Conference Abstracts

ENDOTHELIAL AND SMOOTH MUSCLE CELL INTERACTION VIA FOXM1 MEDIATES VASCULAR REMODELING AND PULMONARY ARTERIAL HYPERTENSION

Dai Z, Zhu MM, Peng Y, Jin H, Machireddy N, Qian Z, Zhang X, Zhao YY

Northwestern University, Chicago, IL, USA

Background: Angioproliferative vasculopathy is a hallmark of pulmonary arterial hypertension (PAH). However, little is known how endothelial cell (EC) and smooth muscle cell (SMC) crosstalk regulates the angioproliferative vascular remodeling. We aimed to investigate the role of EC and SMC interaction and underlying signaling pathways in the development of PAH.

Methods: SMC-specific FoxM1 or Cxcr4 knockout mice, EC-specific

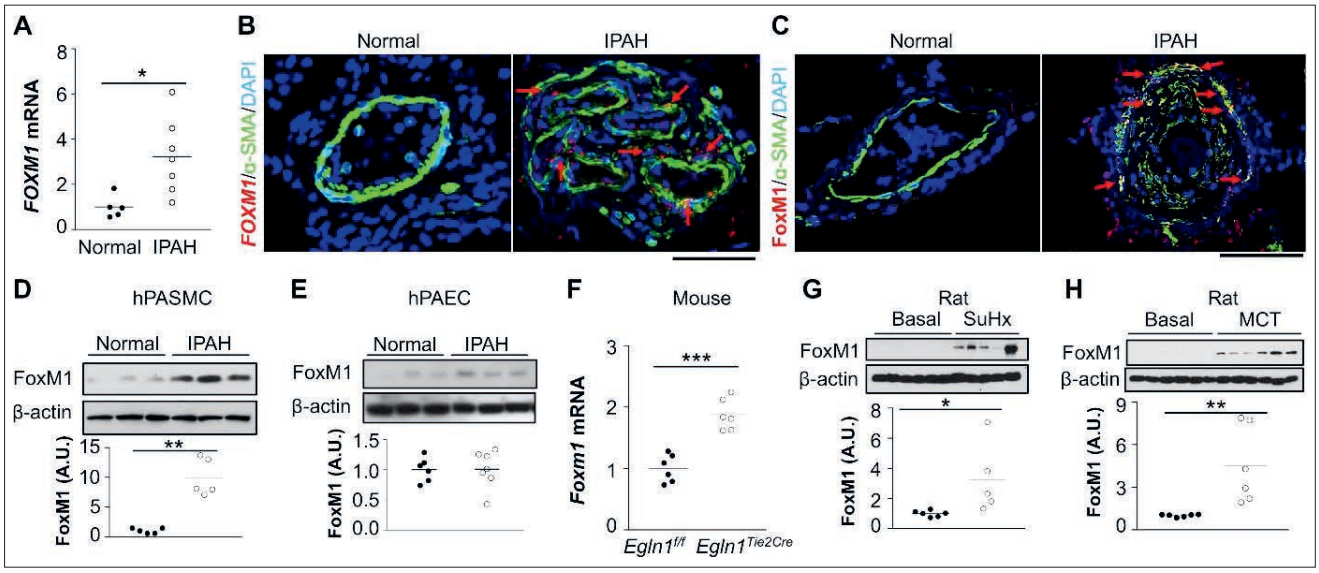


Figure 1: Activation of FoxM1 signaling in lungs of IPAH patients and PH mice and rats.

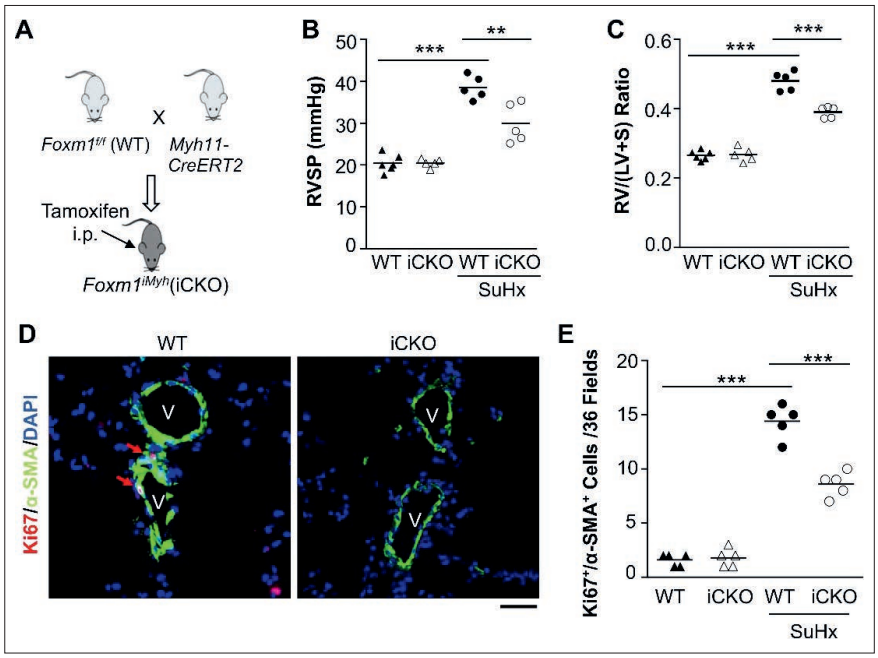


Figure 2: FoxM1 disruption in SMCs inhibits SuHx-induced PH in mice.

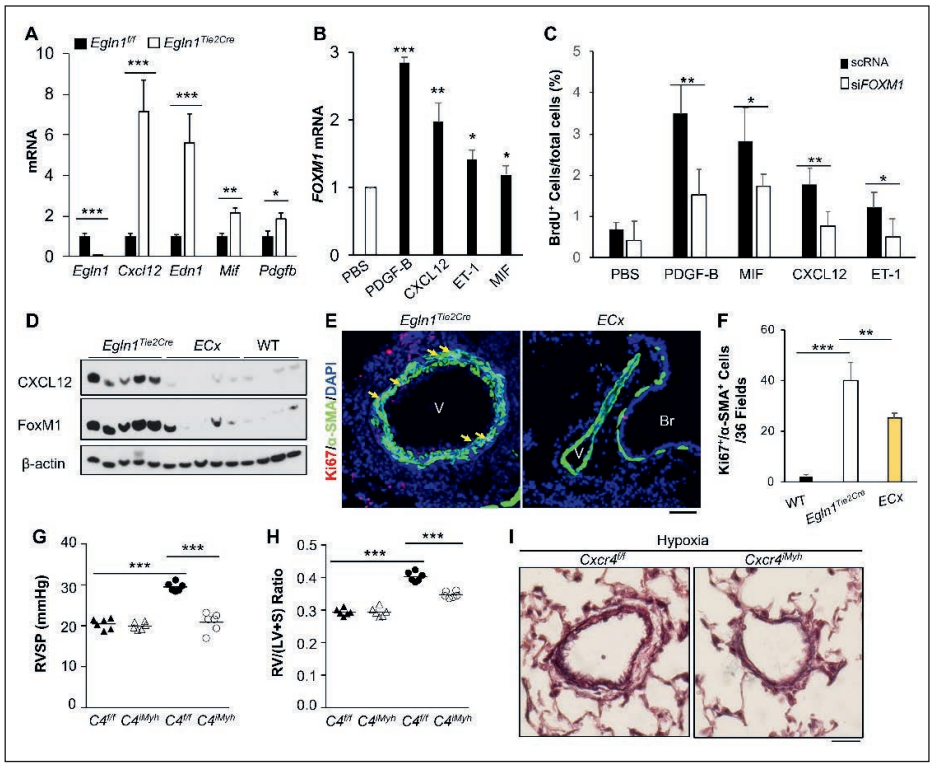


Figure 3: Endothelial and smooth muscle cell interaction mediate FoxM1 upregulation, SMC proliferation, and PH.

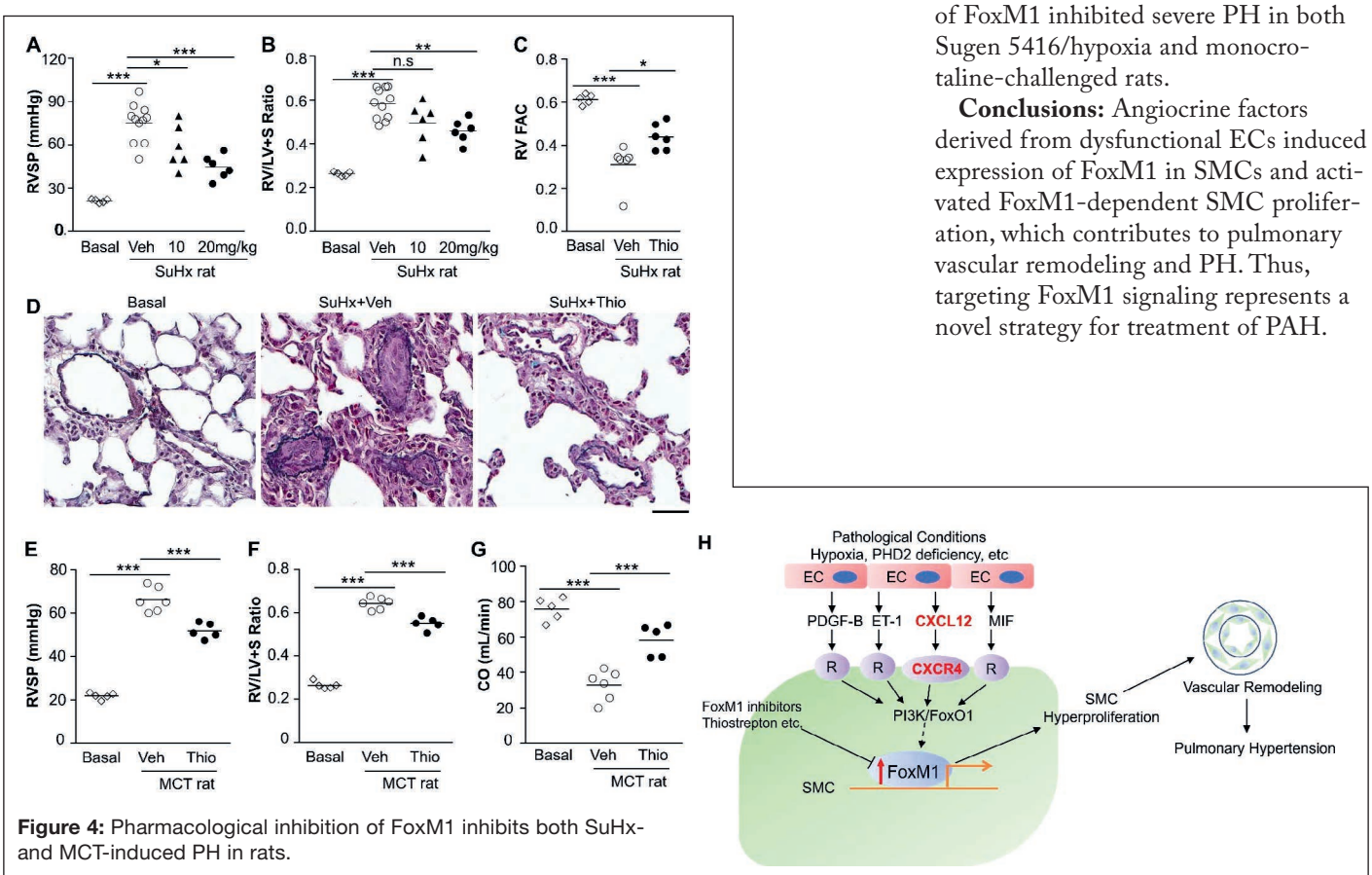


Figure 4: Pharmacological inhibition of FoxM1 inhibits both SuHx- and MCT-induced PH in rats.

FoxM1 or Egln1 knockout mice, as well as EC-specific Egln1/Cxcl12 double-knockout mice were used to assess the role of FoxM1 on SMC proliferation and pulmonary hypertension (PH). Lung tissues and cells from PAH patients were employed to validate clinical relevance. FoxM1 inhibitor Thiostrepton was used in Sugen 5416/hypoxia- and monocrotaline-challenged rats.

Results: FoxM1 expression was markedly upregulated in lungs and pulmonary arterial SMCs of idiopathic PAH (IPAH) patients and 4 discrete PH rodent models. Mice with SMC- (but not EC-) specific deletion of FoxM1 were protected from hypoxia- or Sugen 5416/hypoxia-induced PH. The upregulation of FoxM1 in SMCs induced by multiple EC-derived factors (PDGF-B, Cxcl12, ET-1, and MIF) mediated SMC proliferation. Genetic deletion of endothelial Cxcl12 in Egln1Tie2Cre mice or loss of its cognate receptor Cxcr4 in SMCs in hypoxia-treated mice inhibited FoxM1 expression, SMC proliferation, and PH. Accordingly, pharmacological inhibition of FoxM1 inhibited severe PH in both Sugen 5416/hypoxia and monocrotaline-challenged rats.

Conclusions: Angiocrine factors derived from dysfunctional ECs induced expression of FoxM1 in SMCs and activated FoxM1-dependent SMC proliferation, which contributes to pulmonary vascular remodeling and PH. Thus, targeting FoxM1 signaling represents a novel strategy for treatment of PAH.

THE PPARγ AGONIST PIOGLITAZONE REVERSES PULMONARY ARTERIAL HYPERTENSION AND PREVENTS RIGHT HEART FAILURE THROUGH FATTY ACID OXIDATION

Legchenko E,[1] Chouvarine P,[1] Borchert P,[1] Fernandez-Gonzalez A,[2] Snay E,[3] Mägel L,[4] Mitsialis SA,[2] Meier M,[5] Rog-Zielinska E,[6] Kourembanas S,[2] Jonigk D,[4] Hansmann G[1]

[1]Department of Pediatric Cardiology and Critical Care, and Pulmonary Vascular Research Center (PVRC), Hannover Medical School, Germany; [2]Division of Newborn Medicine, Department of Medicine, Boston Children's Hospital, Harvard Medical School, Boston, MA, USA; [3]Division of Nuclear Medicine, Boston Children's Hospital, Harvard Medical School, Boston, MA, USA; [4]Institute of Pathology, Hannover Medical School, Hannover, Germany; Biomedical Research in Endstage and Obstructive Lung Disease Hannover (BREATH), Hannover, Germany; The German Center for Lung Research (Deutsches Zentrum für Lungenforschung DZL); [5]Institute of Laboratory Animal Science, Hannover Medical School, Hannover, Germany; [6]Institute for Experimental Cardiovascular Medicine, Freiburg, Germany

Background: Pulmonary arterial hypertension (PAH) has a mortality rate of 25% to 60% within 5 years after diagnosis, with right ventricular (RV) failure being the leading cause of death. So far, no intervention could fully reverse PAH or even prevent pressure overload heart failure, in the well-established Sugen 5416/hypoxia (SuHx) rat model that closely resembles human disease. We hypothesized that the PPARγ agonist pioglitazone (Pio) reverses angio-obliterative PAH and prevents heart failure in RV pressure overloaded rats.

Methods: We measured ventricular function and pulmonary artery acceleration time (PAAT) in mice with deletion of PPARγ in cardiomyocytes (cmPPARγ -/-) by magnetic resonance imaging (MRI) and echocardiogram (ECHO). In addition, SD rats were divided into 4 groups, injected either with no agent, vehicle (dimethyl sulfoxide, DMSO), or the VEGFR2 inhibitor SU5416: control normoxia (ConNx); control/hypoxia (ConHx, 1x subcutaneous [SC] DMSO, 3 weeks hypoxia, 6 weeks room air); SU5416/hypoxia (SuHx, SU5416 20 mg/kg/dose SC x1, 3 weeks Hx, 6 weeks Nx); SU5416/hypoxia treated with Pio (SuHx + Pio, SU5416 SC x1, 3 weeks Hx, 6 weeks Nx, including 5 weeks of Pio treatment 20 mg/kg/day by mouth). Hemodynamics, RV/left ventricular (LV) mass and volumes were assessed by cardiac catheterization, MRI, ECHO, RV/LV+S mass ratio. Mitochondrial integrity and lipid accumulation were assessed by 2D/3D electron microscopy and cardiac magnetic resonance spectroscopy. RNA expression studies (mRNASeq, single/array miRNA qPCR) were performed on rat RV and LV (N=3/group), and on laser-capture microdissected explanted heart and lung tissue of idiopathic PAH (IPAH) heart-lung transplantation (HLTx) patients and donors (N=7-10). We measured Pio-regulated mitochondrial function (FAO, ATP production) in rat neonatal ventricular cardiomyocytes (NRCM) by Seahorse.

Results: cmPPARγ -/- mice developed biventricular systolic dysfunction vs controls, in the abscence of PAH (PAAT not significantly different). SuHx rats developed severe PAH and overt RV failure vs ConNx and ConHx. PAH was fully reversed and RV failure prevented by Pio administration (SuHx+Pio): right ventricular systolic pressure (RVSP) (91.1 in SuHx vs 28.8 vs 32.2 vs 34.2 mm Hg). Electron microscopy showed abnormal mitochondrial and T-tubule/SR couplon morphology, and large vacuoles (≈lipid vacuoles) in SuHx rats. Magnetic resonance spectroscopy unraveled inter- and intracellular lipid accumulation in failing RVs, which was not present in controls or SuHx+Pio rats. Consistently, Pio induced FAO and ATP production in cultured NRCM. RNASeq revealed 104 genes with differential expression in SuHx RVs, and 67 genes to be Pio-regulated. Several miRNAs were altered in SuHx RVs and regulated by Pio. Accordingly, altered miR expression was confirmed in human plexiform lesions vs small pulmonary arteries, and RVs of HLTx IPAH patients.

Conclusions: PPARγ deficiency in cardiomyocytes leads to biventricular systolic dysfunction in mice. PPARγ activation by Pio is the first intervention that fully reverses angio-obliterative PAH/pulmonary vascular disease and prevents heart failure in a robust animal model of PAH/RV failure, and as such is an attractive treatment option for clinical PAH that regulates lipid metabolism.

BONE MORPHOGENETIC PROTEIN 9 IS A MECHANISTIC BIOMARKER OF PORTOPULMONARY HYPERTENSION

Nikolic I,[1] Yung LM,[1] Yang P,[1] Malhotra R,[2] Paskin-Flerlage SD,[1] Dinter T,[1] Bocobo GA,[1] McNeil M,[1] Faugno AJ,[3] Lai CSC,[1] Upton PD,[4] Goumans MJ,[5] Zamanian RT,[6] Elliott G,[7] Morrell NW,[4] Chung RT,[8] Channick RW,[9] Roberts KE,[3] Yu PB[1]

[1]Brigham and Women's Hospital and Harvard Medical School, Division of Cardiovascular Medicine, Department of Medicine, Boston, MA, USA; [2]Massachusetts General Hospital and Harvard Medical School, Division of Cardiology, Department of Medicine, Boston, MA, USA; [3]Tufts Medical Center, Division of Pulmonary, Critical Care and Sleep Medicine, Department of Medicine, Boston, MA, USA; [4]University of Cambridge School of Clinical Medicine, Addenbrooke's and Papworth Hospitals, Division of Respiratory Medicine, Department of Medicine, Cambridge, United Kingdom; [5]Leiden University Medical Centre, Department of Molecular Cell Biology and Cancer Genomics Centre Netherlands, Leiden, Netherlands; [6]Stanford University Medical Center,

Division of Pulmonary and Critical Care Medicine, Department of Medicine, Stanford, CA, USA; [7]Intermountain Medical Center and University of Utah, Department of Medicine, Salt Lake City, UT, USA; [8]Massachusetts General Hospital and Harvard Medical School, Gastrointestinal Unit and Liver Center, Department of Medicine, Boston, MA, USA; [9]Massachusetts General Hospital and Harvard Medical School, Division of Pulmonary and Critical Care Medicine, Department of Medicine, Boston, MA, USA

Background: Bone morphogenetic protein 9 (BMP9) is a circulating endothelial quiescence factor with protective effects in pulmonary arterial hypertension (PAH). Loss-of-function mutations in BMP9 receptors and downstream effectors have been reported in heritable PAH. We sought to determine how an acquired deficiency of BMP9 signaling might contribute to PAH.

Methods: Plasma levels of BMP9 and antagonist soluble endoglin (sEng) were measured in Group 1 PAH, Group 2 and 3 pulmonary hypertension (PH), and in patients with severe liver disease without PAH. Mice with CCl4-induced cirrhosis and portal hypertension were assessed for PH and levels of circulating BMP9. The impact of administering the BMP9 ligand trap ALK1-Fc was assessed in wild-type mice.

Results: BMP9 levels were markedly lower in portopulmonary hypertension (PoPH) as compared to controls in a derivation cohort (46 pg/mL [IQR 21-71 pg/mL] vs 210 pg/mL [IQR 190-246 pg/mL], *P*<0.0001), and confirmed in a distinct validation cohort. Across both cohorts diminished BMP9 was present in PoPH but not other etiologies of Group 1 PAH (ROC-AUC=0.91±0.03, *P*<0.0001), but was an independent predictor of transplant-free survival (Cox HR 0.74 per 50 pg/mL increase, 95% CI 0.56-0.97, *P*=0.03) in Group 1 PAH regardless of PoPH status. Diminished BMP9 distinguished PoPH from patients with liver disease without PAH (223 pg/ml [IQR 153-282 pg/mL], *P*<0.0001 vs PoPH), particularly among individuals with mild hepatic dysfunction (Model for End-Stage Liver Disease score ≤10), and was more sensitive for the presence of PoPH among patients with liver disease than echocardiographic RVSP, with equivalent specificity. BMP9 levels were decreased in mice with PH associated with CCl4-induced portal hypertension and cirrhosis, but were normal in other rodent models of PH including monocrotaline-treated rats, and SUGEN-hypoxia-treated rats and mice. Administration of potent BMP9 ligand trap ALK1-Fc severely exacerbated PH and pulmonary vascular remodeling in mice treated with hypoxia vs hypoxia alone (50±8 mm Hg vs 34±1 mm Hg, *P*=0.04).

Conclusions: BMP9 is a sensitive and specific biomarker of PoPH, predicting transplant-free survival and the presence of PAH in end-stage liver disease.

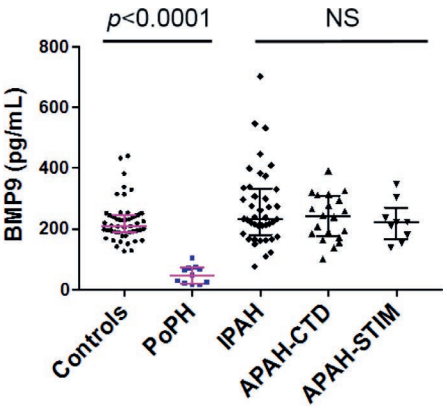


Figure 1: Circulating BMP9 is profoundly decreased in PoPH. Among Group 1 PAH patients, circulating BMP9 was markedly diminished in patients with PoPH; a difference is not observed in other etiologies of Group 1 PAH.

In rodent models, acquired deficiency of BMP9 signaling can predispose to or exacerbate PH, thereby providing a new mechanistic link between PoPH and heritable PAH. These findings raise concerns about the effects of anti-angiogenic therapies targeting BMP9/ALK1 signaling, while describing a novel experimental model of severe PH and remodeling due to acquired loss of BMP signaling.

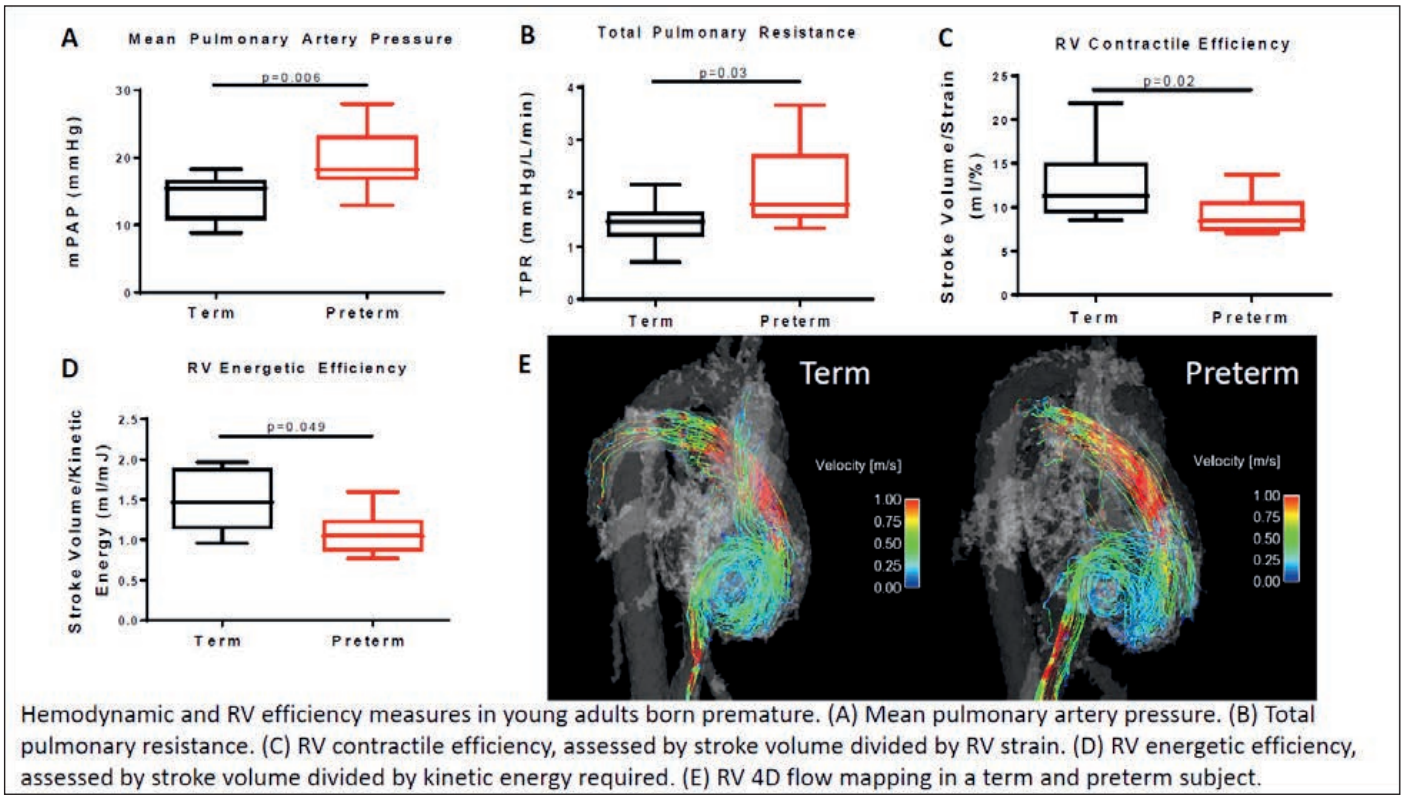
EARLY PULMONARY VASCULAR AND RIGHT VENTRICULAR DYSFUNCTION IN HEALTHY YOUNG ADULTS BORN PREMATURELY

Goss KN, Beshish A, Macdonald J, Barton GP, Mulchrone AM, Chesler NC, Francois C, Wieben O, Eldridge MW

University of Wisconsin, Madison, WI, USA

Background: Preterm birth, or less than 37 weeks completed gestation, affects 1 in 10 live births in the United States. While lung disease is the most frequently recognized complication of prematurity, adults born moderately to extremely preterm have a 3-fold increased risk for the development of pulmonary vascular disease and a 17-fold increased risk for heart failure. The right ventricle (RV) is disproportionately affected, with decreases in RV but not left ventricular (LV) ejection fraction in early adulthood identified in a prior single-center study. Here, we sought to characterize pulmonary vascular and RV function in young adults born prematurely.

Methods: Adults born preterm (n=11; gestational age 28.2±0.8 weeks; current age 27±1 years) were recruited from the Newborn Lung Project at the University of Wisconsin-Madison, a prospective cohort of infants born 1988-1991 with birth weight <1500 g. Controls were recruited from the general population (n=10; age 26±1 years). All subjects were free from adult cardiopulmonary disease. Subjects underwent right heart catheterization with measurement of pulmonary vascular hemodynamics and cardiac magnetic resonance imaging (MRI) on a 3.0T scanner. Cardiac MR measures included assessment of RV function and RV circumferential strain. 4D flow imaging was acquired with a radially undersampled PC VIPR sequence to assess RV kinetic energy, and RV energetic efficiency was defined as total kinetic energy across the cardiac cycle normalized



Hemodynamic and RV efficiency measures in young adults born premature. (A) Mean pulmonary artery pressure. (B) Total pulmonary resistance. (C) RV contractile efficiency, assessed by stroke volume divided by RV strain. (D) RV energetic efficiency, assessed by stroke volume divided by kinetic energy required. (E) RV 4D flow mapping in a term and preterm subject.

to stroke volume. Mann-Whitney tests were performed to assess for statistical significance ($P<0.05$).

Results: Young adults born prematurely had mild elevations in mean pulmonary artery pressure and total pulmonary vascular resistance. Resting RV stroke volume, stroke volume index, and ejection fraction were similar between preterm and term subjects. However, cardiac output and cardiac index were significantly higher among preterm subjects secondary to a higher resting heart rate. RV strain analysis demonstrated increased peak RV circumferential strain among preterm subjects, suggesting a hypercontractile myocardium. When stroke volume was divided by peak RV circumferential strain as a measure of RV efficiency, preterm subjects exhibited reduced RV efficiency. 4D flow analysis also demonstrated a decreased RV energetic efficiency, requiring a higher kinetic energy for a given stroke volume.

Conclusions: Otherwise healthy young adults born prematurely demonstrate mild elevations in pulmonary pressures and vascular resistance, though most fall below current cut points for treatment of pulmonary vascular disease. In addition, they have a hypercontractile, energetically inefficient RV, which may provide mechanistic insight into the increased risk for heart failure in this population. Whether they will require earlier treatment of pulmonary vascular disease in order to maintain RV function warrants further study.