valve stenosis in ~50% of Notch1+/-;Gata5+/- mice and ~80% of Notch1+/-;Gata5-/- mice by 16 weeks of age. Notably, progression of aortic valve stenosis, as measured by an increase in aortic velocity from 6 to 16 weeks, was found in Notch1;Gata5 compound mutant mice. Furthermore, thickened and malformed aortic valves were found by histological examination in Notch1;Gata5 compound mutant mice at 16 weeks and 1 year. Interestingly, thickened and dysmorphic aortic valves were also exhibited in Notch1;Gata5 compound mutant mice at embryonic day (E)18.5 and postnatal day 10, consistent with a congenital phenotype. Of note, the left ventricle appeared to be of normal size by histologic sectioning at E18.5. Immunostaining showed a significant decrease in the expression of NOTCH1 intracellular domain (NICD) and Nos3 in aortic valves of Notch1;Gata5 compound mutant mice as compared to wildtype littermates. In conclusion, our findings demonstrate a novel genetic interaction between Notch1 and Gata5. These new compound mutant mouse models display highly penetrant congenital and progressive aortic valve stenosis with normal left ventricular size and without lethality. This model will be used for unbiased genomic approaches to identify novel therapeutic targets for aortic valve disease initiation and progression.

Poster 31 - Defining the "Usual" Right Ventricle in Hypoplastic Left Heart Syndrome Patients with Statistical Shape Modeling
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Background - Beyond postoperative outcomes of single ventricle palliation, there is great interest in determining the functional status of the systemic right ventricle (RV) in hypoplastic left heart syndrome (HLHS). Current diagnostic imaging such as cardiac magnetic resonance imaging (cMRI) can risk-stratify HLHS patients, however conventional cMRI measurements (including RV size and function) do not consider the heterogenous morphology of the RV. This study utilized statistical shape modeling to determine a mean shape template of the "usual" RV in HLHS, identifying shape modes that may be relevant for guiding therapy.
Methods - This was a retrospective cohort study of HLHS patients utilizing cMRI datsets. 3D end-diastolic models of the RV

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were created from cine, MR angiography and 3D SSFP datasets using Mimics (Materialise, Leuven, Netherlands). The 3D models were then uniformly re-meshed and rigidly aligned using the iterative closest point technique. The mean shape template was computed using an iterative technique based on the Large Deformation Diffeomorphic Metric Mapping (LDDMM) implemented into the open-source software Deformetrica. Shape modes (deviations from the mean shape template) were computed using the Principal Component Analysis (PCA) technique implemented into in-house MATLAB software.
Results - 21 HLHS studies (body surface area 1.52±0.42, age 17.2±7.4) were included. The cohort had RV ejection fraction 45±7% and indexed RV end-diastolic volume 115±27 mL/m2. From PCA analysis, 20 independent principal component modes were extracted, with 95% of shape variance achieved by Shape mode 8. Shape mode 4, which was associated with prominence and bulging of the RV outflow tract relative to the RV body, correlated with indexed RV end-diastolic volume ($r=0.745$, $p<0.001$), indexed RV end-systolic volume ($r=0.503$, $p<0.001$) and indexed RV stroke volume ($r=0.639$, $p<0.001$).
Conclusions - Statistical shape modeling can be applied to HLHS and other forms of systemic RVs. Deriving a mean shape template of the "usual" RV" and shape mode variations may serve as advanced biomarkers that determine RV dysfunction in HLHS.

Poster 32 - Evaluating anti-LYST LNA siRNA as a strategy for preventing TEVG stenosis
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Congenital heart disease is the leading cause of death due to birth defects in the newborn period. Non-degradable synthetic vascular grafts represent a critical tool in the surgical management of CHD. Yet, they are associated with complications including thromboembolism and stenosis, and importantly, these grafts do not have growth capacity. Tissue-engineered vascular grafts (TEVGs) offer an autologous tissue replacement with growth potential; however, the development of stenosis has challenged their clinical use. Recent work in our lab has identified the lysosomal trafficking regulator (LYST) protein as a key contributor to the progression of stenosis. Indeed, mice with mutations in the murine homolog, Lyst, demonstrated reduced occlusion of vascular grafts compared to wild-type controls. The critical role of Lyst in the formation of stenosis suggests its potential as a therapeutic target for preventing graft complications in the clinic.
To further evaluate this therapeutic target, we have developed a locked nucleic acid (LNA) siRNA that targets the Lyst gene. We utilized murine bone marrow-derived macrophages and primary murine fibroblasts to validate the efficacy of the siRNA in knocking down Lyst gene expression. In vitro treatment with the siRNA significantly reduced Lyst expression in mouse fibroblasts ($p = 0.0256$) and macrophages ($p = 0.0197$). To investigate the efficacy of siRNA treatment in vivo, we implanted unseeded TEVGs as interposition inferior vena cava conduits in C57Bl/6J mice. Mice received a 1.0 mg/kg injection of either anti-LYST siRNA or a scrambled siRNA negative control encapsulated in lipid nanoparticles either intravenously or directly onto the graft during surgery. At two weeks post-implantation, mice were imaged with micro-CT to evaluate graft patency. In vivo treatment with the siRNA did not affect the formation of stenosis compared to negative control mice. A challenge in interpreting these results is the unclear host cell contribution to graft inflammation and subsequent formation of stenosis. To further elucidate the critical cell types involved in Lyst-mediated stenosis, we have generated a mouse model with conditional Lyst knockout in macrophages. Pending experiments are designed to determine whether mice with Lyst-deficient macrophages experience decreased incidence of stenosis compared to wild-type controls. Here, we present an initial phenotypic and genotypic characterization of this novel mutant."

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Poster 34 - Single-cell transcriptomics reveals impaired human cardiac cell lineage determination and cardiomyocyte proliferation due to NOTCH1 deficiency
Mingtao Zhao*[1]

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NOTCH1 pathogenic variants are implicated in multiple types of congenital heart defects including hypoplastic left heart syndrome (HLHS). However, mechanisms by which these pathogenic variants cause ventricular abnormalities in HLHS remain unknown. Here, we have utilized CRISPR/Cas9 genome editing to knock out NOTCH1 in human induced pluripotent stem cells (iPSCs). Ventricular cardiomyocyte differentiation is impaired, whereas atrial cardiomyocyte determination is promoted in NOTCH1 homozygous knockout (N1KO) iPSCs. NOTCH1 deficiency leads to defective proliferation of early human cardiomyocytes, and transcriptomic analysis indicates that pathways involved in cell cycle progression and mitosis are downregulated in N1KO cardiomyocytes. Single-cell transcriptomic analysis reveals that epicardial and second heart field progenitors are more prevalent at the expense of first heart field progenitors in N1KO cell populations. We conclude that NOTCH1 is essential for human ventricular cardiomyocyte differentiation and proliferation through balancing cell fate determination of cardiac mesoderm and modulating cell cycle progression. Our study provides valuable insights into the mechanisms by which NOTCH1 pathogenic variants lead to ventricular hypoplasia in HLHS.

Poster 35 - Characterization of cellular changes following hemodynamic induced single ventricle physiology in the chick embryo
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Congenital heart defects are the leading cause of death in infants under one year of age. Untreated hypoplastic left heart syndrome (HLHS) is responsible for over one quarter of neonatal cardiac fatalities. Because genetic causes only account for an estimated 20% of cases, understanding the role that environmental factors play in disease pathogenesis is crucial. To date, there exist two animal models for the HLHS four chamber phenotype--a genetic mouse model and mechanical chick model. The flow-induced left atrial ligation (LAL) model restricts flow into the developing left ventricle before the completion of cardiac looping and septation. Abnormal hemodynamic and strain patterns result in a hyperplastic single ventricle physiology. While changes in the resulting HLHS phenotype's function, trabeculation and tissue structure have been extensively characterized, these characterizations have not been extended to the single cell level. Here, we assess how the impact of LAL on cellular phenotypes propagates from one hour post-insult to multiple days post-insult, when cardiac septation has taken place and the HLHS phenotype is apparent.
Methods - We incubated G. domesticus chick embryos up until HH31 (for controls) and HH23 for embryos undergoing left atrial ligation. In order to obtain cellular suspensions, hearts were dissected out and the cardiac tissue dissociated. A suture was tied over the developing left atrium of HH23 (Day 4) embryos and pulled tightly enough to arrest flow. LAL embryos were subsequently re-incubated and allowed to develop up until HH31 (Day 7), with cardiac cells isolated at one hour post-intervention for a small subset followed by 24 hour increments for subsequent LAL cell populations. Cells underwent regular imaging to characterize their structure and spreading patterns before being subjected to a custom nano-sensor to further characterize membrane properties.
Results & Conclusions - LAL harvested cardiac cells displayed lower rates of cell-substrate spreading and lower rates of multicellular patterning as compared to controls. We hypothesized that changes in the LAL cell population were brought about by instantaneous changes in wall shear stress that occurred immediately following ligation as well as the sustained absence of flow necessary to further activate mature cardiomyocyte phenotypes. Investigation into nuclear envelope mechanics is underway and may further clarify changes between LAL cell populations and that of controls, as the cells progress

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towards the severe HLHS phenotype. Ultimately, understanding the cellular-level mechanisms underlying single ventricle etiology paves the way for development of further interventions in the formation of congenital heart defects.

Poster 36 - Single ventricle in heterotaxy syndrome: an anatomic study
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Aim of the study - Heterotaxy syndrome is associated with abnormal left/right patterning of the embryo. Our aim was to evaluate, in a cohort of foetal heart specimens with heterotaxy, the incidence of single ventricle, and to describe its morphology.
Material and Methods - We analyzed 80 fetal specimens with heterotaxy, divided in right or left isomerism according to the internal morphology of the atrial appendages (extent of the pectinate muscles) or, when the appendages were not isomeric, to the morphology of the bronchi. Among them, 30 were found to have right isomerism (RI), 50 left isomerism (LI). A ventricle was defined as hypoplastic when it did not extend to the apex of the heart with an hypoplastic atrioventricular valve which would have precluded biventricular surgical repair.
Results - Single ventricle was found in 43/80 heart specimens (53.7%), 20/30 with RI, 23/50 LI (67% vs 46%, p=ns). The single ventricle was morphologically right in 29 (hypoplastic left ventricle), left in 14 (67.4% vs 32.5%, p=0.03). Hypoplastic left ventricle was more frequent in patients with RI than in those with LI (50% vs 28%, p<0.05), while the incidence of hypoplastic right ventricle was not different in RI and LI (16.7% vs 18%, p=ns). Hypoplastic left heart syndrome (extreme left ventricular hypoplasia with atrioventricular valve atresia or extreme hypoplasia, and atresia or severe stenosis of the arterial valve originating from the left ventricle) was more frequent in RI than in LI (23.3% vs 6%, p<0.03). All hearts with RI had a common atrioventricular junction with complete atrioventricular canal, vs 69.5% of hearts with LI.
Conclusion - Single ventricle morphology is present in more than half of foetuses with heterotaxy. Morphologically right single ventricle (hypoplastic left ventricle) is twice more frequent than morphologically left single ventricle in the cohort as a whole and is found predominantly in RI. The association of hypoplastic left heart syndrome with heterotaxy is not rare (10/80) and is also more frequent in RI. These results underline the severity of cardiac malformations associated with heterotaxy and explains the worse prognosis after Fontan-type surgery in these patients. Experimental research on animal models is now warranted to analyze the contribution of left/right patterning in the etiology of single ventricle heart defects.

Poster 37 - Prediction of Ventricular Growth in Single Ventricle Fetuses using Circulating Cell-Free miRNAs in Maternal Blood
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Single-ventricle heart defects (SVHD), such as hypoplastic left heart syndrome (HLHS) and pulmonary atresia with intact ventricular septum (PA-IVS), are congenital heart defects where one of the heart's ventricles is underdeveloped, causing abnormal hemodynamics in newborns that could be lethal if left untreated. Although medical interventions have improved the survival of infants into adulthood, many patients still suffer from cardiac complications and morbidities. The earliest detection time for fetal SVHD is at around 20 weeks by echocardiogram; however, by this time, the defect has already developed enough to be manifested and visualized. Thus, in this study, we sought to discover novel biomarkers in the maternal blood of

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pregnant women that potentially signal SVHD pathogenesis of their fetuses. Our supposition on the feasibility of this study is that human adult cardiomyocytes, such as the case with the pregnant mother, do not possess the ability to proliferate. On the other hand, cardiomyocytes in the developing fetus abundantly divide to form the full-functioning heart. Thus, we hypothesize that small secretory signaling molecules from fetal proliferating cardiomyocytes are released in the maternal circulatory system and can serve as indicators of abnormal ventricular growth in the developing SVHD fetuses. In this study, we used both clinical and in vitro approaches to identify possible cell-free miRNAs released from the developing fetal heart in the maternal systemic circulation. Blood plasma of pregnant women with healthy and SVHD fetuses were screened for differentially expressed cell-free miRNAs and was calibrated against miRNA signatures observed in an induced pluripotent stem cell-derived cardiomyocyte (iPSC-CM) model from healthy and SVHD donors. Using this combinatorial approach, we identified two miRNAs that were elevated in women gestating SVHD babies and in proliferating iPSC-CMs from SVHD patients compared to healthy controls. We expect to further characterize and establish these circulating cell-free miRNAs in the maternal blood as a potential non-invasive biomarker for the prediction of abnormal fetal cardiac development leading to SVHDs. Results from this study will lay foundation in changing the way we screen and diagnose SVHDs, which could lead to alternative strategies for early fetal intervention to improve clinical outcomes.

Poster 38 - The role of RNA-binding protein QKI in ventricular wall development
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QKI is a unique RNA-binding protein within hnRNP K-homology domain family. Recently, we have found a rare de novo mutation of QKI in a Hypoplastic Left Heart Syndrome (HLHS) patient with an in-frame 88-amino acid deletion that effectively removes the critical Src-PTK binding site. By analyzing a QkiLacZ reporter mouse line, QKI expression is found in the developing heart as early as E7.5 and remained in both cardiomyocytes and endocardial cells. This expression pattern is well maintained in the adult mouse heart. QkiLacZ/LacZ mutant embryos die around E10.5 and show multiple developmental defects, including severe cardiac developmental defects with hypoplastic ventricular wall and collapsed endocardial structure. To determine the role of QKI in human cardiac development, we have generated mutant human embryonic stem cells (hESCs) that are deficient in QKI (QKIdel) using CRISPR/Cas9 genomic editing technology. hESCs-QKIdel maintains normal self-renewal activity and pluripotency. Transcriptomic analysis at single-cell resolution (scRNA-seq) further demonstrates that hESCs-QKIdel can efficiently differentiate into cardioprogenitor cells. However, these mutant cardioprogenitors have a slightly reduced level of proliferative activity and fail to produce functional cardiomyocytes. Bulk RNA-seq and replicate multivariate analysis of transcript splicing (rMATS) demonstrates dramatically altered pre-mRNA splicing events in key genes involved in myofibrillogenesis. More interestingly, endocardial cells derived from hESCs-QKIdel also exhibit a significant defect in proliferative activity, which is independent of cardiomyocyte defect, confirming that QKI is involved in cardiogenic events likely via both developing cardiomyocytes and endocardial cells. This finding was further confirmed by a series of conditional Qki knockout model.

Poster 39 - PCBP1 is an important alternative splicing regulator for cardiac development
Yao Wei Lu*[1], Zhuomin Liang[1], Haipeng Guo[1], Tiago Fernandes[1], Xiaoyun Hu[1], Ramon A Espinoza-Lewis[1], Douglas Cowan[1], John D Mably[2], Hong Chen[1], Da-Zhi Wang[1,2]

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Proper development of the heart relies on a tightly regulated, yet diverse, pattern of gene expression that is governed by transcriptional, post-transcriptional, and translational processes. Congenital heart disease (CHD) is responsible for most common birth defects and is also a leading cause of morbidity and mortality in children. While the genetic causes for some

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types of CHD have been identified, the molecular basis for the rest remains elusive. Poly(rC)-binding protein 1 (Pcbp1) is an RNA-binding protein that regulates RNA processing as well as post-transcriptional and translational processes in a variety of biological systems. We hypothesize that Pcbp1 plays a critical role in regulating heart development by governing Notch and unfolded protein response (UPR) pathways and mediating proper Aars2 gene splicing. Germline deletion of Pcbp1 results in lethality before embryonic day (E) 8.5. We generated a cardiac-specific deletion of Pcbp1 by crossing Pcbp1-Flox with cTNT-Cre mice (Pcbp1-cKO) and found that 50% of the Pcbp1-cKO mice die perinatally. Embryonic hearts from Pcbp1-cKO mice displayed ventricular non-compaction and abnormal ventricular apex formation. Deep RNA sequencing of Pcbp1-cKO hearts revealed alteration of gene expression profiles consistent with delayed ventricular maturation and dysregulation of Notch and UPR pathways. Interestingly, loss of Pcbp1 in cardiomyocytes disrupts alternative splicing of many important genes, including Aars2, a gene associated with a congenital mitochondrial cardiomyopathy. Pcbp1 deficiency resulted in the generation of an Aars2 exon16-skipping variant, leading to its premature termination. eCLIP-seq showed that Pcbp1 primarily binds to CU-rich motifs within the 3'UTR, distal introns and CDS regions of targets, and interacts with regions of the Aars2 transcript near exon 16. Using CRISPR/Cas9 technology, we knocked in loxP sites flanking exon 16 of Aars2 (Aars2-Flox) and crossed the floxed mice with cTNT-Cre mice to generate a cardiac-specific exon 16 deletion mutant of Aars2 (Aars2-cKO). Intriguingly, the Aars2-cKO embryonic hearts exhibit abnormalities that phenocopy features observed of Pcbp1-cKO hearts, including ventricular non-compaction. Accordingly, the transcriptome from hearts of Pcbp1-cKO and Aars2-cKO display high concordance and share striking commonality in the dysregulated pathways. These studies establish a novel function for Pcbp1 in heart development through the regulation of the Notch and UPR pathways. Additionally, we determined that Pcbp1 is crucial for Aars2 gene splicing, deficiency of which is associated with congenital cardiomyopathy. Our findings suggest that modulation of Pcbp1 in developing hearts may offer a novel therapeutic approach for the treatment of congenital heart defects.

Poster 40 - Mice harboring a novel de novo missense RBFOX2 variant associated with hypoplastic left heart syndrome display abnormal heart development

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Hypoplastic Left Heart Syndrome (HLHS) is a complex form of congenital heart disease, characterized by severe underdevelopment of the left ventricle, stenosis or atresia of the mitral and aortic valves, and hypoplasia of the ascending aorta and aortic arch. Despite advances in surgical and palliative strategies leading to improved neonatal survival outcomes, HLHS is still associated with early mortality and long-term morbidity. Elucidation of HLHS etiology is compounded by its complex inheritance mechanism and heterogenous genetic landscape, with studies indicating a multigenic etiology. We performed exome sequencing on a parent-offspring trio in which the proband was affected with HLHS and identified a novel de novo missense variant in the gene, RBFOX2, that was bioinformatically predicted to be pathogenic. The pathogenicity of this variant is supported by exome sequencing of large patient cohorts which identified pathogenic loss-of-function RBFOX2 variants in patients with HLHS. RBFOX2 belongs to a family of RNA-binding proteins that is broadly expressed in all tissues and regulates splicing and transcription of many targets, including those involved in heart development. The RBFOX2 missense variant demonstrated normal nuclear localization in vitro. In order to determine the in vivo pathogenicity of the RBFOX2 variant, we generated a knock-in mouse model using CRISPR/Cas9 technology. RBFOX2KI/KI mice were found to be embryonic lethal. Morphological analysis revealed 100% of homozygous mutants exhibit gross developmental defects. Normal Mendelian ratios are found at E9.5 but homozygous mutants display severe growth retardation from E9.5 to E11.5. Histologic analysis demonstrated underdevelopment of the ventricular chambers starting at E9.5. Analysis of E9.5 RBFOX2KI/KI embryos suggests minimal expression of RBFOX2 in the developing heart with reduced expression of cardiac troponin T, a marker of

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cardiomyocyte differentiation. Overall, these mutant mice, harboring a clinically relevant human HLHS variant in RBFOX2, display highly penetrant hypoplastic hearts with significant growth retardation. The use of this new murine model will pave the way for better cellular and molecular characterization of the deficits in RBFOX2 function that contribute to HLHS.

Poster 42 - Ribosomal protein genes as a novel class of Congenital Heart Disease candidates regulating cardiac growth and proliferation in concert with cardiac transcription factors

Rolf Bodmer*[1], Tanja Nielsen[1], Anias Kervadec[1], X.-X. Zeng[1], Analyne Schroeder[1], Jeanne Theis[2], Timothy Olsen[2], Karen Ocorr[1], Alexandre Colas[1], Georg Vogler[1]

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Hypoplastic Left Heart Syndrome (HLHS) is characterized by an underdeveloped left ventricle and is the most severe Congenital Heart Disease (CHD). Its etiology is poorly understood but is likely oligogenic. Sequencing efforts identified thousands of putative human disease variants, however, establishing genotype-phenotype relationships remains challenging. To address this, we have performed high-throughput in vivo functional analyses of candidate genes using the fly heart, human iPSC-cardiomyocytes (hiPSC-CMs), with validation in zebrafish to interrogate their potential contributions in CHD/HLHS. Whole-genome-sequencing of HLHS proband-parent trios with poor clinical outcome and GO enrichment analysis of prioritized genes revealed an over-representation for ribosomal protein (RP) genes. In patient-derived iPSC-CM carrying an RPS15A variant proliferation was reduced compared to the parents. Knockdown of variant-carrying RP genes in generic hiPSC-CMs also reduced proliferation and triggered a transcriptomic response consistent with nucleolar stress. In adult flies, RP knockdown resulted in partial or complete heart loss or reduced contractility. Consistent with nucleolar stress, reducing RpS15Aa function increased the size of cardiomyocyte nucleoli. In zebrafish, rps15a knockdown led to reduced CM numbers and/or contractility, and defective heart looping. Probing for cardiac-specific RP functions, we found conserved, synergistic interactions between RPS15A and cardiac transcription factors tinman/Nkx2.7 and dorsocross/Tbx5a/TBX5 in Drosophila, zebrafish, and hiPSC-CMs. RPS15A knockdown-induced defects were significantly reversed (1) by p53 co-knockdown in hiPSC-CMs and zebrafish, or (2) by YAP/yorkie overexpression or myc co-knockdown in flies.
We conclude that RP genes play a critical role in cardiac growth/ CM proliferation, likely in conjunction with cardiogenic genes, thus representing a potential novel class of genetic effectors in CHD/HLHS.

Poster 43 - Single-cell genomics study of Fontan-associated liver disease

Liming Pei*[1,2], Po Hu[1], Juanjuan Zhao[1], Wenbao Yu[1], Benjamin Wilkins[1], Aidan Bauer[1], Kai Tan[1], Jack Rychik[1]

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The Fontan operation is the current strategy of care for single ventricle congenital heart disease (SVCHD) in which there is only one functional ventricle. Although operative survival has improved over the years, it is now evident that these surviving SVCHD patients are facing new life-threatening challenges: severe complications resulting from the Fontan operation, including one of the most evident consequences, hepatic fibrosis, that is now recognized as Fontan-Associated Liver Disease (FALD). However, the fundamental mechanisms underlying FALD remain little understood. Here we generate the first single cell atlas of FALD by studying normal and early-stage Fontan livers with multiomic snRNA-ATAC-seq. Fontan livers exhibit

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cell type-specific gene expression changes, affecting mostly central hepatocytes but also significantly endothelial cells and hepatic stellate cells. In particular, central hepatocytes exhibit profound metabolic changes. Therefore, FALD is not only a hepatic fibrosis disease, but also features severe dysregulation of liver metabolism.

Poster 44 - High-throughput cardiac in vivo platform to functionally validate genome-wide candidate genes for congenital heart disease
Georg Vogler*[1], Jeanne L. Theis[2], Marco Tamayo[1], Karen Ocorr[1], Alexandre Colas[1], Timothy M. Olson[2], Timothy J. Nelson[2], Rolf Bodmer[1]

[1]Sanford-Burnham Medical Research Institute
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Patient-specific genomics is now a major diagnostic tool for the understanding of congenital heart disease (CHD) with the potential to predict the outcome following medical intervention. The wealth of genetic information for each patient is in stark contrast to the available functional data on affected genes and gene-gene interactions, presenting the major hurdle in leveraging patient-specific genomics. To close this genotype-to-phenotype gap we adapted an in vivo Drosophila heart model to allow rapid functional characterization of cardiac gene function and interaction at high spatio-temporal resolution. Following image-acquisition, the phenotypic characterization of cardiac parameters (e.g. contractility and heart size) is fully automated and unbiased. The simplicity of the Drosophila genome is a unique feature of this genetic model organism, which has the benefit of permitting the rapid construction of CHD gene networks, and focus on gene networks affected in patients with hypoplastic-left heart syndrome (HLHS). Our platform complements alternative approaches, such as patient-derived iPS-cardiomyocyte cultures and genetically more complex vertebrate model systems. We present our approach to fully functionally characterize hundreds of candidate genes for HLHS, many are required for normal heart structure and function, with possible implications for future diagnostics and care of HLHS.

Poster 45 - Validation of Candidate HLHS Genes in the Zebrafish Heart Model
Karen Ocorr*[1], X.-X. Zeng[1], Tanja Nielsen[1], Katya Marchetti[1], Ingolf Reim[2], Analyne Schroeder[1], Jeanne Theis[3], Timothy Olsen[3], Alexandre Colas[1], Georg Vogler[1], Paul Grossfeld[4], Rolf Bodmer[1]

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Hypoplastic Left Heart Syndrome or HLHS accounts for 7-9% of congenital heart diseases and is known to have a genetic etiology. However, the genetics and cellular mechanisms causing this disease have not been delineated, likely because this disease is due to interactions among multiple genetic variants. We had identified and preliminarily tested a large number of candidate genes based on sequencing and genomic data from HLHS patients at the Mayo Clinic and at UCSD-Rady Children's Hospital. Using the Zebrafish cardiac development model we have now validated several high value HLHS genes.

One of these genes encoding the transcription factor ETS1 is part of a deletion found in Jacobsen's syndrome patients, who exhibit a high incidence of HLHS. We found that reducing function of ETS1 (pointed or pnt, in the fly) caused misspecification of cardioblasts in the Drosophila heart. CRISPR KO of ETS family genes (ETS1, ETS2 and ETV2) in the embryonic fish heart caused significant reductions in chamber size associated with delayed development of myocardial cells. In addition, we found that KO hearts exhibited reduced contractility and bradycardia resulting in significant reductions in cardiac output.

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Ribosomal Protein genes, and in particular RPS15A, were identified from whole-genome-sequencing of HLHS proband-parent trios at the Mayo Clinic. Surprisingly, cardiac KD of RPS15a caused a "No heart" phenotype (flies can live for a short time without a heart since they get oxygen through a separate system). CRISPR KO in fish caused a significant reduction in the number of myocardial cells that that was more pronounced in the ventricle. Importantly, we identified genetic interactions between RPS15a with the cardiac transcription factors NKX 2.7 (fish homolog of NKX 2.5) that reduced cardiac contractility, measured as fractional area change. In addition, co-KD of RPS15a and NKX2.7 caused bradycardia and 2:1 heart block at the atrioventricular canal. RPS15a also interacted with TBX5 to cause bradycardia.

Identification of genes and genetic interactions that produce similar phenotypes in human, fly and fish will make it possible to delineate conserved genetic pathways underlying HLHS, providing new diagnostic tools and potential targets for therapy.

Poster 46 - The Effect of Upstream Flow Mixing on the Hemodynamic Assessment of Total Cavopulmonary Connections
Zhenglun Alan Wei*[1]

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Introduction
Total cavopulmonary connections (TCPCs) are widely used to alleviate single ventricle physiology, and TCPC hemodynamics has been linked to patients' outcomes. For simplicity, the current assessment of TCPC hemodynamics, whether in vivo, in vitro, or in silico, focuses on the flow field inside TCPC. However, immediately upstream of the TCPC, blood flow mixes streams between the inferior vena cava (IVC), hepatic veins (HVs) and between the two brachiocephalic veins (BVs). The flow mixing results in sophisticated inflow conditions of TCPC, yet there is little knowledge of its effect on TCPC hemodynamics. Therefore, this study aims to quantify this effect on clinically relevant hemodynamic metrics in TCPC
Materials and Methods
We developed a CFD platform that incorporates the spatial and temporal velocity profiles from in vivo measurements to inlets of the TCPCs (including IVC, HVs, SVC, and BVs). Transient patient-specific simulations were conducted using ANSYS Fluent. Blood is treated as a single-phase, non-Newtonian fluid with a density of ρ=1060 kg-m-3. The dynamic viscosity of blood $\eta((\dot{\gamma}))$ is modeled with a Carreau model experimentally measured from Fontan patients. Primary hemodynamic metrics from CFD simulation include hepatic flow distribution (HFD) and power loss (PL). These two metrics are clinically relevant and correlated with Fontan long-term complications. This study involved four patients who underwent Fontan surgery. For each patient, HFD and PL were obtained for the full model (with all upstream vessels) and transitionally simplified model.
Results and Discussion
The results reveal that flow mixing induces swirling flow in TCPC. Both IVC and SVC flow mixing have a negligible impact (<1%) on TCPC PL. Intriguingly, the SVC mixing itself induces a substantial PL while the FP one does not. For HFD assessment, while the SVC mixing has limited impact (<1%), the FP one could result in a 23% discrepancy due to merging of the IVC and HV flows.
Conclusions
We successfully used a novel CFD platform to examine the effect of upstream flow mixing on the TCPC hemodynamics. We observed no effect on PL but moderate effects on HFD because IVC and HV flow streams mix inside FP. Future studies are suggested to acquire patient-specific IVC and HV flows for reliable HFD assessment. Nevertheless, a cohort study is warranted to examine the generalization of these findings.
Acknowledgements
ANSYS software was provided through an Academic Partnership between ANSYS, Inc. and the Biomedical Modeling and Simulation Lab at UMass Lowell

SVIM 2022 Breakout Sessions

Friday, October 7 at 9:45-10:45 AM

Leading Self, Team, & Institution

Led by: Jennifer Askey, PhD | Academic Coach from Academic Impressions

Leadership comes in a variety of forms, whether you are leading your own life and career and/or your organization, lab, team, or discipline. Fortunately, being an effective leader doesn't have to be one-size fits all. In this interactive session, academic coach Dr. Jennifer Askey, will guide participants through identifying their leadership style so they can maximize their "working genius" and minimize frustrations.

Minimally Invasive Monitoring to Ensure an Enduring Heart Transplant

Led by: Enduring Hearts
Moderators: Shelley Miyamoto, MD and Palak Shah, MD
Featured Speakers: Palak Shah, MD, Margaret Samyn, MD, Brian Feingold, MD, Marius George Lingararu, MD

Pediatric heart transplant has enjoyed improved outcomes from the early days with infant transplant now often lasting more than two decades. Our outcomes have really not significantly changed, however, since the 1980s. The largest threat to allograft survival is the immune system causing rejection. This breakout features some of the new and innovative methods being developed to detect rejection. It will end with a visionary review of the novel applications of artificial intelligence to integrate multiple modalities in patient management decisions.

New Frontiers in Single Ventricle Gene Regulation

Led by: Susan Liao, PhD | New York University
Featured Speakers: Deepak Srivastava, MD, Muge Kuyumcu-Martinez, PhD

Our understanding of both normal cardiac development and single ventricle molecular etiology focuses largely on regulation of gene expression at the transcriptional level. However, groundbreaking genetic, developmental, and epidemiological studies have identified key roles for RNA-based regulation of gene expression in SV models and HLHS patients. This session will (1) highlight these advances to make the case that understanding RNA-based regulatory mechanisms will lead to improved understanding of cardiac development and single ventricle genotype-phenotype relationships, (2) foster discussion among pioneers on ongoing work in understanding RNA-based regulation, and (3) introduce powerful novel resources (experimental tools, computational methods, and databases) that will empower the Additional Ventures community to investigate RNA-based regulation.

Patient and Family Perspectives on Research Directions

Panel Moderator: Diane Pickles | Additional Ventures
Featured Panelists: B. Arman Aksoy, PhD, Tawanna Williams, CPC, Taylor Houlihan, Jameson Rich

In this breakout session, Research Directions: Patient and Family Perspectives, we will have an opportunity to hear from single ventricle patients and family members about their experiences, challenges, concerns, and hopes. From this discussion, we will gain a more profound understanding of the research priorities of the single ventricle community and discover new connections that may drive novel research approaches and directions.

SVIM 2022 Breakout Sessions

(Friday, October 7 at 9:45-10:45 AM)

Brain Health Outcomes in Single Ventricle Heart Disease Patients Across Lifespan

Led by: Ashok Panigrahy, MD | University of Pittsburgh, Nadine Kasparian, PhD | Cincinnati Children's Hospital, Caitlin Rollins, MD | Boston Children's Hospital
Featured Speaker: Kathryn Leigh Humphreys, PhD

Single-ventricle physiology (SVP) patients now are showing significantly increased longevity over time with increased risks to brain health across lifespan. Factors that could contribute to increased potential for cognitive impairment in SVHD include the following: (a) brain dysmaturation beginning in utero; (b) subsequent acquired injury occurring in early childhood from staged surgeries; and (c) medical complications related to SVHD itself, as new arrhythmias, heart failure, and other issues which may develop during emerging adulthood. This session will feature focused presentations and moderated round table discussions about contemporary topics related to SVHD brain health including: (1) associations between brain imaging findings, neurodevelopmental outcomes, and mental health outcomes; (2) neuroinformatic topics related to data-mining and harmonization to foster data-sharing and leveraging existing funded consortium/registries; (3) social determinants of brain health in SVHD across lifespan and impact on design and execution of research studies.

The Obstacle is the Way – Translating Clinical Challenges into Therapeutic Opportunities

Led by: Danielle Gottlieb Sen, MD | Johns Hopkins Children's Center
Featured Panelists: Chris Breuer, MD, Danielle Gottlieb Sen, MD, Bret Mettler, MD, George Nicholson, MD

This is a small group session designed for scientists to gain exposure to clinical needs and obstacles that could potentially provide raw material for novel research of single ventricle congenital heart disease. Panelists will provide a surgical perspective on three stage palliation of single ventricle CHD, as well as the care of single ventricle patients. By focusing on what remains unknown and suboptimal in our care provision, we will identify and discuss productive areas for translational research.

Saturday, October 8 at 9:15-10:15 AM

Computational Fluid Dynamics: Making it a reality in everyday practice

Led by: Mark Fogel, MD | Children's Hospital of Philadelphia, David Hoganson, MD | Boston Children's Hospital, Alison Marsden, PhD | Stanford University
Featured Speakers: Mark Fogel, MD, David Hoganson, MD, Vijay Govindarajan, PhD, Alejandro Roldán-Alzate, PhD

Surgical planning for the Fontan operation using computational fluid dynamic modeling has been around for over a decade and has contributed to our understanding of the hemodynamics of the Fontan pathway and has helped some hundred patients throughout the world. Metrics produced from this technique such as powerless and pathway resistance have been associated with exercise performance, quality of life and the development of liver fibrosis and holds the promise to intervene to improve these morbidities. Virtual surgical planning has also spawned some different configurations of the Fontan pathway such as the "Y" graft option and has cured pulmonary arteriovenous malformations. Nevertheless, in 2022, the computational modeling remains a "boutique" technique that is not widely used for a couple of reasons such as specialized expertise and the time needed perform it. This session will discuss what computational modeling for the Fontan is, its advantages clinically, why it has not come into widespread use and attempt to find solutions to bringing it to the masses.

SVIM 2022 Breakout Sessions

(Saturday, October 8 at 9:15-10:15 AM)

Innovations for Enduring Heart Transplantation

Led by: Enduring Hearts
Session Moderators: Anne Halpin, MD and Stephanie Nakano, MD
Featured Speakers: Jennifer Conway, MD, Anne Halpin, MD, Stephanie Nakano, MD, Jane O, MD

This session will focus on the areas being investigated to improve the longevity of the transplanted heart in the recipient. First, we will review the innovative research being done to reduce the injury to the donor heart during procurement and transportation to the recipient. ABO incompatible heart transplants have now been routine in certain populations for over two decades. We will review what this has taught us about transplant tolerance and how it informs future research. We will finish by discussing the advances in immune tolerance and the role of the thymus. Each session will have be followed by an opportunity to ask questions of the speakers.

Developing Vision for Your Career

Led by: Jennifer Askey, PhD | Academic Coach from Academic Impressions

During this interactive breakout session, academic coach, Dr. Jennifer Askey, will guide participants through crafting a professional vision based on your own definition of success. Then, she will guide participants through working backwards from that vision to align their daily work with their vision, so they can live their vision on a daily basis. Participants will complete individual work using a guided worksheet, as well as participate in small group discussion.

Model Organisms in Single Ventricle: What's the Best Approach?

Led by: Luis Hernandez Nunez, PhD | Harvard University
Featured Speakers: Benoit Bruneau, PhD, Anthony Firulli, PhD, Mengmeng Huang, PhD, Luis Hernandez Nunez, PhD, Stephanie Lindsey, PhD, Georg Vogler, PhD

Studying the mechanisms, origins, or treatments of single ventricle disease benefits from the use of model organisms. In this breakout session, we will discuss the potential advantages and limitations of using zebrafish, chickens, and murine models to study congenital heart disease. Panelists will briefly explain their research and the audience will propose questions and discussion topics. This is an opportunity for basic scientists to discuss with engineers and physicians about the knowledge gaps with higher priority that can be addressed with their model organisms. Similarly, this is an ideal opportunity for physicians and engineers to learn about the potential contributions of model organism approaches to more applied areas of single ventricle disease research.

Registries and Data in Single Ventricle: What's out there? And what's in there?

Led by: Jack Rychik, MD | Children's Hospital of Philadelphia, Alexander Opotowsky, MD | Cincinnati Children's Hospital
Featured Panelists: Kristin Burns, MD, Yves D'Udekem, MD, Nadine Kasparian, PhD, Shelby Kutty, MD, Alexander Opotowsky, MD, Diane Pickles, Rahul Rathod, MD, MBA, Jack Rychik, MD, Kurt Schumacher, MD

An open panel discussion considering existing and emerging multi-center clinical and translational data sources for research on single ventricle heart disease. Here, we'll discuss what currently exists, and is on the horizon for registries in single ventricle. Panelists will discuss data sharing and interdisciplinary harmonization, financial and access models, sustainability, and emerging possibilities for data integration and analysis, including advances in imaging and machine learning-associated research strategies.

SVIM 2022 Breakout Sessions

Sunday, October 9 at 9:15-10:15 AM

An Open-Source Pipeline for Bioprinting Perfusable Tissues

Led by: Zachary Sexton, Jessica Herrmann, Mark Skylar-Scott, PhD, Alison Marsden, PhD | Stanford University

This workshop session will facilitate skills development in open-source technologies aimed at generating vasculature for engineered tissues. Breakout attendees will be introduced to SimVascular, a patient-specific blood flow simulation and analysis software package and use its tool for automated microvascular generation. Attendees will be able to produce their own vascular models within minutes—a process otherwise requiring hours of computer aided design (CAD). Vascular models will be printed via open-source embedded sacrificial extrusion 3D printing technology with portable low-cost bioprinters. Basic parameters for quality microvascular bioprinting will be provided during the guided demo. Remaining workshop time will provide an overview of simulations that can be computed to test blood flow within generated vascular models. Attendees will leave with software capable of generating, fabricating, and simulating blood flow within microvascular networks. This workshop represents the first complete design-fabrication-testing framework for vascular bioprinting and seeks to provide standardized practices in scalable tissue engineering to advance Additional Ventures' curative solutions roadmap.

Surgically Palliated Single Ventricle: Pre- and Post-transplant Patient Care

Led by: Enduring Hearts
Moderator: Kurt Schumacher, MD, George Nicholson, MD
Featured Speakers: Pranava Sinha, MD, Kathleen Simpson, PhD, Kurt Schumacher, MD, Sharon Chen, MD

This breakout session will bring it all together as we focus on the many things that make the single ventricle a unique group of patients when they are considered for transplant. We will start by discussing the challenges of mechanical support in patients will single ventricle anatomy and discuss some of the innovations occurring in the field. We will then discuss the impact of the Fontan physiology on the liver as the patient ages and the considerations for combined heart and liver transplant. This will be followed by a discussion of the other affected areas and interventions that can make these patients better transplant candidate. We will review the outcomes of transplants in single ventricle patients and finish with a panel discussion and the opportunity for the audience to ask questions.

Bolstering Your Communication Toolkit: Strategies for Success in Science and Engineering

Led by: Jay Humphrey, PhD | Yale University

One of the best ways to learn to communicate effectively is to listen carefully to good speakers. In this session, attendees will be asked to recall presentations that they heard throughout SVIM 2022 and to identify characteristics of a "good" presentation, both for the domain expert and for those in allied fields. We will then dive deeper to identify what this individual did well: what made their presentation memorable, what made it compelling? Consider the different contributions of personal style, including body language, and the effective use of audio-visual aids. Finally, we will seek to identify particular elements of effective presentation styles that attendees can adopt and practice.

SVIM 2022 Breakout Sessions

(Sunday, October 9 at 9:15-10:15 AM)

Defining the Mechanistic Basis for the Anatomic Subtypes of Single Ventricle

Led by: Paul Grossfeld, MD | University of California San Diego

The molecular and cellular mechanisms underlying single ventricle heart defects are poorly understood. In this session we will discuss the anatomic subtypes of HLHS, the evidence implicating a critical role for the endocardium in the pathogenesis of at least some of the more common anatomic subsets of HLHS, and how this may relate to the pathogenesis of other single ventricle defects including pulmonary atresia/intact ventricular septum (sometimes viewed as a "mirror image" of HLHS). One fascinating area of discussion will be which factors cause left versus right heart pathology during early stages of heart development. It is anticipated this will be a highly interactive discussion, in which recent unpublished data and ideas for future research directions will be shared, fostering a much-needed multi-disciplinary and collaborative approach to this complex set of disorders.

Artificial Intelligence for Single Ventricle Disease: What are the challenges and opportunities?

Led by: Markus Rottmann, PhD | Northwestern Medicine

Artificial intelligence (AI) is poised to disrupt and revolutionize medical fields via data-driven solutions to improve patient outcomes. Here, we'll discuss insights on how AI-based techniques impact and improve health care for single ventricle disease patients with opportunities including: disease diagnosis using AI in cardiac imaging, AI-based electrogram analysis and monitoring, benefits of AI in digital pathology in histopathological analyses, AI in early symptom prediction and identification of high-risk patients, AI in clinical workflow augmentation and hospital optimization, and AI in drug and gene treatment development. We'll also discuss insights on how AI tools have the potential to suffer from a host of shortcomings, including: inapplicability outside of the training domain and bias, and complex evolving relationship between clinicians and AI tools.