

Distinct Biochemical Profile in Human Thoracic Aneurysms with Associated Valve Dysfunction:

Integrative Phosphoproteomic Analysis from the MultiTAAD Multiomic Database

M Elbatarny, U Kuzmanov, D Eliathamby, JCY Chung, C Simmons, AO Gramolini, M Ouzounian, on behalf of MultiTAAD investigators

No Disclosures



UNIVERSITY OF
TORONTO



UHN

Peter Munk
Cardiac Centre



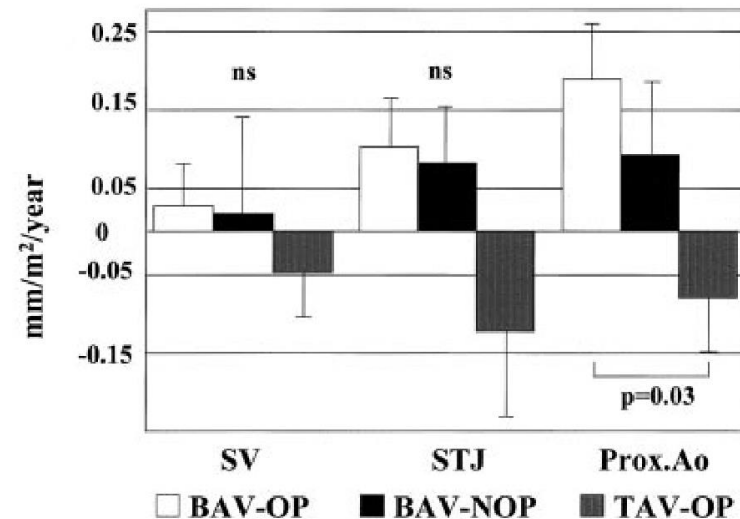
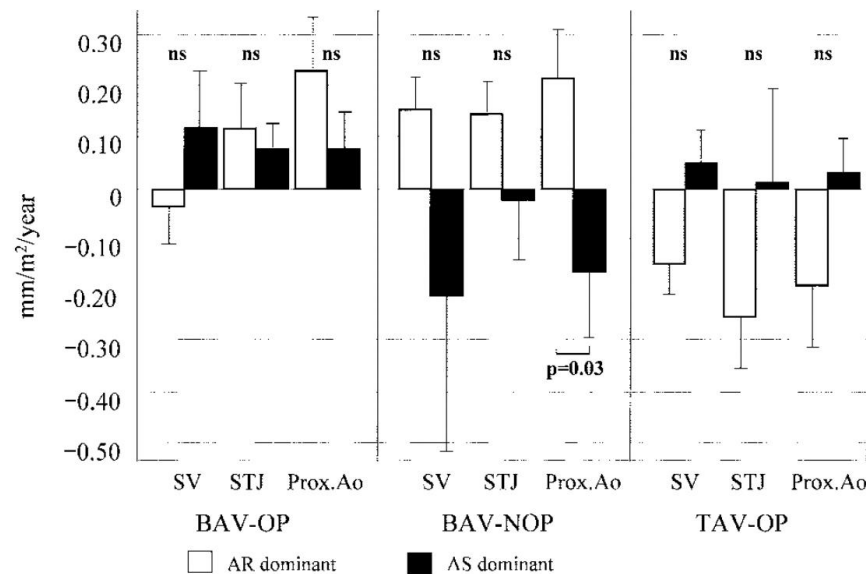
Proximal Aortopathy is Frequently Associated with *Aortic Valve Dysfunction*

Impact of valve function on aneurysm phenotype unknown

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**AVR fails to prevent progressive aortic dilation:
*especially in AR-associated aneurysms***



Hemodynamics may not be the full story...

Likely additional underlying molecular mechanisms

Objective

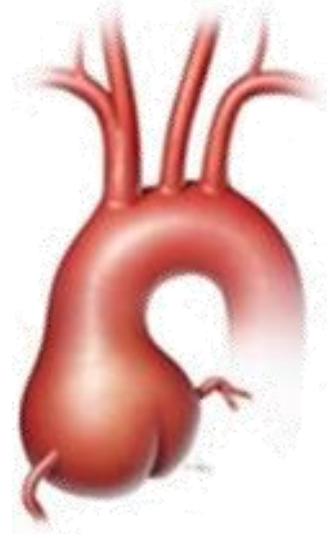
Compare *molecular profiles* of *human aortic aneurysm* tissues associated with different *valve functional phenotypes*



Normal

N=176

Human Aortic
Tissues



Proximal Aortic
Aneurysm

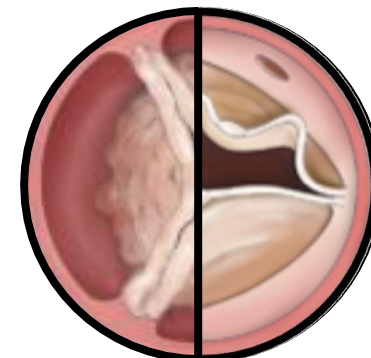


Regurgitation



Stenosis

Integrative proteomic & phosphoproteomic analysis



Mixed

Subanalysis: MultiTAAD *Multiomics of Thoracic Aneurysm & Dissection Database*

WHOLE GENOME
SEQUENCING

PROTEOMICS

PHOSPHO-
PROTEOMICS

CLINICAL DATA

SPATIAL RNA
SEQUENCING

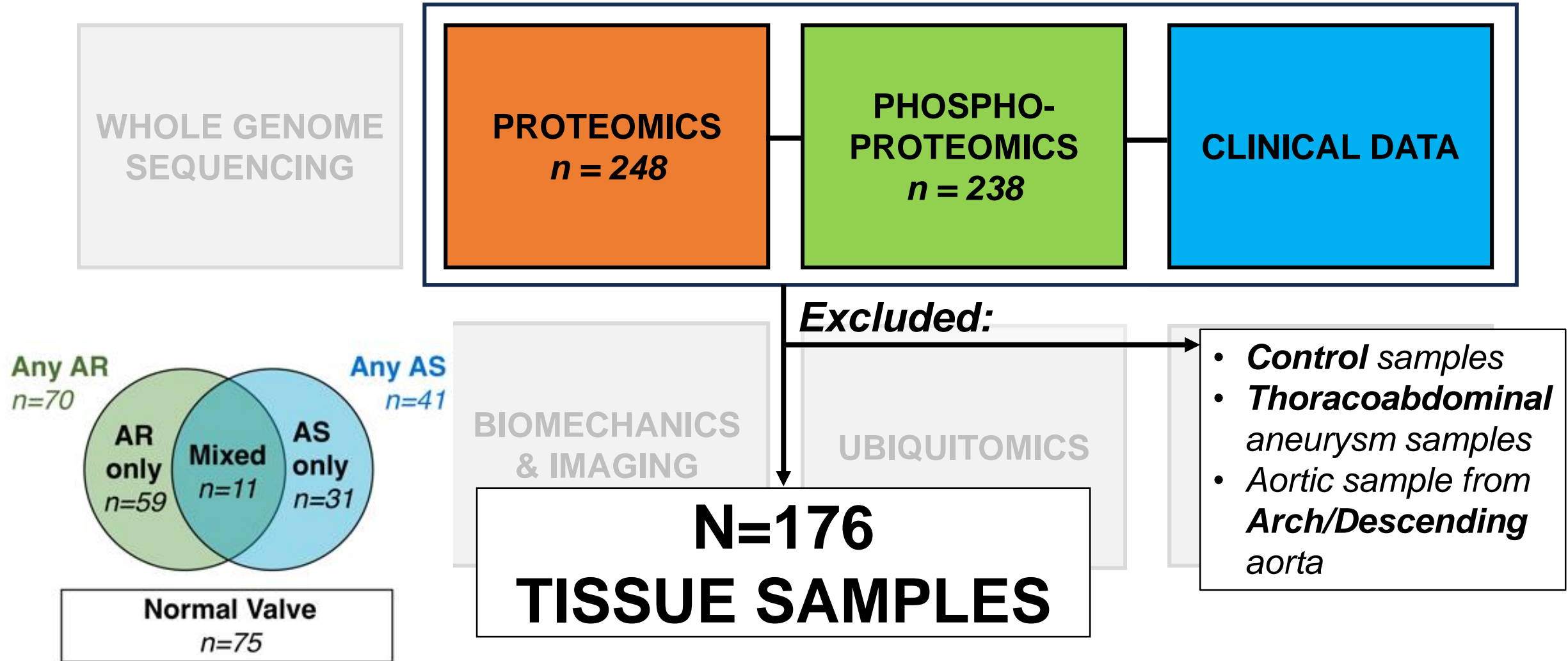
BIOMECHANICS
& IMAGING

UBIQUITOMICS

ACETYLOMICS

670 human aortic tissue samples analyzed to date
232 unique individuals; **2** centres

MultiTAAD *Multiomics of Thoracic Aneurysm & Dissection* Database



Proteomic & Phosphoproteomic Methods

Sample Preparation

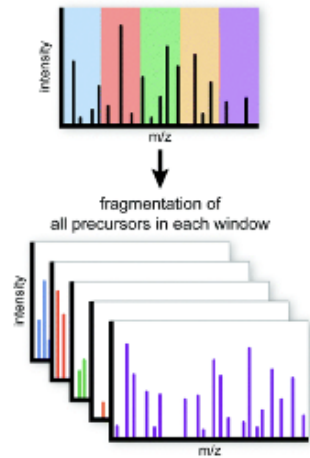
Polytron Homogenization in Urea
Reduction, Alkylation, Digestion
C18 desalting (Zip Tip)

Custom Library
Generation

Quantitative MS

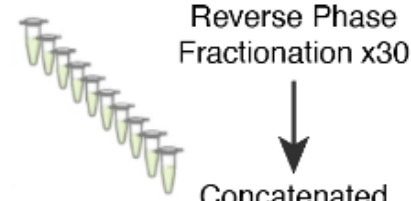
Data Independent Acquisition

EvoSep LCMS

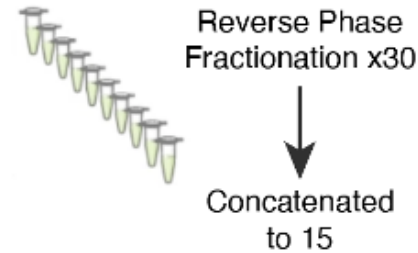


DIANN identification
quantification

Pool aliquot samples 1-100



HEK Cell Lysate

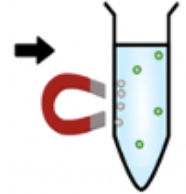


Sample Preparation

Polytron Homogenization in Urea
Reduction, Alkylation, Digestion
C18 desalting (Zip Tip)

**Two-Step
Phosphopeptide
Enrichment**

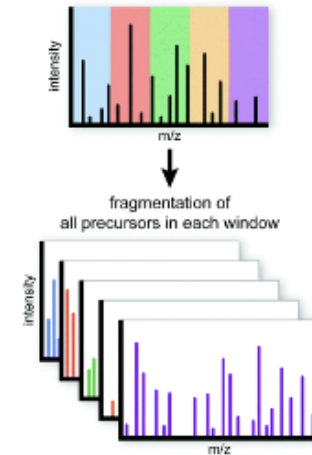
1. Fe/Zr
2. Ti



Quantitative MS

Data Dependent Acquisition

EvoSep LCMS



MaxQuant identification
quantification

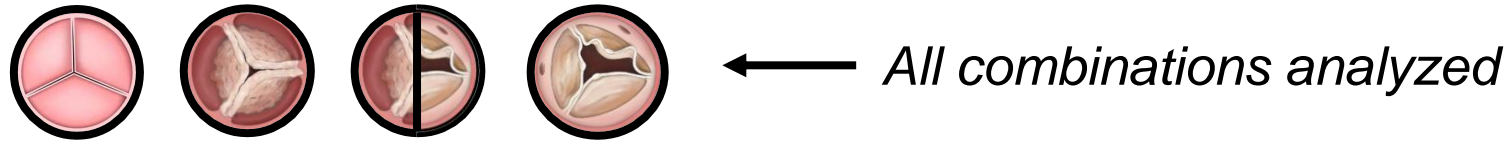
Analysis

Proteomics
(All Individual Proteins)

Phosphoproteomics
(All Phosphorylation Sites)



Directly Compared Aneurysm proteomic & phosphoproteomic profiles by valve morphology (*Perseus V2.6.0*)

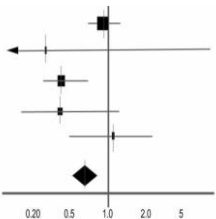


The **biological relevance of differential markers** identified via functional enrichment analyses (*PantherDB, X2KWeb*)



The CUCIKOO Workgroup

In silico site-specific kinase prediction (*GPS 6.0*)



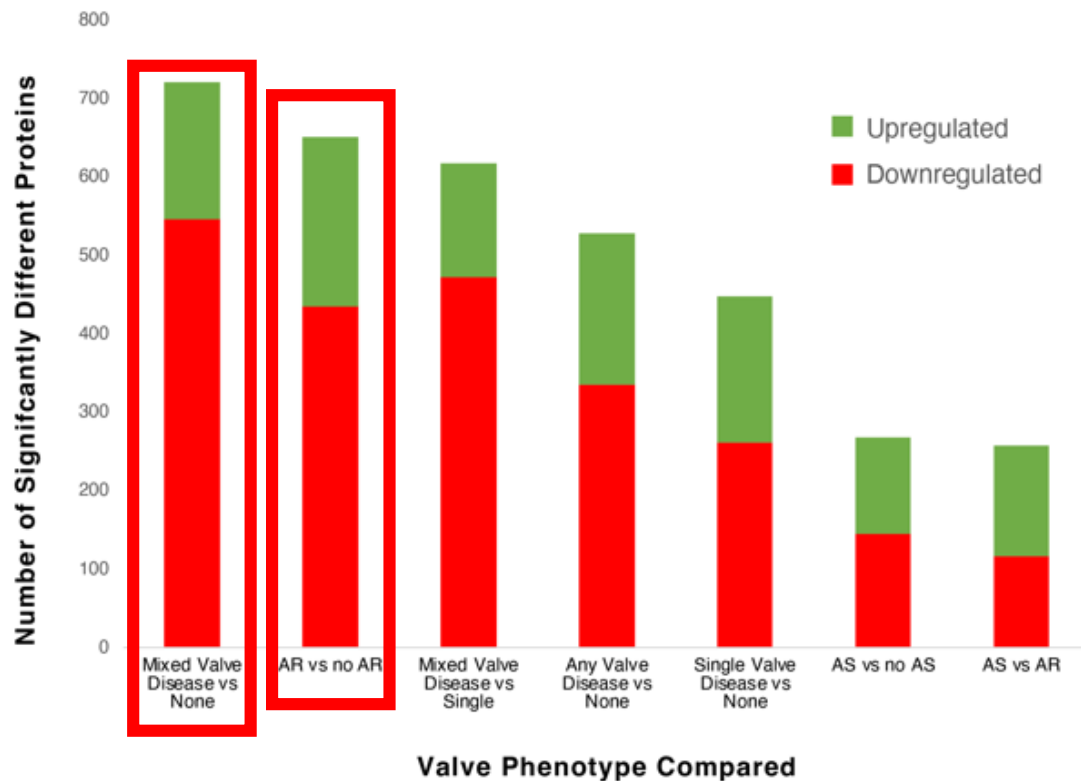
Statistical adjustment for clinical & imaging covariates (*multivariable regression*)

Results: Mixed Valve Disease Aortic & Regurgitation Confer Unique Phenotypes

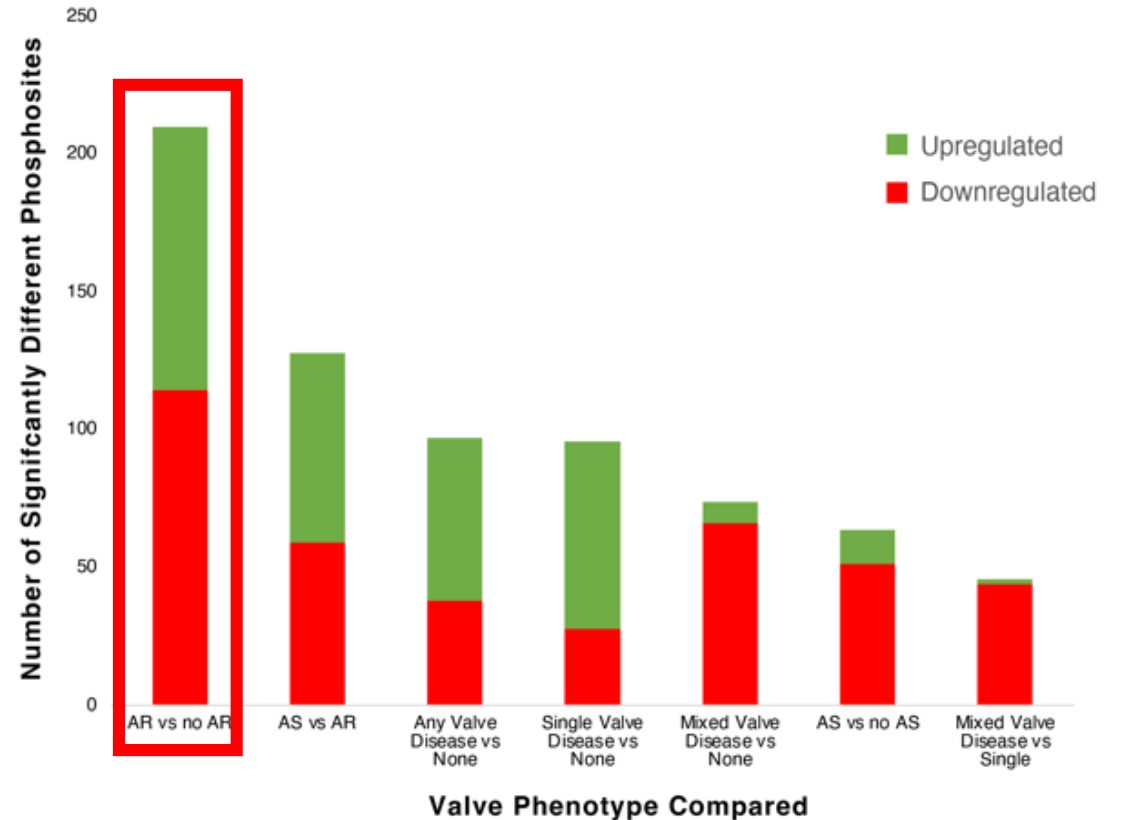
Total Proteins Quantified **6094**

Phosphosites Quantified **2125**

Significantly Different by Valve Type



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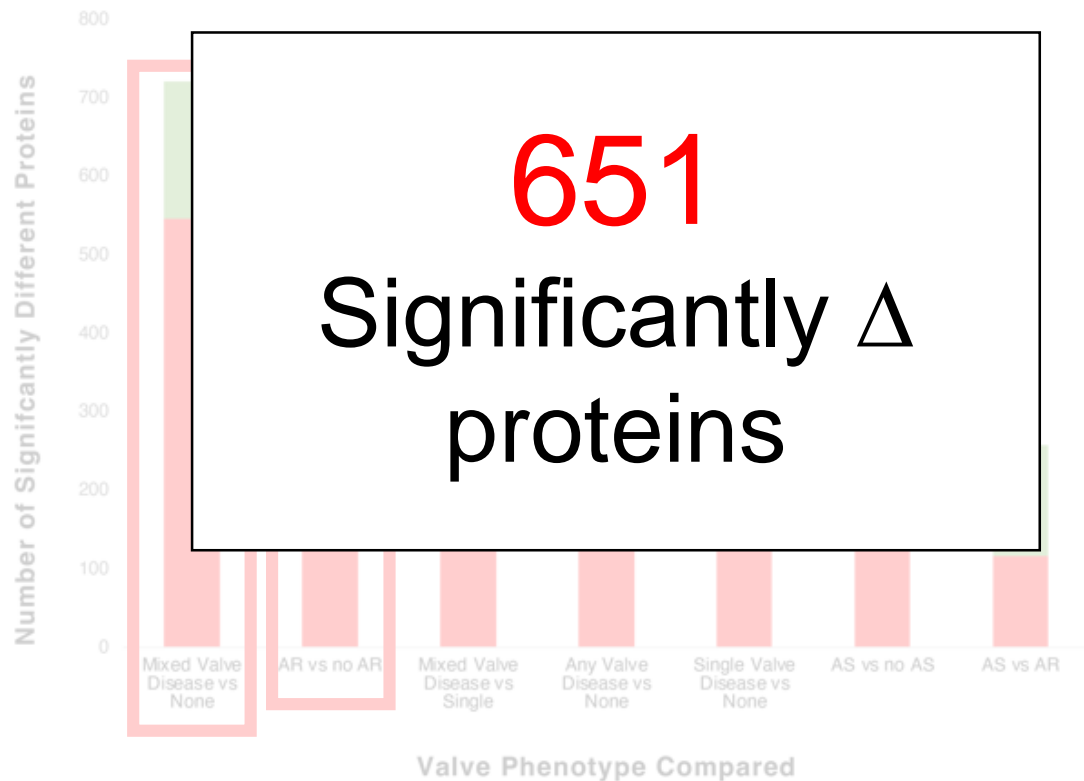


Results: Mixed Valve Disease & Aortic Regurgitation Confer Unique Phenotypes

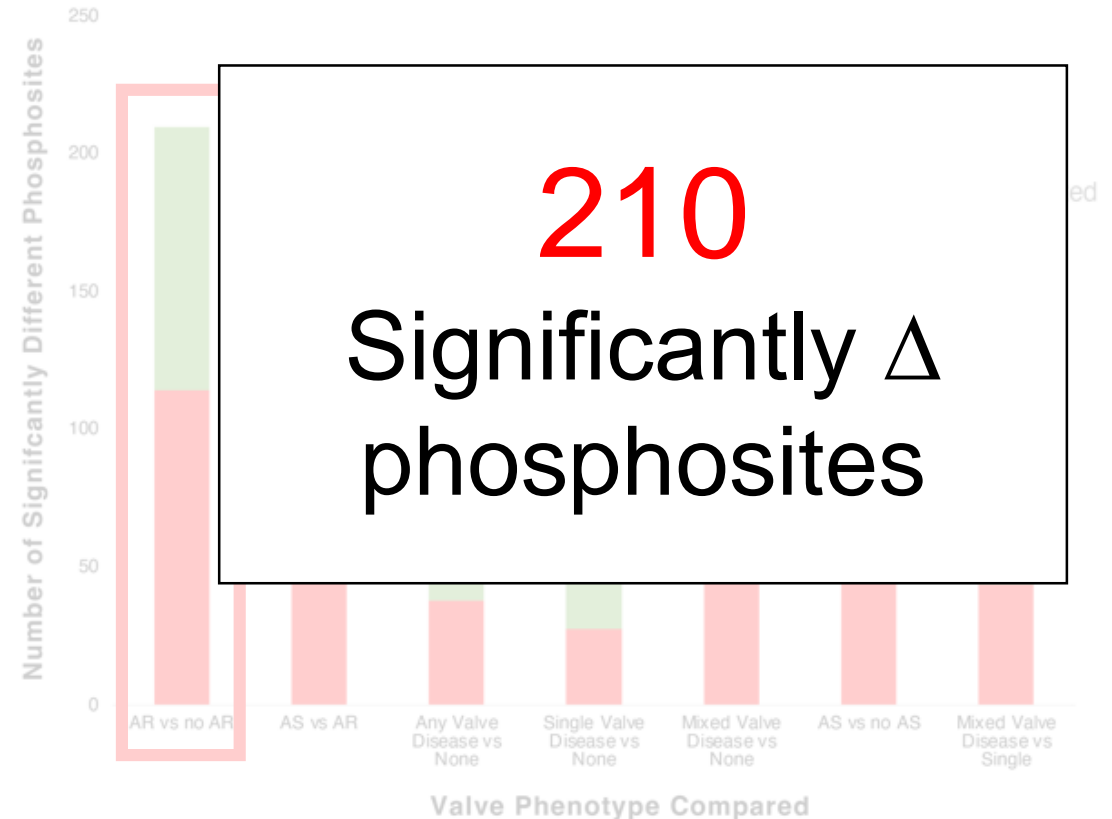
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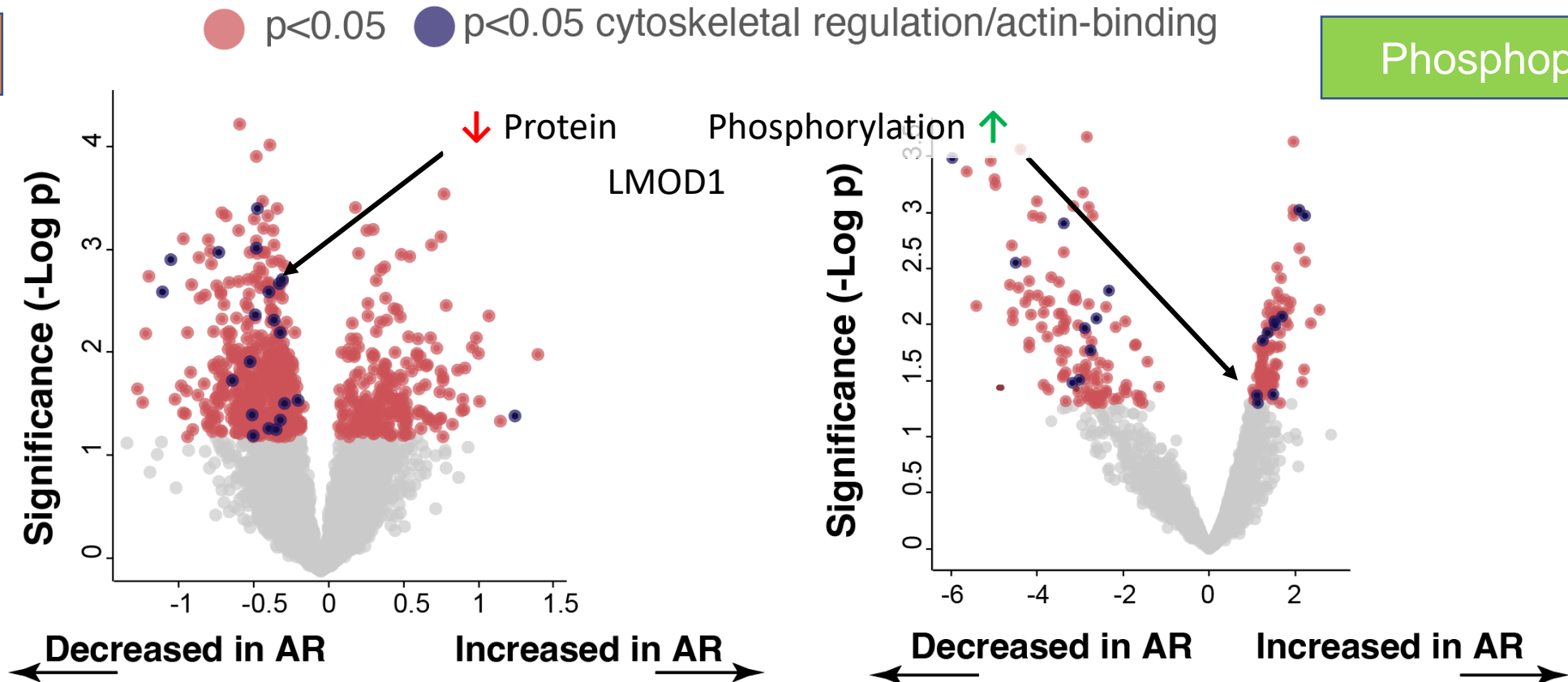
Significantly Different by Valve Type



Altered Proteome, Phosphorylation patterns in AR-associated Aneurysms

Proteome

Phosphoproteome

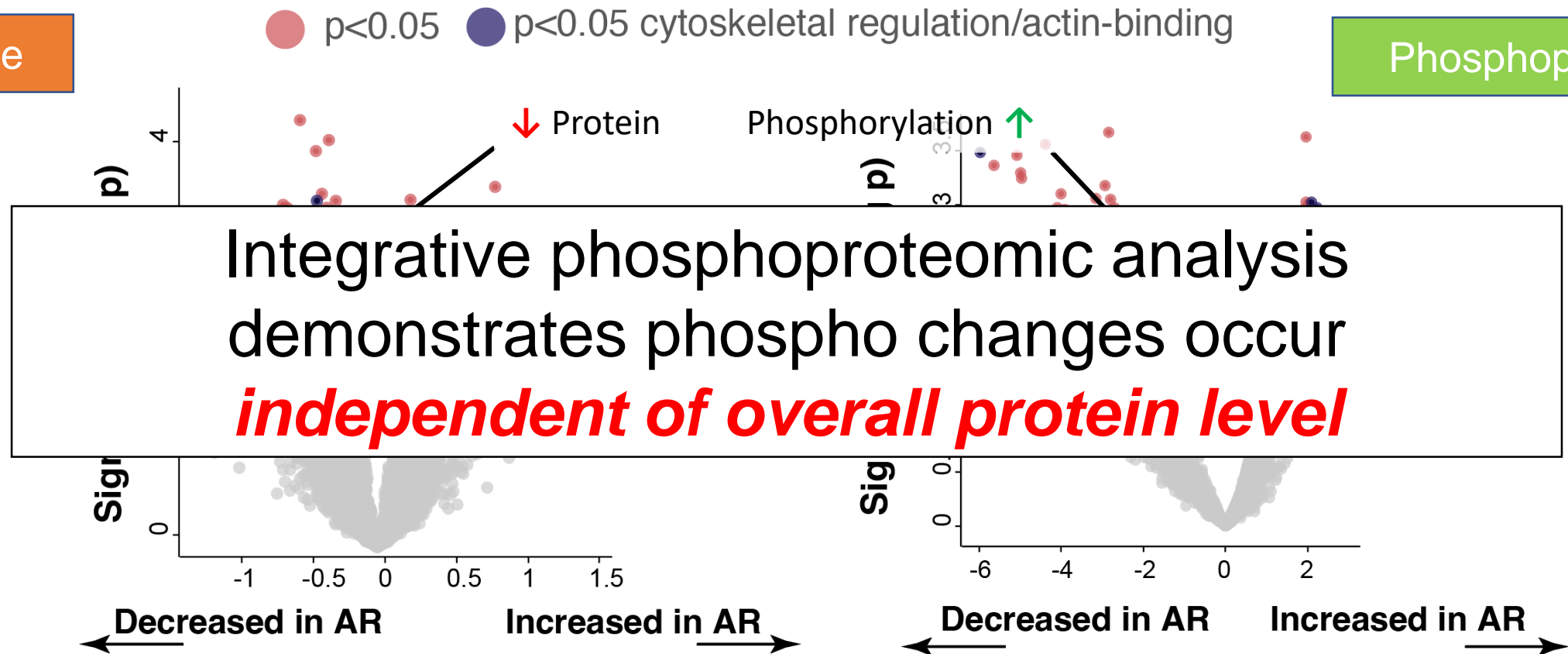


**Cytoskeletal regulatory *protein levels* are *downregulated* in AR
but *phosphorylation levels* are altered *bidirectionally***

Altered Proteome, Phosphorylation patterns in AR-associated Aneurysms

Proteome

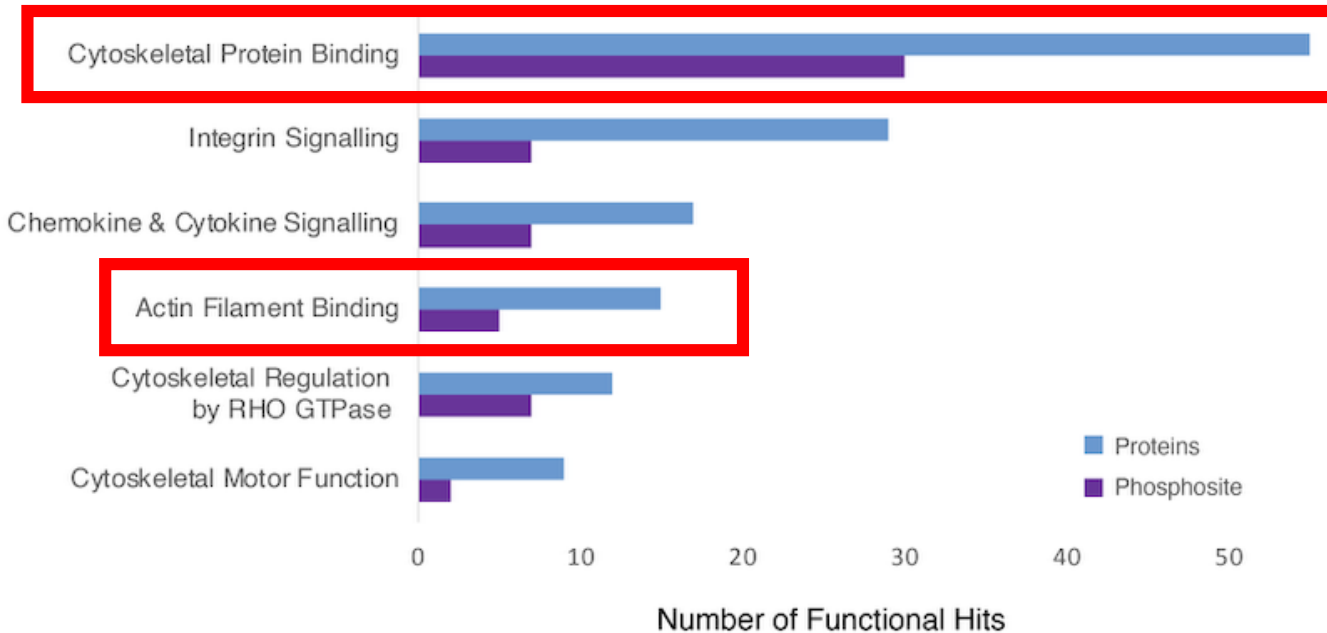
Phosphoproteome



Cytoskeletal regulatory ***protein levels*** are ***downregulated*** in AR
but ***phosphorylation levels*** are altered ***bidirectionally***

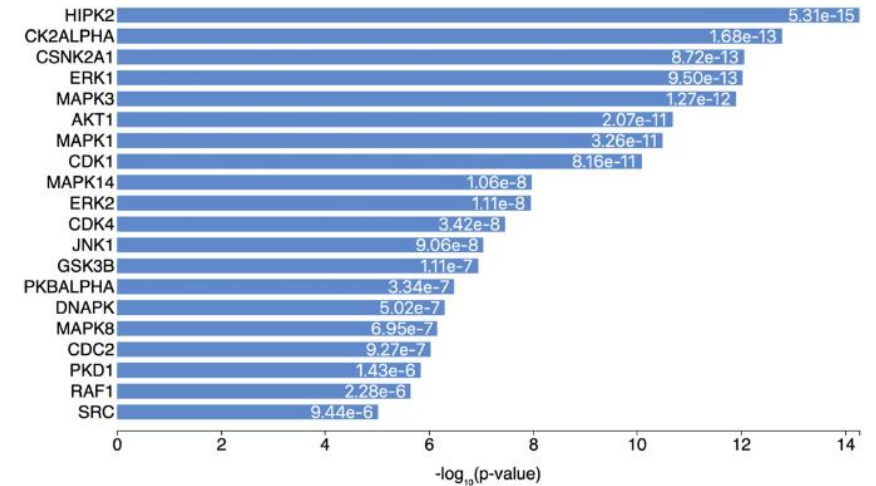
Biological Function, Kinases, and of AR-associated Proximal Aneurysms

GO Terms in Aortic Regurgitation-associated Aortopathy vs None



Cytoskeletal regulatory activities define differences in phenotype among AR-associated aortic aneurysms

Top Predicted Kinases in AR-associated aneurysms based on **All Top Differential Phosphorylation Sites**



Top Predicted Kinases for **Individual Sites**

ID	Position	Code	Kinase	PSP	Score
LMOD1	19	S	TKL	SEDPDIDSLLETLSP	0.3752
LMOD1	19	S	STE	SEDPDIDSLLETLSP	0.0372
LMOD1	19	S	PKL	SEDPDIDSLLETLSP	0.0356
LMOD1	19	S	RGC	SEDPDIDSLLETLSP	0.025
LMOD1	19	S	CK1	SEDPDIDSLLETLSP	0.0103
LMOD1	19	S	CMGC	SEDPDIDSLLETLSP	0.0004
LMOD1	19	S	CAMK	SEDPDIDSLLETLSP	0.0003
LMOD1	19	S	AGC	SEDPDIDSLLETLSP	0.0001

***AR-Aneurysms Do Have Distinct
Molecular Profiles***

***But... driven by clinical
characteristics?***

Clinical Characteristics Compared by AR Status

	No AR	AR	P
Age	58.7 (14.4)	56.5 (17.8)	0.36
Male	78 (72.9)	57 (81.4)	0.26
BMI	28.17 (5.4)	28.60 (6.0)	0.62
BSA	2.03 (0.3)	1.97 (0.3)	0.13
HTN	73 (68.2)	46 (65.7)	0.85
DLP	51 (48.1)	19 (27.5)	0.011
DM2	10 (9.4)	6 (8.7)	1
Smoking	32 (52.5)	14 (37.8)	0.23
AS	31 (29.0)	11 (15.7)	0.07
EF	60.5 (6.4)	58.4 (5.7)	0.07
AVR	18 (16.8)	18 (25.7)	0.21
VSR	34 (31.8)	9 (12.9)	0.007
Bentall	28 (26.2)	31 (44.3)	0.019
RAA	49 (45.8)	33 (47.1)	0.98
Hemiarch	14 (13.1)	16 (22.9)	0.14
Extended Arch	14 (13.1)	7 (10)	0.70
Elephant Trunk	9 (8.4)	2 (2.9)	0.24
Ross	2 (1.9)	7 (10)	0.04
Bicuspid	39 (36.4)	26 (37.1)	1
Diameter			
Root, Indexed	22.2 (3.9)	22.2 (5.2)	0.98
Ascending, Indexed	22.6 (5.19)	25.2 (4.7)	0.001

Clinical Characteristics

AR group

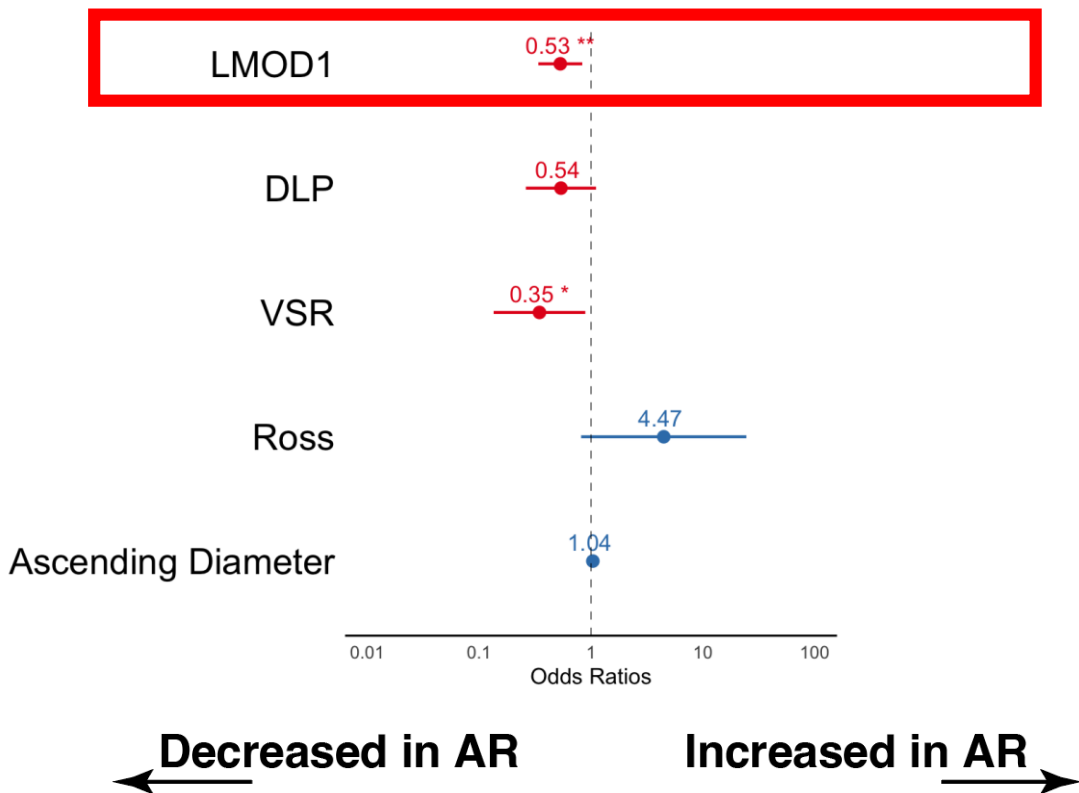
↓ *dyslipidemia*

↑ *Root Replacements*

↑ *Ascending Diameters*

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Cytoskeletal Regulatory Markers Remain Significantly Associated with Aneurysm Phenotype *after adjustment*



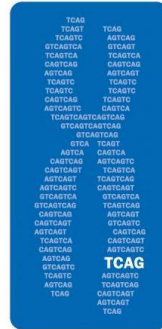
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Root, Indexed			
Ascending, Indexed			

Cytoskeletal molecular changes are **independently** associated with AR in proximal aneurysms

Conclusions

- **1st multi-proteomic analysis** of aortopathy by aortic valve function on a **large cohort of human samples** **closer to phenotype vs DNA/RNA**
- Valve dysfunction, **especially AR**, is associated with **altered aortic tissue biochemistry** → **loss of cellular contractility**
 - Differences observed at both the proteomic and **independently** phosphoproteomic level
- **Incorporate valve functional parameters** in personalized surgical management of aneurysm
 - **Earlier aneurysm repair for AR patients?** Surveillance studies required

Acknowledgements



Senior Leadership

Collaborators

Maral Ouzounian

Sylvain Meloche,
Scott Weed, Boris Hinz,
Grith Lykke Sørensen,
Kevin Lachapelle
Richard Leask

Anthony Gramolini

Bo Wang

Raymond Kim

Jennifer CY Chung

Craig Simmons

Valli Subasri

Biomechanics

Daniella Eliathamby

Gramolini Lab

Uros Kuzmanov Allen Teng

Wenping Li Cristine Reitz

Omar Hamed Linda Lu

MultiTAAD Investigators

Artificial Intelligence

Vivian Chu, Rashmi Nedadur

Clinical Samples

Karamvir Deol, Rifat Islam,
Amy Tanzania + 7 students

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