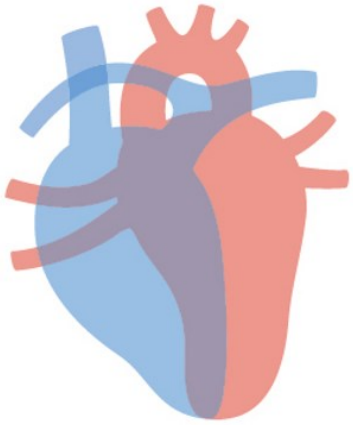
UNIVERSITY OF KENTUCKY

AORTIC SYMPOSIUM



October 21, 2023

Healthy Kentucky Research Building

UNIVERSITY OF KENTUCKY
AORTIC SYMPOSIUM

October 21, 2023 / Healthy Kentucky Research Building 150

8:00	**Check in and breakfast**	
	Session 1	
8:30	**Zamaneh Kassiri MSc, PhD** University of Alberta	*Targeting Adams (Disintegrin and Metalloproteinases) as Potential Therapy for Aortic Aneurysm*
9:00	**Scott LeMaire MD** Baylor College of Medicine	*Fluoroquinolones and Aortic Disease: An Update on the Evidence*
9:30	**Break**	
9:45	**Ronald Dalman MD** Stanford University	*What Diabetes Is Teaching Us About AAA Disease*
10:15	**Scott Damrauer MD** University of Pennsylvania	*All Aortic Aneurysms Are Not Equal: Lessons from the Genetics of Abdominal and Thoracic Aortic Aneurysms*
10:45	**Saha Aortic Center Awards**	
	Session 2	
11:00	**Poster Session**	
11:45	**Lunch**	
	Session 3	
12:15	**Early Career Presentations**	
	Samantha Xu MPH Baylor College of Medicine	**John DePaolo MD, PhD** University of Pennsylvania	**Xufang Mu** University of Kentucky
12:45	**Jean Marie Ruddy MD** Medical University of South Carolina	*Tension-induced SGK-1 Signaling in AAA*
1:15	**Break**	
1:30	**Phillip Owens PhD** University of Cincinnati	*The Role of The Microbiome and Circulating TMAO in the Epidemiology and Pathology of AAA*
2:00	**Scott Cameron MD PhD** Cleveland Clinic	*New Biomarkers and Mechanisms of Arterial Aneurysmal Disease*
2:30	**Follow Up Discussion and Closing Remarks**	

Featured Speakers

Zamaneh Kassiri, MSc, PhD

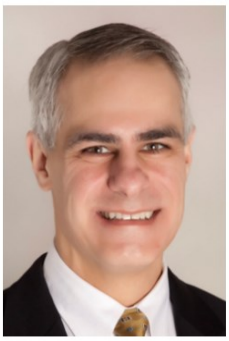
Professor, Department of Physiology

Director of Graduate Studies (Physiology)

Department of Physiology

Canada Research Chair (Tier 1)-Cardiovascular Extracellular Matrix

University of Alberta



Scott LeMaire, MD

Jimmy and Roberta Howell Professor of Cardiovascular Surgery

Vice Chair for Research, Michael E. DeBakey Department of Surgery

Professor of Molecular Physiology and Biophysics

Director of Research, Division of Cardiothoracic Surgery

Baylor College of Medicine

Scott Damrauer, MD

Associate Professor of Surgery

Associate Professor of Genetics

Perelman School of Medicine

University of Pennsylvania

Attending Vascular Surgeon

Corporal Michael Crescenz VA Medical Center

Associate Director of the Penn Medicine BioBank

Ronald Dalman, MD

Dr. Walter C. and Elsa R. Chidester Professor of Surgery

Associate Dean for Stanford Medicine for Market Development and Outreach

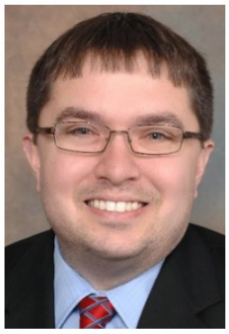
Stanford University School of Medicine

Jean Marie Ruddy, MD

Associate Professor of Surgery
Division of Vascular Surgery

Medical Director of Vascular Laboratory

Vice Chair for Research, Dept of Surgery
Medical University of South Carolina



A. Phillip Owens III, PhD

Associate Professor

Heart, Lung and Vascular Institute

The University of Cincinnati

Scott Cameron, MD, PhD

Section Head of Vascular Medicine

Assistant Staff

Department of Cardiovascular Medicine

Cleveland Clinic Foundation

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Early Career Presenters

Samantha Xu MPH

Baylor College of Medicine

John DePaolo MD, PhD

University of Pennsylvania

Xufang Mu

University of Kentucky

Poster Presenters

Anu Aggarwal
Post Doc
Cleveland Clinic Foundation

Yasir Alsiraj
Faculty
University of Kentucky

Tyler Benson
Post Doc
University of Cincinnati

Daniëlle Coenen
Post Doc
University of Kentucky

John DePaolo
Resident/Fellow
University of Pennsylvania

David Graf
Undergraduate
University of Kentucky

Sohei Ito
Post Doc
University of Kentucky

Yanming Li
Faculty
Baylor College of Medicine

Bowen Li
Post Doc
University of Kentucky

Shayan Mohammadmoradi
Post Doc
University of Kentucky

Xufang Mu
Graduate Student
University of Kentucky

Alex Pettey
Graduate Student
University of Kentucky

Anthony Spuzzillo
Graduate Student
University of Cincinnati

Samantha Xu
Medical Student
Baylor College of Medicine

Contribution of platelet endo- and exocytosis to the initiation and progression of aortic aneurysms

Daniëlle M. Coenen[1], Sidney W. Whiteheart[1]

[1]Department of Molecular and Cellular Biochemistry, University of Kentucky College of Medicine, Lexington, KY, USA

Background: Aortic aneurysms present as a widening and often catastrophic rupture of the aorta. While precipitated by an atherothrombotic and inflammatory environment, the underlying pathological mechanisms are unclear and adequate treatment strategies are lacking. Although antithrombotic drugs have been implied as a therapy, few studies have defined how platelets contribute to aortic aneurysms.
Aim: To investigate the role of platelet endo- and exocytosis in aortic aneurysm initiation and progression.
Methods: Aneurysm formation was induced in mice defective in platelet α-granule biogenesis (Nbeal2$^{-/-}$ and Serglycin$^{-/-}$), endocytosis (Arf6$^{-/-}$ and VAMP2/3$^{\Delta}$), and exocytosis (Munc13-4^{Jinx}) using: **1**) *i.p.* injection of an adeno-associated viral (AAV) vector expressing a gain-of-function mutation (D377Y) of mouse proprotein convertase subtilisin/kexin type 9 (PCSK9); **2)** western diet; and **3**) continuous, subcutaneous infusion of angiotensin II. *In vivo* ultrasound measurements were performed before and during infusion to assess aneurysm formation in both thoracic and abdominal aortic regions. At 4 and 12 weeks, or after rupture, aortas were harvested and analyzed *ex vivo*.
Results: During the study, 30% (3/10, p=0.0014) of the Nbeal2$^{-/-}$ and 21.4% (3/14, p=0.0145) of the Munc13-4^{Jinx} mice suffered thoracic and/or abdominal aortic rupture, compared to 0% (0/26) of wildtype mice. In the survivors, the inner diameter of neither the thoracic nor the suprarenal abdominal aorta of the α-granule-deficient Nbeal2$^{-/-}$ mice changed over time. However, *ex vivo*, Serglycin$^{-/-}$ mice showed a clear, more distributed, widening of the thoracic and descending aorta instead of local aneurysm formation with an increased maximum outer aortic diameter of the ascending/arch region compared to wildtype. Interestingly, Munc13-4^{Jinx} mice had an all-or-nothing phenotype; presenting with early-stage, massive aneurysm formation (females) or rupture (males), or showing a more distributed and atherosclerotic phenotype similar to Serglycin$^{-/-}$ mice. Animals with endocytosis-deficient platelets survived the study and at early stages, the differential increase in the average inner diameter of both the thoracic and suprarenal abdominal aorta was higher in female Arf6$^{-/-}$ mice compared to wildtype. Similarly, VAMP2/3$^{\Delta}$ mice had the largest inner thoracic diameter increase after 28 days of infusion. Arf6$^{-/-}$ and VAMP2/3$^{\Delta}$ mice showed a smaller maximum outer diameter of the descending aorta than wildtype mice.
Conclusions: Our data show that platelet endo- and exocytosis exert significant but perhaps contrasting effects on aortic aneurysm initiation and progression. By comparing the phenotypes of these strains, more detailed mechanistic insights into the role of platelets in aortic aneurysms seems possible.

Supported by the AHA (1020159), NIH/NHLBI (HL150818), and the VA.

Dynamic Phenotypic Transition of Smooth Muscle Cells During Human Ascending Thoracic Aortic Disease Progression: From Compensation in Aortic Aneurysm to Decompensation in Aortic Dissection

Yanming Li, PhD[1]; Chen Zhang, MD[1]; Hernan G. Vasquez, PhD[1]; Yang Li, PhD[1]; Abhijit Chakraborty, PhD[1]; Kimberly Rebello, MD[1]; Samantha Xu, MPH[1]; Lin Zhang, BS; Hisashi Sawada, MD, PhD[4,5]; Zhen Zhou, MD[3]; Rui Chen, PhD[1]; Yumei Li, PhD[1]; Steven B. Eisenberg, MD[7]; Hong S. Lu, MD, PhD[4,5]; Lisa A. Cassis, PhD[6]; Joseph S. Coselli, MD[1, 2]; Alan Daugherty, PhD, DSc[4,5]; Dianna M. Milewicz, MD, PhD[3]; Ying H. Shen, MD, PhD[1, 2, *]; Scott A. LeMaire, MD[1, 2, *]

From [1]Division of Cardiothoracic Surgery, Michael E. DeBakey Department of Surgery, Baylor College of Medicine, Houston, TX; [2]Texas Heart Institute, Houston, TX; [3] Division of Medical Genetics, Department of Internal Medicine, McGovern Medical School, The University of Texas Health Science Center at Houston, Houston, TX; [4]Saha Cardiovascular Research Center, University of Kentucky, Lexington, KY; [5]Department of Physiology, University of Kentucky, Lexington, KY; [6]Department of Pharmacology and Nutritional Sciences, University of Kentucky, Lexington, KY; [7]Department of Cardiothoracic and Vascular Surgery, The University of Texas Health Science Center at Houston, Houston, TX

Presenting author: Yanming Li, Instructor
Word counts: 249 words

Abstract

Introduction: The regulation of progression from ascending thoracic aortic aneurysm (ATAA) to dissection (ATAD) remain poorly understood. We examined the dynamics in smooth muscle cells (SMCs) of aortic tissues from ATAA and ATAD patients and further examined mechanisms in a mouse model.

Methods: Single-cell RNA sequencing (scRNA-seq) analyses were performed in ascending aortic tissues from individuals without aortic disease (n=8), patients with ATAA (n=9), and acute ATAD (n=9). Further, scRNA-seq and scATAC-seq were performed in ascending aortic tissues from mice infused with angiotensin II (AngII); scRNA-seq analyses were performed on SMC-specific *Tgfbr2*$^{-/-}$ mice.

Results: Six SMC subtypes were identified in human aortic tissues. We observed a profound SMC transition from contractile in control to extracellular matrix (ECM)-contractile phenotype in ATAA, however, was reduced in ATAD tissues. Instead, ATAD showed significant SMC transition to inflammatory phenotype. Consistently, many genes in ECM organization and muscle contraction, collectively termed compensatory response, were upregulated in ATAA but downregulated in ATAD tissues compared with controls, whereas inflammatory response and cell death pathway genes were significantly upregulated in ATAD tissues. Cell-cell interaction, regulatory network, and motif analysis suggested that TGF-β-MEF2C signaling contributed to the induction of the ECM-producing SMCs. MEF2C^{+} SMCs exhibited transition towards ECM-producing SMCs in both mouse and human aorta. SMC-specific *Tgfbr2*$^{-/-}$ mice showed compromised SMC transition to ECM-producing phenotype.

Conclusions: Our study reveals SMC phenotypic transitions to ECM-producing SMCs (a compensatory response) in ATAA and to inflammatory and pro-death SMCs (a decompensatory status) in ATAD. TGF-beta-MEF2C promoted the compensatory response in SMCs of the ascending aorta.

Optimal Timing and Duration of BAPN Intervention to Augment Angiotensin II-induced Aortopathies in Adult Male C57BL/6J Mice

Bowen Li,[1] Michael K. Franklin,[1] Deborah A. Howatt,[1] Sohei Ito,[1]
Hisashi Sawada,[1,2,3] Alan Daugherty,[1,2,3] Hong S. Lu[1,2,3]

[1]Saha Cardiovascular Research Center, [2]Saha Aortic Center, [3]Department of Physiology, University of Kentucky

Background: Infusion of angiotensin II (AngII) leads to modest dilatations of the ascending aorta and a low incidence of abdominal aortic aneurysms (AAAs) in adult male C57BL/6J mice. β-aminopropionitrile (BAPN), a lysyl oxidase (LOX) and LOX-like protein inhibitor, induces aortic aneurysms and rupture in 3-4-week-old C57BL/6J mice. In this study, we examined the effects of BAPN intervention at selected intervals on aortic aneurysms and rupture in adult male C57BL/6J mice infused with AngII.

Methods and Results: Male C57BL/6J mice (~11 weeks of age) were randomized to 4 groups (N=10/group) that were all infused with AngII (1,000 ng/kg/min) for 4 weeks. The groups were: (1) No other drug, (2) BAPN co-administration for the first 2 weeks [AngII+BAPN(1-2)], (3) BAPN co-administration for the last 2 weeks[AngII+BAPN(3-4)], and (4) BAPN co-administration for the entire 4 weeks [AngII+BAPN(1-4)]. During the study, no mice died in Group 1. One of 10 mice in AngII+BAPN(1-2) group and 6 of 10 mice in AngII+BAPN(1-4) group died of aortic rupture, as confirmed by necropsy (P=0.04; Log-Rank test). Three of 10 mice in AngII+BAPN(3-4) died of aortic rupture. However, 2 mice died prior to BAPN administration. Maximal diameters of the ascending, descending thoracic, and suprarenal abdominal aortic regions were measured after termination. The maximal diameters of the ascending aorta were significantly larger in AngII+BAPN(1-2) and AngII+BAPN(1-4) groups, compared to Group 1 (P=0.01 and 0.02, respectively; Kruskal-Wallis one-way ANOVA on Ranks followed by Dunn's method). No differences were found in the maximal diameters of the descending thoracic region among the 4 groups. The maximal diameters of the suprarenal aorta measured using ex vivo images were significantly larger in AngII+BAPN(1-2) group (P=0.001) or AngII+BAPN(1-4) group (P=0.004), compared to Group 1 (Kruskal-Wallis one-way ANOVA on Ranks followed by Dunn's method). The maximal diameters of both the ascending and suprarenal aortic regions were not different between AngII+BAPN(3-4) and Group 1 and between AngII+BAPN(1-2) and AngII+BAPN(1-4) groups.

Conclusion: BAPN administration throughout 4 weeks augmented AngII-induced aortic rupture. BAPN administration during the first two weeks attenuated aortic rupture, despite prominent dilations of both the ascending and suprarenal aortic regions.

IDENTIFICATION OF A MOLECULE TO MODULATE A PLATELET OLFACTORY RECEPTOR AND LIMIT THROMBOSIS

Anu Aggarwal, Cleveland Clinic, Cleveland, OH; Nancy Wang, Josyula V Prasad, Cleveland Clinic, Cleveland, OH; Courtney Jennings, Matthew Godwin, Cleveland Clinic, Cleveland, OH; Rohan Bhandari, Washington Univ, St. Louis, MO; Mariya Ali, Young J Shim, Cleveland Clinic, Cleveland, OH; Keith McCrae, Shaun Stauffer, Scott J Cameron, Cleveland Clinic, Cleveland, OH

We found the expression of the Olfactory Receptor, OR2L13 which is a G protein-coupled receptor in human platelets. OR2L13 activation by an olfactory ligand L-Carvone limits platelet activation. Our goal was to find a potent ligand of OR2L13 using an 8K bioactive non-olfactory ligand library and deciphering their role as an anti-thrombotic. 169 molecular hits >25% threshold in an OR2L13-expressing reporter cell line for cAMP production were found. By counter screen (cell line without OR2L13 receptor), 12 ligands were identified for OR2L13 and 6 (CCF0054500, CCF0054432, CCF0053070, CCF0052249, CCF0051970, CCF0058399) made endogenous cAMP. Healthy platelet-rich plasma (n=7) was incubated with ligands for 30 minutes, then subjected to Light Transmission Aggregometry. 3/6 ligands inhibited platelet aggregation through the P2Y12 receptor and so were further validated by flow-cytometry using washed healthy platelets (n=8, attenuation of agonist-activated platelets surface P-selectin expression) for the following receptors: ADP, TRAP-6, U46619 and CRP. CCF0054500 suppresses platelet activation through P2Y12, PAR-1, Thromboxane and GPVI receptors. Whole blood (n=5) traversing collagen-coated capillaries reduced thrombosis when compared to vehicle under arterial sheer stress with CCF0054500. CCF0054500 also inhibited in-vivo thrombosis in the IVC ligation model in FVB mice. The size of the thrombus was 8.2±2.6 mg as compared to the DMSO-treated group with a thrombus size of 15.9±5 mg (P=0.035). In a laser arterial injury model (cremaster muscle), CCF0054500 decreased platelet-mediated thrombus size compared to vehicle (3.1x109 vs. 8.9x109 RFU, P=0.0003) without affecting fibrin formation. Like neurons, platelets have a fully functional OR signal transduction cascade. Platelet OR2L13 operates as a negative regulator of platelet reactivity and thrombosis in response to non-odorant surface agonists (biochemical activation) and under shear stress (biomechanical activation).

Regeneration of Elastic Fibers Following Aortic Dissections

Sohei Ito, Hong S. Lu, Alan Daugherty, Hisashi Sawada

Background
Aortic dissection (AD) is a life-threatening vascular disease displaying the disruption of the extracellular matrix (ECM). Elastic fibers are a major component of ECM and confer structural integrity to the aortic wall. Normally, elastic fibers are thought to not be synthesized in adulthood. However, it remains unknown whether and how elastic fibers are synthesized following ADs.

Methods and Results
AD was induced by administration of β-aminopropionitrile (BAPN, 0.5% wt/vol) to 4-week-old male C57BL/6J mice. Thoracic aortas were collected after 4 or 12 weeks of BAPN administration. False lumen formation lined with fresh hematoma was observed at 4 weeks of BAPN administration, indicating development of acute ADs. The false lumen developed striking remodeling by 12 weeks, suggesting AD progression to the chronic phase. RT-qPCR revealed that mRNA abundance of tropoelastin, the precursor of elastic fibers, was increased significantly during the chronic phase of ADs, but not during the acute phase. Of interest, in situ hybridization demonstrated the presence of tropoelastin mRNA in the vascular wall of the false lumen, and elastic fibers were formed in these lesions in chronic ADs, as evidenced by Verhoeff iron hematoxylin staining. Ultrasonography showed that aortas with AD lesions had higher elasticity compared to intact aortas. These data suggest that elastic fibers were newly synthesized during the progression of ADs, which provided mechanic functions to the vascular wall. Bulk RNA sequencing identified 7,264 differentially expressed genes (DEGs) between chronic ADs and control aortas. Among the1,173 DEGs, including tropoelastin, were increased in chronic ADs. Transcription factor enrichment analysis identified 120 transcription factors as potential regulators for the 1,173 DEGs. Alignment analysis further filtered these molecules and identified ETS variant transcription factor 5 (Etv5) which could be aligned in both the promoter and enhancer regions of tropoelastin. Of note, immunostaining showed that Etv5 was distributed in the vascular wall of the false lumen, which was spatially coincident with tropoelastin mRNA.

Conclusion
Chronic ADs exhibit vascular remodeling with new elastic fiber formation; the AD lesions are accompanied by enhanced Etv5 mRNA and protein.

Role of the X chromosome gene, *Kdm5c*, in sexual dimorphism of AngII-induced aortopathies

Julianne Sharpe, Victoria English, Heba Ali, Mark Ensor, Lisa Cassis, Yasir Alsiraj
Department of Pharmacology and Nutritional Sciences, College of Medicine, University of Kentucky, Lexington, Kentucky

Background: Aortopathies, including thoracic and abdominal aortic aneurysms, are sexually dimorphic diseases occurring at a greater prevalence and severity in males than females. Due to the limited treatment options, aortopathies are life threatening conditions resulting in high morbidity and mortality from aortic rupture. Recently, we demonstrated that an XX sex chromosome complement protected female mice from AngII-induced aortopathies. In addition, we showed that a gene on the X chromosome known to escape X-inactivation in mice and humans, namely *Kdm5c*, was more highly expressed in aortas of XX than XO female mice. In this study, we hypothesized that gene dosage effects of *Kdm5c*, an X chromosome gene that escapes X inactivation, protects females against aortopathies.

Methods: We bred *Kdm5c* floxed heterozygous females ($Kdm5c^{fl/+}$) to germ-line Cre expressing transgenic male mice (B6. Ctg(CMV-cre)1Cgn/J) to obtain female mice that are either wild-type ($Kdm5c^{+/+}$) or globally hemizygous for this gene ($Kdm5c^{+/-}$). In terms of gene dosage, the $Kdm5c^{+/-}$ female mouse is analogous to an XY male mouse that has only one copy of this gene. At two months of age, female mice of each genotype (on a C57BL/6 genetic background) were injected intraperitoneally with an adeno-associated virus carrying a gain of function mutant of proprotein convertase subtilisin/kexin type 9 (AAV-PCSK9) to induce hypercholesterolemia (which augments susceptibility to aortopathies). Mice were fed Western diet through study endpoint. One week after AAV injection, mice were infused with AngII (1,000 ng/kg/min) by osmotic minipump for 28 days and we monitored the disease development via ultrasound.

Results: Surprisingly, at baseline prior to AngII infusion, $Kdm5c^{+/-}$ females exhibited significantly dilated ascending and abdominal aortic lumen diameters compared to $Kdm5c^{+/+}$ females. Following AngII infusion, internal diameters of the ascending and abdominal regions of aortas from $Kdm5c^{+/-}$ females were increased, but this effect was not observed in $Kdm5c^{+/+}$ females. Furthermore, at study endpoint, maximal external diameters of ascending and abdominal aortas were significantly greater in AngII-infused $Kdm5c^{+/-}$ females compared to *Kdm5c*+/+ females. As an additional measure for aortopathy, we quantified aortic weights and aortic arch area; both of these parameters were significantly greater in hemizygous $Kdm5c^{+/-}$ than $Kdm5c^{+/+}$ females.

Conclusion: These results suggest that gene dosage effects of the X chromosome gene, *Kdm5c*, protect females from the development of aortopathy. Future studies should identify downstream targets regulated by the X chromosome gene, *Kdm5c*.

Single Cell RNA Sequencing Reveals a Lineage-specific Response to Angiotensin II in Smooth Muscle Cells in the Ascending Aorta of Mice

David B. Graf[1-3], Hisashi Sawada[1,3], Hong S. Lu[1,3], Alan Daugherty[1,3]

Affiliations:
[1]Saha Cardiovascular Research Center, College of Medicine, University of Kentucky, KY.
[2]College of Engineering, University of Kentucky, KY.
[3]Saha Aortic Center, College of Medicine, University of Kentucky, KY.

Background:
Ascending thoracic aortic aneurysm is a life-threatening disease with no options for pharmacologic treatments. The major cell type populating the media of this region is smooth muscle cell (SMC). SMCs in this region are derived from 2 embryonic origins; cardiac neural crest (CNC) and second heart field (SHF). SHF-derived cells play a vital role in the pathophysiology of angiotensin II (AngII)-mediated thoracic aneurysm. The role of CNC-derived cells and functional differences between the two origins remain unknown.

Methods and Results:
Mef2c-Cre +/0 mT/mG mice were infused with AngII (1,000 ng/kg/day) for 3 days and ascending aortas were harvested. Aortic samples were also harvested from Mef2c-Cre +/0 mT/mG mice without AngII infusion as a control. After tissue digestion to create a single-cell suspension, FACS sorting was performed to separate cells based on their origin using mTotamto and mGFP signals. mGFP proteins were present on Mef2c-Cre positive cells indicating the cells were derived from the SHF, while cells with mTomato signal were not derived from the SHF (nSHF). After sorting cells by origin, single-cell RNA sequencing was performed to determine transcriptomic differences between origins. The “Seurat” R package was used to integrate the sequencing data and a two-way ANOVA analysis for the interaction between origin and infusion. This analysis identified 3,703 differentially expressed genes (DEGs) and hierarchical clustering found 4 major subclusters among these DEGs. Gene ontology analysis revealed that these subclusters were associated with cellular respiration, mitochondria function, p53 signaling, and RNA splicing. One of the clusters exhibited a unique transcriptional response to AngII infusion for cells derived from the SHF. In this cluster, there were 295 DEGs, all of which had significantly higher abundance in the SHF versus nSHF origin in response to AngII infusion. The most upregulated gene was *Tnnt2*, which regulates muscle contraction. The top 10 upregulated genes also included *Loxl2*, a key enzyme for elastic fiber development. Of note, other LOX family members, such as *Lox* and *Loxl1* were downregulated in SHF-derived SMCs compared to nSHF-derived SMCs in response to AngII infusion.

Conclusions:
AngII infusion alters the transcriptome of aortic SMCs in a lineage-specific manner. AngII upregulates genes related to muscle contraction and extracellular matrix development specifically in SHF-derived SMCs

TITLE: Using a polygenic risk score to improve clinical risk modeling for aortic dilation

Authors: John DePaolo, MD, PhD,[1] Gina Biagetti, MD,[2] Renae Judy, MS,[1] Grace J. Wang, MD,[2] Nimesh Desai, MD, PhD,[3] Wilson Y. Szeto, MD,[3] Joseph E. Bavaria, MD,[3] Michael G. Levin, MD,[4,5] Scott M. Damrauer, MD,[2,4,5,6]

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Abstract

Background:

Ascending thoracic aortic dilation is a complex trait that involves modifiable and non-modifiable risk factors and can lead to thoracic aortic aneurysm and dissection. Clinical risk factors have been shown to predict ascending thoracic aortic diameter. Polygenic scores (PGS) are increasingly used to assess clinical risk for multifactorial diseases. The degree to which a PGS can improve aortic diameter prediction in diverse populations is not known. In this study we tested the extent to which the addition of a PGS to clinical prediction algorithms improves the prediction of aortic diameter in a diverse biobank.

Methods

The patient cohort comprised 6,790 Penn Medicine Biobank (PMBB) participants with available echocardiography and clinical data linked to genome-wide genotype data. Linear regression models were used to integrate PGS weights derived from a large genome wide association study of thoracic aortic diameter in the UK biobank and were compared to the performance of the standard and a reweighted variation of the recently published AORTA Score.

Results:

Cohort participants were 56% male, 31% genetically similar to the African reference population (AFR), and had a median age of 61 years (IQR 52-70) with a mean ascending aortic diameter of 3.4 cm (SD 0.5). Compared to the AORTA Score which explained 30.6% (95% CI 29.9% to 31.4%) of the variance in aortic diameter, AORTA Score + PGS explained 33.1%, (95% CI 32.3% to 33.8%), the reweighted AORTA score explained 32.5% (95% CI 31.8% to 33.2%), and the reweighted AORTA Score + PGS explained 34.9% (95% CI 34.2% to 35.6%). When stratified by genetic ancestry, model improvement was consistent among individuals genetically similar to the European reference population (EUR). However, among AFR individuals model improvement from the standard AORTA Score explanation of variance (28.3%, 95% CI 27.0% to 29.6%) was attenuated; the AORTA Score + PGS explained 28.8% (95% CI 27.6% to 30.1%) of the variance, the reweighted AORTA Score explained 28.6% (95% CI 27.3% to 30.0%) of the variance, and the reweighted AORTA Score + PGS explained 29.3% (95% CI 28.1% to 30.6%) of the variance. This performance disparity was observed in respective model area under the receiver operator characteristic curve (AUROC) analyses as well as model sensitivity and specificity.

Conclusions:

We demonstrated that inclusion of a PGS to the AORTA Score results in a clinically meaningful performance enhancement among EUR individuals. However, there was no corresponding significant improvement among AFR individuals. While the standard AORTA Score offers a clinically useful screening mechanism to identify individuals at risk of aortic dilation, inclusion of a currently available PGS may exacerbate healthcare disparities and should be avoided at this time.

Deficiency of Plasminogen Activator Inhibitor-1 Augments Angiotensin II-induced Cardiac and Thoracic Aortic Pathologies

Alex Pettey[# 1-3], Sohei Ito[# 2, 3], Deborah Howatt[2, 3], Michael Franklin[2, 3], Nancy Zhang[4], David Graf[2, 3, 5], Hisashi Sawada[1-3], Hong S. Lu[1-3], Alan Daugherty[1-3]

Affiliations:

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[3]Saha Aortic Center, College of Medicine, University of Kentucky, KY.
[4]Trinity College of Arts and Sciences, Duke University, NC.
[5]College of Engineering, University of Kentucky, KY.
[#]Contributed equally.

Background:

Thoracic aortopathies, including aneurysm, dissection, and intramural hemorrhage, pose grave risks due to potential ruptures and limited treatments. Angiotensin II (AngII) is a critical contributor to aortopathy. Plasminogen activator inhibitor-1 (PAI-1) is the most elevated protein in thoracic aortas of mice infused with AngII for 3 days, prior to overt pathology. Underscoring its key role, PAI-1 increases drastically in aortic walls of human thoracic aortopathies, yet its contribution to the disease remains unknown.

Methods and Results:

To determine the contribution of AngII infusion to plasma PAI-1 levels, wild type mice were infused with saline (control) or AngII (1,000 ng/kg/min) for three days. AngII infusion led to increased plasma PAI-1 concentrations (median 6.7 ng/mL; range 0.9-9.4 ng/mL) compared to controls, in which plasma PAI-1 was not detected ($P = 0.03$ by Mann-Whitney U test). To investigate the role of PAI-1 in thoracic aortopathy, whole-body PAI-1 deficient mice (PAI-1-/-) or their wild type littermates (PAI-1+/+) were infused with AngII for 28 days. External and luminal diameters in the thoracic aorta were similar between genotypes. However, in male mice, PAI-1 deficiency exacerbated aortic remodeling, defined histologically. Medial thickening and adventitial hemosiderin deposition, indicative of intramural hemorrhage, were pronounced in PAI-1-/- mice infused with AngII. Additionally, pathology was grossly evident by discoloration occupying 20% of the aortic surface in PAI-1-/- mice compared to 3% in PAI-1+/+ mice ($P = 0.002$ by Mann-Whitney U test). PAI-1 deficiency also resulted in a 35% decrease in ascending aortic wall distensibility, an inverse measure of vascular stiffness, determined by ultrasound ($P = 0.035$ by Student's t-test). Next, to investigate the acute phase of aortopathy, PAI-1-/- mice were infused with AngII for 7 days. Intramural hemorrhage severity was increased in both male and female PAI-1-/- mice, defined by overt hemorrhage in the aortic wall. Unexpectedly, PAI-1-/- mice also displayed an increased incidence of hemorrhage in ventricular tissue at 7 days of AngII ($P = 0.002$ in males and $P < 0.001$ in females by Fisher's exact test) and increased ventricular tissue remodeling defined by overt discoloration at 28 days of AngII infusion. Additionally, systolic blood pressure was decreased by 24 mmHg (median) in female PAI-1-/- mice infused with AngII, compared to PAI-1+/+ mice ($P = 0.04$ by Mann-Whitney U test). To determine the source of PAI-1 in thoracic aortopathy, PAI-1 was deleted in smooth muscle cells (SMCs), the most abundant aortic cell type. SMC-specific PAI-1 deficient mice were infused with AngII for 28 days. Notably, measures of dilatation and tissue remodeling were similar between genotypes.

Conclusions:

PAI-1 deficiency exacerbated AngII-induced hemorrhage and remodeling in both ventricular and thoracic aortic tissues in mice. SMC-specific PAI-1 deficiency did not alter aortic or cardiac tissue remodeling, indicating that aortic-derived PAI-1 is not a key regulator of AngII-induced pathology. Future research will investigate the source of pathologically elevated PAI-1 and the mechanisms driving aberrant tissue remodeling in PAI-1-/- mice.

Platelet-Derived Transforming Growth Factor-β Ameliorates AAA in a Murine Model

Anthony Spuzzillo, Bailey Stone, Tyler W. Benson, Seth Brunner, Caris Wadding-Lee, and A. Phillip Owens III.

Division of Cardiovascular Health and Disease; Department of Internal Medicine; Heart, Lung, and Vascular Institute; University of Cincinnati, Cincinnati, OH 45267

Background: Abdominal aortic aneurysm (AAA) is a dilatation of the infrarenal aorta and is defined as a >50% increase in diameter from baseline. Platelets are rapidly recruited to the forming intraluminal thrombus in AAA, stabilizing the aortic wall, and preventing rupture. Adhesion and activation of platelets releases a mix of inflammatory mediators such as transforming growth factor β (TGFβ), which mediates cellular proliferation, inflammatory signaling, and fibrosis. Importantly, while TGFβ neutralization results in increased rupture and disease progression in mouse models of AAA, the source of protective TGFβ signaling remains unknown. We hypothesized that platelet-derived TGFβ1 is the source of protective signaling and will attenuate aortic growth in murine AAA models.

Methods and Results: *Tgfβ1* floxed mice were obtained from Jackson Labs and bred with the platelet-specific Cre line platelet factor 4 (*Pf4*). Male *Tgfβ1-Pf4*$^{Cre-}$ (n = 7) and *Tgfβ1-Pf4*$^{Cre+}$ (n = 8) were subjected to laparotomy and topical elastase application (5µl 10 mg/mL porcine pancreatic elastase for 5 minutes). Ex vivo aortic diameter at day 28 showed *Tgfβ1-Pf4*$^{Cre+}$ mice had augmented abdominal aortic diameters compared to *Pf4*$^{Cre-}$ mice (*β1-Pf4*$^{Cre-}$: 1.70 ± 0.12 mm; *β1-Pf4*$^{Cre+}$: 2.37 ± 0.25 mm; P<0.01). Additionally, 25% (2/8) of Cre+ males died of an infrarenal aortic rupture with no deaths occurring in the control group. Similar results were found in female mice with platelet specific TGFβ1 depletion in the elastase model (*β1-Pf4*$^{Cre-}$: 1.38 ± 0.08 mm; *β1-Pf4*$^{Cre+}$: 2.21 ± 0.38 mm; P = 0.03).

Conclusion: Our results further support the view that TGFβ signaling plays a protective role in AAA. Furthermore, we identify TGFβ1 as a key isoform and platelets as an important source of TGFβ1 in aneurysm mitigation. Future studies will investigate potential cellular targets and underlying mechanisms of this protective phenotype.

Vascular Smooth Muscle Cell–specific Deletion of Protein Kinase R-like Endoplasmic Reticulum Kinase Attenuates Microbiome-enhanced Abdominal Aortic Aneurysm

Tyler W. Benson, Caris A. Wadding-Lee, Anthony R. Spuzzillo, Jessica E. Merland, Seth I. Brunner, A. Phillip Owens III.

BACKGROUND: We have previously linked the microbiome-derived metabolite trimethylamine N-oxide (TMAO) to abdominal aortic aneurysm (AAA) in both associative human and mechanistic mouse studies. TMAO is reported to bind protein kinase R-like endoplasmic reticulum kinase (PERK), resulting in selective activation of the unfolded protein response (UPR). Concordantly, our previous RNA sequencing results indicate that TMAO augments PERK-mediated UPR pathways in the aneurysm wall. Yet, the afflicted cell types and underlying mechanisms of how TMAO–mediated PERK activation drives AAA pathogenesis remain unknown.

METHODS & RESULTS: As global deletion of PERK is embryonically lethal, *Ldlr*$^{-/-}$ mice with conditional global *Perk* deletion were generated by crossing hemizygous male CAGCre-ER (B6.Cg-Tg(CAG-Cre/Esr1*)5Amc/J, Jackson Labs) to female *Perk* floxed mice (*Eif2ak3*$^{tm1.2Drc}$*/J*, Jackson Labs). Subsequently, both male and female CAGCre-ER positive (CAG^{CRE+}) and CARCre-ER negative (CAG^{CRE-}) mice were fed tamoxifen diet for 14 days. Surprisingly, CAG^{CRE+} died (14/24) during tamoxifen feeding compared with no deaths (0/14) in CAG^{CRE-} mice. A significant reduction in blood glucose after 10 days of tamoxifen diet accompanied by a pronounced reduction in pancreas size was observed in CAG^{CRE+} compared to CAG^{CRE-} mice. Next, given that global PERK deletion is lethal at any age and the pronounced role of VSMCs in AAA progression, *Ldlr*$^{-/-}$ mice with VSMC-specific deletion of *Perk* were produced by breeding hemizygous male mice expressing Cre under the control of the Transgelin promoter (*B6.Cg-TG(Tagln-cre)1Her/J*, Jackson Labs) to female *Perk* floxed mice (*Eif2ak3*$^{tm1.2Drc}$*/J*, Jackson Labs). Specific deletion of PERK in VSMCs was validated by western blot. Male mice were subjected to angiotensin II (AngII) infusion via osmotic mini pump (1,000 ng/kg/min; 28 days), while female mice underwent laparotomy and application of topical elastase (10 mg/mL porcine pancreatic elastase 5 minutes) to induce AAA. To augment circulating TMAO levels, mice were fed a high choline diet (0.2% total cholesterol + 1.2% choline supplementation) for one week prior to and throughout the study. Male mice with VSMC specific PERK knockout demonstrated significantly reduced AngII-induced aortic dilation (Cre+: 0.90 ± 0.048 mm; Cre-: 1.57 ± 0.156 mm; P = 0.001) and rupture induced death

relative to control. Similarly, female mice with VSMC specific PERK knockout displayed significantly reduced aortic diameter (Cre+: 1.17 ± 0.069 mm; Cre-: 2.65 ± 0.432 mm; P = 0.002) and rupture induced mortality as compared to control.

CONCLUSIONS: These results indicate that while global deletion of *Perk* is lethal, VSMC specific knockout of PERK blunts AAA formation in two independent murine models. Further studies are needed to elucidate the mechanism of PERK mediated ER stress in AAA, which may reveal novel therapeutic targets for AAA treatment.

Unraveling the Impact of VAMP8 Deficiency on Aortopathies: A Fresh Perspective on Platelet Involvement

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Introduction and Objective: Platelet activation and its secreted mediators promote thrombus formation and the accumulation of inflammatory cells, which may play an important role in the development of aortopathies by destroying the structural integrity and stability of the vessel wall. This study first explores the role of angiotensin II (AngII) in platelet activation and pivots on the soluble N-ethylmaleimide-sensitive factor activating protein receptor (SNARE) protein, the vesicle associated membrane protein 8 (VAMP8) – the primary vesicle membrane (v-SNARE) and a major facilitator of platelet secretion.

Approach and Results: Washed platelets from saline or AngII-infused mice (28 days) exposed to thrombin (0.05 U/mL) showed elevated P-selectin, suggesting AngII-driven platelet activation. Hematological studies showed increased mean platelet volume (MPV) and platelet distribution width (PDW) in the AngII group. Washed platelets from wildtype (WT) mice, treated with varying AngII doses, also exhibited elevated P-selectin and Jon/A levels. Additionally, abdominal aortas from 28-day AngII-infused mice, revealed platelets abundance at elastin break sites and false lumen formation compared to saline-infused counterparts.

Given the profound impairment in platelet cargo secretion due to VAMP8 deficiency, we investigated its role in aortopathies. Using RNA-seq data from washed platelets of VAMP8$^{-/-}$ mice vs. WT, we analyzed the molecular signature of VAMP8 deficiency. Gene ontology analysis revealed that the major differences in enriched genes between the two groups involved triglyceride biosynthetic process and platelet calcium homeostasis. Further, ApoE$^{-/-}$ mice crossed with VAMP8$^{-/-}$ mice exhibited a substantial reduction in atherosclerotic lesion development when compared to the control group while plasma cholesterol remained consistent across all groups. Additionally, proprotein convertase subtilisin/kexin type 9 (PCSK9)-induced hypercholesterolemic VAMP8$^{-/-}$ or WT male mice fed a Western diet were infused with AngII (1,000 ng/kg/min) for 4 weeks and aortic *ex vivo* and *en face* analysis indicated that the VAMP8 deficiency profoundly attenuated AngII-induced aortic aneurysms and atherosclerosis compared to control group.

Conclusion: Our findings reveal that VAMP8 deficiency markedly mitigates aortic aneurysm and atherosclerosis, thereby proposing a novel perspective to explore the influence of platelet cargo secretion on the inception of vascular complications.

Background: A family was recently identified with an inherited bleeding disorder of unknown cause, characterized by a prolonged bleeding time but otherwise normal hemostatic parameters. Genetic analyses identified the Tyr242Cys mutation in fragment 2 of prothrombin, a region removed upon its activation to thrombin. Thrombin is the serine protease responsible for making blood clots, and thrombin deficiency is incompatible with life. Coagulation factor Xa cuts prothrombin at two specific sites (Arg271, Arg320) to generate thrombin. We hypothesize that addition of thrombomodulin will reduce anticoagulant dependence on thrombomodulin.

Methods: Calibrated Automated Thrombography (CAT) was utilized to measure thrombin activation in plasma from affected family members and healthy controls. Purified proteins were used to determine clotting rates in family members and controls.

Results: Thrombin generation was reduced in platelet-poor and platelet-rich plasma in the samples of all patients after tissue factor was added or contact pathway initiation. The variant is very close to the Arg271 activation site, and therefore we hypothesized impaired release of the activation fragments resulting in limited clotting. Thrombin generation is corrected through the mixture of Prothrombin-Wauwatosa plasma with plasma depleted of either PC or the cofactor protein S. In control samples, addition of thrombomodulin reduced thrombin generation samples containing Prothrombin-Wauwatosa, consistent with reduced anticoagulant dependence on thrombomodulin.

Conclusions: Findings support a novel mechanism in which Prothrombin-Wauwatosa activation results significant anticoagulant activity and reduced clotting time.

Shifts in Glycolytic Phenotype in Smooth Muscle Cells of Sporadic Aortic Aneurysms and Acute Dissections

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[2]Texas Heart Institute, Houston, TX.
[3]Cardiovascular Research Institute, Baylor College of Medicine, Houston, TX.
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[5]Saha Aortic Center, University of Kentucky, Lexington, KY.
[6]Department of Physiology, University of Kentucky, Lexington, KY.
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*co-senior authors

ABSTRACT

Introduction: Ascending thoracic aortic aneurysms (ATAA) and their progression to acute dissection (ATAD) are associated with high risk of mortality. Because metabolic pathways can regulate cell phenotype and disease progression, we investigated the transcriptomic profile of glyocoysis in smooth muscle cells (SMCs) in human aortic tissue and its potential involvement in promoting an inflammatory phenotype in SMCs of aortic aneurysms and dissections. We hypothesized that glycolytic activity in SMCs is elevated in ATAA and ATAD tissues compared to healthy control aortic tissues.

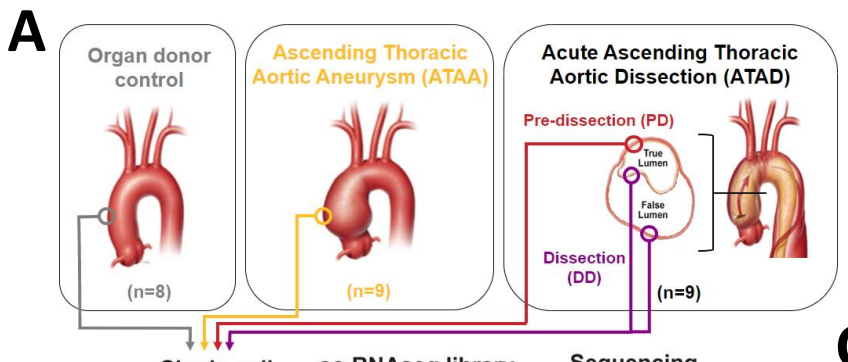
Methods: We performed single cell RNA sequencing (scRNA-seq) analysis of ascending aortic tissue from 9 patients with ATAA without dissection, 9 patients with ATAD (dissected and non-dissected areas collected separately), and 8 organ donor control subjects (Fig A). Within the SMC clusters analyzed (Fig B-C), we identified differentially expressed glycolytic genes between control, ATAA, and ATAD patients. Single-cell flux estimation analysis (scFEA) was performed to estimate metabolic flux variation in glycolytic activity in SMCs. scRNA-seq analyses and single-cell assay for transposase accessible chromatin using sequencing (scATAC-seq) were performed in ascending aortic tissues from wild-type (WT) mice infused with angiotensin II (AngII) or saline (control) and *Sting*$^{-/-}$ mice infused with AngII (Fig G-H, J-L).

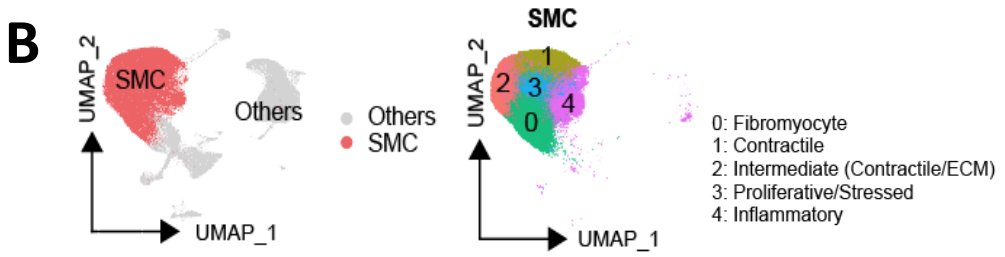
Results: Compared to control, glycolytic genes (e.g., *ENO1, HK1*) and glycolytic activity in SMCs were progressively upregulated from control to ATAA to ATAD (Fig D-F). We also observed progressive induction of lactate production gene *LDHA* from control to ATAA and ATAA to ATAD that was consistent with greater lactate accumulation in scFEA analysis (Fig D-F). In the angiotensin-induced AAD mouse model, AngII infusion increased the expression of glycolytic genes in SMCs (Fig I). Additionally, scATAC-seq analyses revealed elevated chromatin accessibility/gene activity of glycolytic genes (e.g., *Ldha*) in SMCs of Ang II-infused mice (Fig M-

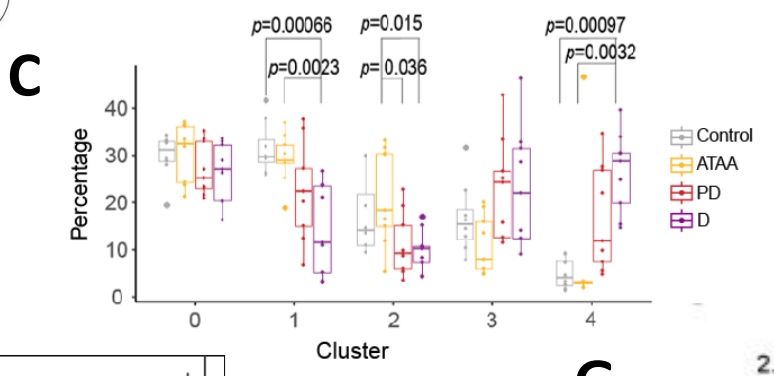
N), suggesting potential regulation at the epigenetic level. Furthermore, correlation analysis revealed that the activity of most glycolytic genes (e.g., *Ldha*) were positively associated with the motif activity of Nfkb1 and Nfkb2, which are well-established pro-inflammatory transcriptional factors (Fig O-P). Finally, knocking down stimulator of interferon genes (*Sting)*, an upstream trigger of NFkB, partially prevented Ang II-induced upregulation of glycolytic genes in SMCs (Fig I).

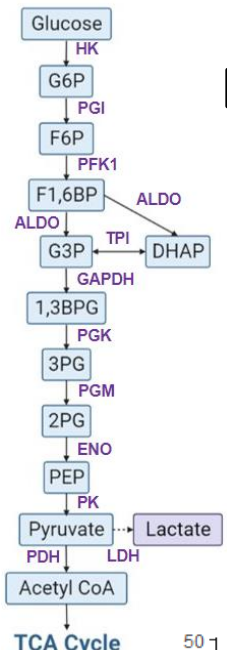
Conclusion: Our data suggest glycolytic activity and lactate production in SMCs is progressively increased from control to ATAA to ATAD. Activation of STING pathway and subsequent activation of NFkB pro-inflammatory signaling may play a critical role in epigenetic induction of these genes. Investigating the upstream and downstream regulators are key to understanding this metabolic shift in aortic disease progression.

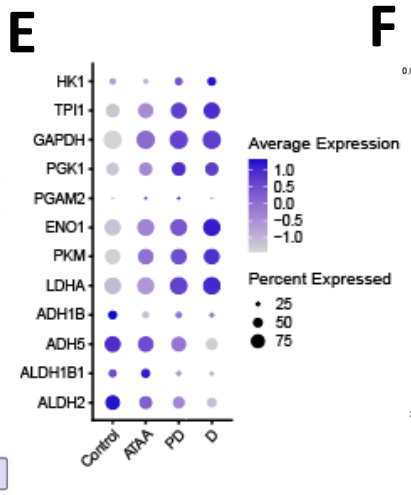
Figure:

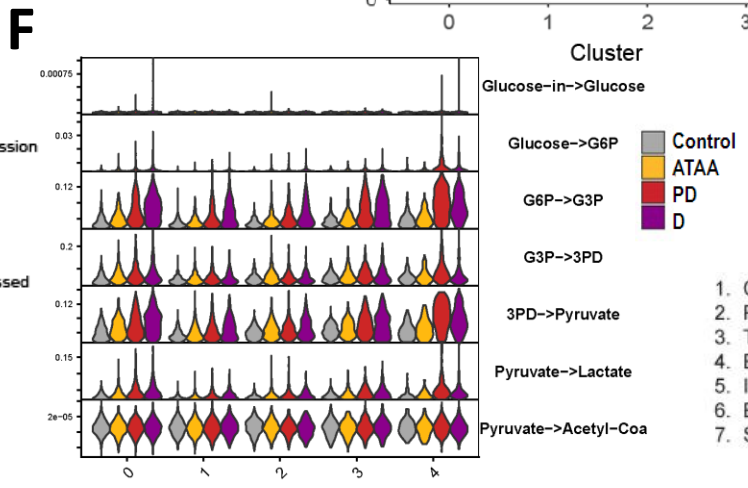

A
Organ donor control
(n=8)
Ascending Thoracic Aortic Aneurysm (ATAA)
(n=9)
Acute Ascending Thoracic Aortic Dissection (ATAD)
Pre-dissection (PD)
True Lumen
False Lumen
Dissection (DD)
(n=9)
Single-cell suspension
sc-RNAseq library
(Control/Disease)
Sequencing

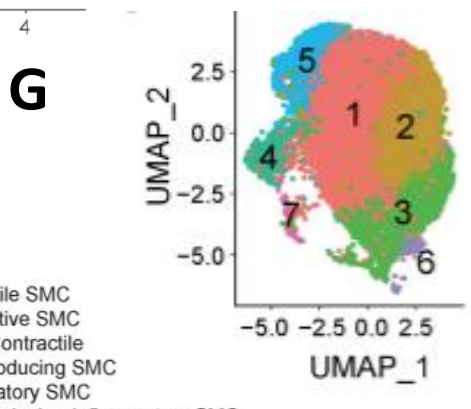

B
SMC
Others
UMAP_2
UMAP_1
Others
SMC
0: Fibromyocyte
1: Contractile
2: Intermediate (Contractile/ECM)
3: Proliferative/Stressed
4: Inflammatory

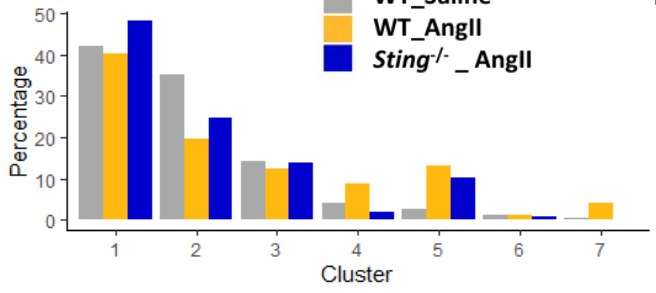

C
p=0.00066
p=0.0023
p=0.015
p=0.036
p=0.00097
p=0.0032
Percentage
Cluster
Control
ATAA
PD
D

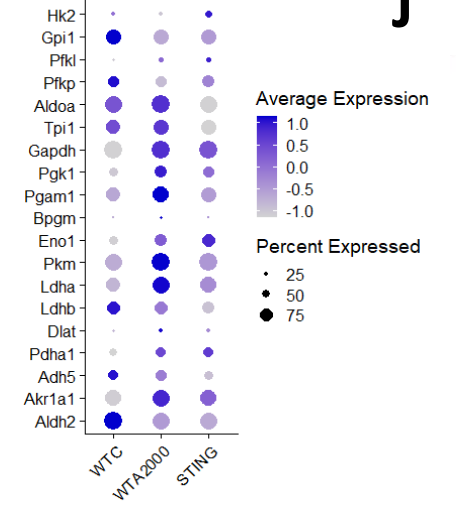

D
Glucose
HK
G6P
PGI
F6P
PFK1
F1,6BP
ALDO
ALDO
G3P
TPI
DHAP
GAPDH
1,3BPG
PGK
3PG
PGM
2PG
ENO
PEP
PK
Pyruvate
Lactate
PDH
LDH
Acetyl CoA
TCA Cycle


E
HK1
TPI1
GAPDH
PGK1
PGAM2
ENO1
PKM
LDHA
ADH1B
ADH5
ALDH1B1
ALDH2
Control
ATAA
PD
D
Average Expression
1.0
0.5
0.0
−0.5
−1.0
Percent Expressed
25
50
75


F
Glucose−in−>Glucose
Glucose−>G6P
G6P−>G3P
G3P−>3PD
3PD−>Pyruvate
Pyruvate−>Lactate
Pyruvate−>Acetyl−Coa
Control
ATAA
PD
D


G
UMAP_2
UMAP_1
1. Contractile SMC
2. Proliferative SMC
3. Tnnt2+Contractile
4. ECM-Producing SMC
5. Inflammatory SMC
6. ECM Producing-Inflammatory SMC
7. Stressed SMC


H
WT_Saline
WT_AngII
Sting-/- _ AngII
Percentage
Cluster


I
Hk1
Hk2
Gpi1
Pfkl
Pfkp
Aldoa
Tpi1
Gapdh
Pgk1
Pgam1
Bpgm
Eno1
Pkm
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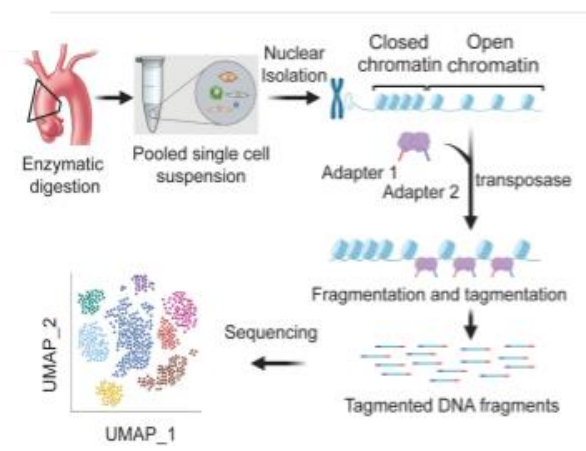
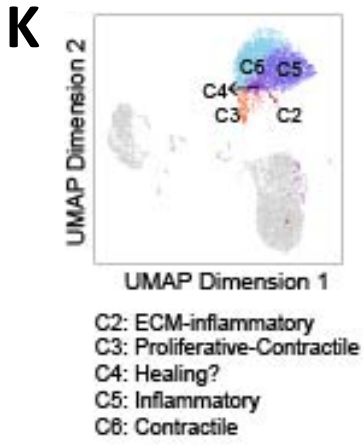
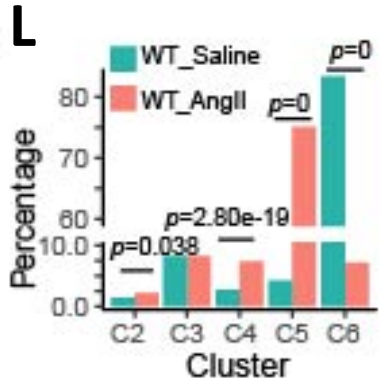
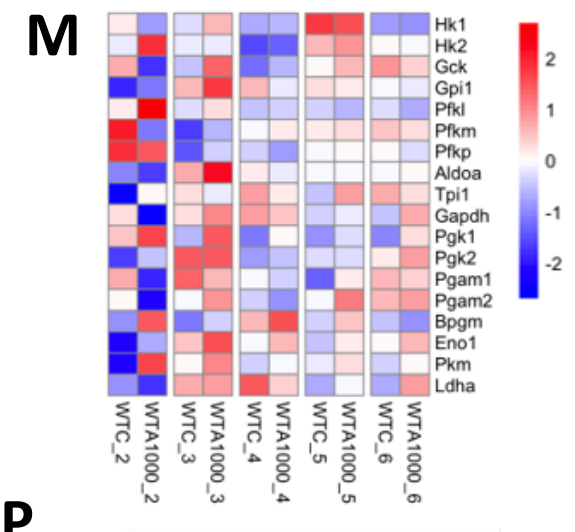
Adh5
Akr1a1
Aldh2
WTC
WTA2000
STING
Average Expression
Percent Expressed


J
Enzymatic digestion
Pooled single cell suspension
Nuclear Isolation
Closed chromatin
Open chromatin
Adapter 1
Adapter 2
transposase
Fragmentation and tagmentation
Sequencing
Tagmented DNA fragments
UMAP_2
UMAP_1


K
UMAP Dimension 2
UMAP Dimension 1
C2: ECM-inflammatory
C3: Proliferative-Contractile
C4: Healing?
C5: Inflammatory
C6: Contractile


L
WT_Saline
WT_AngII
p=0
p=0
p=2.80e-19
p=0.038
Percentage
Cluster


M
Hk1
Hk2
Gck
Gpi1
Pfkl
Pfkm
Pfkp
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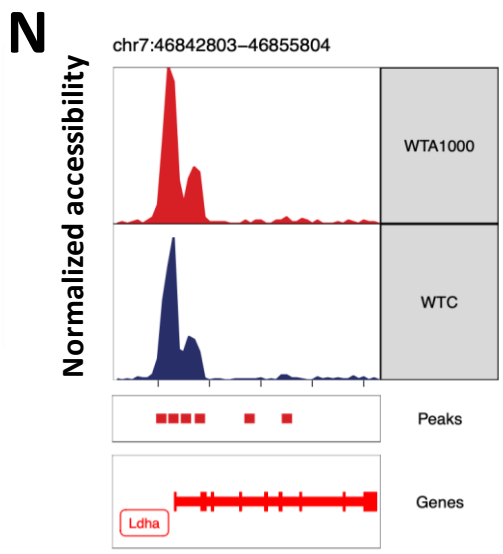
Pkm
Ldha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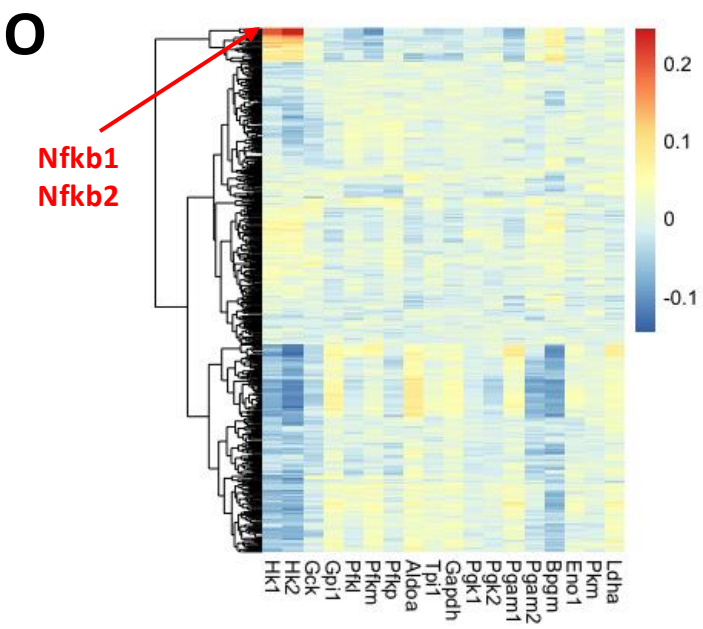
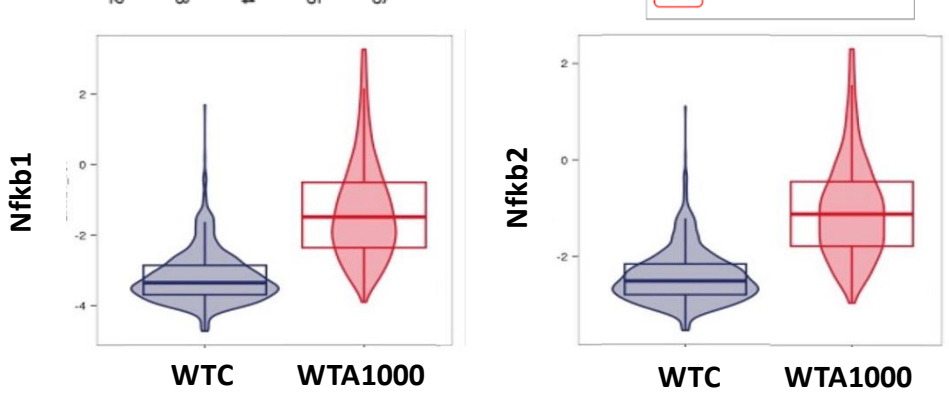
chr7:46842803−46855804
Normalized accessibility
WTA1000
WTC
Peaks
Genes
Ldha


O
Nfkb1
Nfkb2


P
Nfkb1
Nfkb2
WTC
WTA1000

- **Objective** - Male sex is a well-established risk factor for abdominal aortic aneurysms but the underlying mechanisms remain to be fully understood. Our lab reported aldosterone and high salt (Aldo-salt) induced aortic aneurysms in an age-dependent manner in male mice but whether it shows sex dimorphisms like humans is unknown. The current study tested the hypothesis that male mice are more susceptible to Aldo-salt induced aortic aneurysms through androgen mediation and investigated the potential mechanisms.

- **Approaches and Results** - Ten-month-old male and female mice were used in all experiments. We first demonstrated the sex differences in the Aldo-salt model with 70% incidence in male mice (7/10) compared to complete protection in female mice (0/9). Next, we showed androgen and androgen receptor (AR) mediated the process by several independent experiments: 1) orchiectomy provided strong protection (13.3%, 2/15) compared to sham control (69.2%, 9/13); 2) Exogenous DHT administration to orchiectomized mice restored the aneurysms formation (55%, 6/11); 3) inhibition of AR function by drug decreased total incidence compared to control (2/11 vs. 7/11). To dissect the mechanism, we conducted bulk RNA sequencing in aortas from 3 groups of mice given Aldo-salt 1) intact male mice; 2) orchiectomized mice; and 3) orchiectomized mice with DHT. Through bioinformatic analyses, we found PD-1 signaling pathway was significantly enriched in orchiectomized mice compared to control and reversed by DHT addition. Next, we found a significantly higher expression of PD-1 in spleen from orchiectomized mice than sham mice with Aldo-salt presence, at both protein and mRNA levels. Mechanistically we showed AR could bind to PD-1 promoter region and repressed PD-1 expression. Finally, we injected αPD-1 antibody or control IgG antibody to orchiectomized mice during the Aldo-salt administration to confirm phenotypes. Results showed that neutralization of PD-1 partially abolished the orchiectomy protection (0/8 vs. 5/12).

- **Conclusions** - Our results demonstrated that male mice are more susceptible to aldosterone and high salt-induced AAA. Androgen and its receptor mediate the high susceptibility in male mice partially through PD-1 signaling pathway.