Heart Valve Mechanobiology and Underlying Cell Physiology

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Penn Cardiovascular Institute & Institute for Translational Medicine and Therapeutics
GOAL & FUNDING

Our goal is to build and strengthen bridges between research at Clemson and medical practice at GHS. Funding is provided through the Harriet and Jerry Dempsey CU/GHS Bioengineering Professorship awarded to Dr. Dan Simionescu and the Harriet and Jerry Dempsey CU/GHS Industrial Engineering Professorship awarded to Dr. Kevin Taaffe.
Heart Valve Diseases: the next cardiac epidemic

1. A translational approach to complex cardiovascular diseases based on human biospecimens


3. Strategies for therapeutic intervention and diagnosis
Heart Valve Diseases: the next cardiac epidemic

- > 5 M pts are diagnosed with HVD each year in the US;
- HVD can occur in any single valve or a combination of the four valves, but AV and MV are the most common;
- Myxomatous mitral valve disease (MMVD) is expected to occur in approximately 7.2 million individuals in the US and 144 million worldwide
- Without an AVR, as many as 50 % of pts with severe AS will not survive > 2 years after the onset of symptoms
- The predicted survival of inoperable patients with severe AS who are treated with standard non-surgical therapy is lower than with certain metastatic cancers
- 80,000-100,000 AVR procedures are performed every year in the U.S
Heart Valve Diseases: the next cardiac epidemic

![Projected AVR Procedures Worldwide](chart)

**Projected U.S. Population 65 and Older**

Currently available data do not support the use of statins to improve outcomes and to reduce disease progression in non-rheumatic calcific aortic valve stenosis.

**TABLE 2. Published prospective randomized trials**

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<tr>
<td>n (n on statins + ezetimibe)</td>
<td>155 (77)</td>
<td>1873 (944)</td>
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<tr>
<td>Follow-up</td>
<td>2.09 y</td>
<td>4.35 y</td>
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<tr>
<td>Grade, AS at baseline</td>
<td>Mild to severe</td>
<td>Mild to moderate</td>
</tr>
<tr>
<td>Primary endpoints</td>
<td>$V_{max}$ ($P = 0.95$)</td>
<td>Composite ($P = 0.59$)</td>
</tr>
<tr>
<td></td>
<td>$AVC$ ($P = 0.80$)</td>
<td></td>
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<tr>
<td>Secondary endpoints</td>
<td>Composite ($P = 0.19$)</td>
<td>$AVA$, $V_{max}$ ($P = 0.83$)</td>
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<tr>
<td></td>
<td>Death from cardiovascular causes ($P = 1.0$)</td>
<td>Aortic valve events ($P = 0.73$)</td>
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<tr>
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<td>Aortic valve replacement ($P = 0.17$)</td>
<td>Ischemic events ($P = 0.02$)</td>
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<tr>
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<td>Hospitalization for severe AS ($P = 0.73$)</td>
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<tr>
<td></td>
<td>Death from any cause ($P = 0.73$)</td>
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</tr>
<tr>
<td></td>
<td>Hospitalization for any cause ($P = 0.84$)</td>
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Pro statins: No

Abbreviations: n, number of patients; $V_{max}$, peak transaortic velocity; AVC, aortic valve calcium; AVA, aortic valve area; SALTIRE, Scottish Aortic Stenosis and Lipid Lowering Trial, Impact on Regression; SEAS, Simvastatin and Ezetimibe In Aortic Stenosis Study.
Heart Valve Diseases: the next cardiac epidemic

Pulmonary valve

Aortic valve

Mitral valve

Tricuspid valve

65

75 (years)
Heart Valve Diseases: the next cardiac epidemic
Heart Valve Diseases: the next cardiac epidemic

No molecular-based tools are available to risk-stratify patients for the risk of heart valve disease or associated co-morbidities.
Penn Cardiac Bioregistry (PCB)

CT Surgery → PENN CARDIAC BIOREGISTRY

- Aortic Valve Disease
  - Aortic Valve Sclerosis
  - Aortic Valve Insufficiency
- Bioprosthetic Valve Degeneration
- Thoracic Aortic Disease
- Mitral Valve Prolapse
- Vascular Remodeling, MI, CAD, etc.

Retrospective and Prospective Clinical Studies

REDCap
Penn Cardiac Bioregistry (PCB)

- **Tissue Collection:**
  - Cardiac Procedures at Penn
  - Heart Transplant Program
  - Valley Hospital
  - Bioserve

- **Specimen Collected**
  - Plasma, Serum, and Buffy Coat
    - Blood collected prior to incision or heparinization
    - Stored at -80°C
  - Heart Valves and Ascending Aorta
    - Collected in Operating Room after resection
    - Processing
      - Stored at -80°C
      - Prepared for Cell Isolation
      - Paraffin Embedded
      - Prepared for Biomechanical testing
Penn Cardiac Bioregistry (PCB)

PCB eDatabase (410 Fields)

• Patient Information Collected
  • General Information
    • gender, age, BMI, BSA, etc.
  • Medical History
    • HTN, HLD, CAD, DM, medications, prior surgeries, etc.
  • Imaging Reports (Preop Echo, Periop Echo, CT)
  • Lab Values
  • Surgical Procedure

• Information stored in REDCap Database
Aortic Valve Sclerosis: Towards a common definition

- Prevalence of AVSc: 25% to 30% in patients older than 65 and 40% in older than 75 years of age
- AVSc = higher risk of cardiovascular events and increased
- It can progress to moderate or greater aortic stenosis in up to 10% of patients over time
- Identification of high-risk patients at early stages of opens new perspectives for appropriate timing of therapeutic intervention in future clinical trials.

Modified from Otto et al. 2009
Aortic Valve Sclerosis: Towards a common definition

Control

Aortic Sclerosis

Aortic Stenosis

Asymptomatic Stages

Symptomatic Stage

Pathological Stages

Continuum of Disease where pathological and symptomatic stages only partially overlap
Aortic Valve Sclerosis: Towards a common definition

- Irregular, non-uniform thickening of portions of the aortic valve leaflets or commissures, or both;
- Thickened portions of the aortic valve with/or without an appearance suggesting calcification (ie, bright echoes);
- Non-restricted or minimally restricted opening of the aortic cusps; and peak continuous wave Doppler velocity across the valve < 2 m/s.

Ca^{2+} Score: 1 - 4

Symptomatic Stage

Pathological Stages

Tissue and primary cell lines are generally not available to investigators as these patients are asymptomatic.
Aortic Valve Sclerosis: Towards a common definition
A biomechanical model to test subclinical CAVD stages
Oxidative stress accumulation is associated with reduced antioxidant enzymes expression and increased DNA damage in AVSc.
Impaired DNA damage response (DDR) in non-calcified, asymptomatic, patients with aortic valve sclerosis.
Oxidative stress results in unresolved DNA damage in AVSc-derived VICs
Oxidative stress modulates AVSc-derived VIC transdifferentiation via AKT signaling pathway.


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Adenoviral transduction of antioxidant enzymes (SOD and CAT) rescues VICs from an impaired DNA damage response and reduces the expression of early markers of VICs activation.
Adenoviral delivery of catalase reduces H2O2-mediated in vitro calcification of VICs


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Early modifications in the leaflet’s microenvironment modify the mechanobiological response of VICs via matricellular regulation of valve cell (dys)function.

**Matricellular Signalling**
The ECM signals through adhesion receptors (e.g., collagen, elastin fragments, biglycan, tenasin-C, etc. with valve interstitial cells).

**Matricrine signalling**
The ECM sequesters and localizes growth factors (e.g., fibronectin and TGFβ1; biglycan and TNF-α, etc.)

**Mechanical signalling:**
- **Matrix elasticity**: The intrinsic elasticity (stiffness) of the ECM regulates cell function and modulates cell response to other microenvironmental stimuli.
- **External forces**: The ECM transmits external forces (e.g., shear stress, pressure, stretch) to cells.

Chen & Simmons
Early modifications in the leaflet’s microenvironment modify the mechanobiological response of VICs via matricellular...
OPN-CD44 functional interaction as a hallmark of early stages of calcific aortic valve disease

OPN-CD44 functional interaction as a hallmark of early stages of calcific aortic valve disease

CD44 and OPN protect human sclerotic VICs from calcification induced by BMP4


American Heart Association.
Akt phosphorylation induced by CD44/OPN is required to protect human sclerotic VICs from calcium deposition.
Implications for therapy

MPs are functionally regulating AV remodeling and are ideal targets as they bi-directionally regulates both VICS and ECM architecture.
Early modifications in the leaflet’s microenvironment modify the mechanobiological response of VICs via matricellular
Early modifications in the leaflet’s microenvironment modify the mechanobiological response of VICs via matricellular
Mechano-trasduction and Matricellular Proteins

A) Specimen
B) Tensile stretch (15% strain, 1 Hz)
C) Static
D) Dynamic
E) BMP4
F) OPN
G) CTGF
H) Wall shear stress magnitude (dynes/cm²)

Figure 6
(a) High shear rate (1000 rpm), high viscosity (1.2 MPa)
(b) Low shear rate (500 rpm), high viscosity (1.1 MPa)
A TE approach to induce AVSc-derived VICs to osteogenic transdifferentiation
Noggin attenuates BMP4-induced activation of human VICs in asymptomatic AVSc

Implication for clinical care:

1. Ex vivo AVSc tissue can be induced to biomineralization and to express markers of osteogenic transdifferentiation

2. Our TE model allows mechanistic intervention on VICs activation and testing different molecules to try to halt the progression from AVSc to severe AVS
Remodeling of BAV Cusp and Impact of surgical repair on cusps architecture

Lindman, B. R. et al. (2016) Calcific aortic stenosis
Nat. Rev. Dis. Primers doi:10.1038/nrdp.2016.6
AI vs AS: micro-regional architecture

Implications for therapy

BAV AS- and TAV AS-derived VICs are not intrinsically different

Microarchitecture impact CAVD progression

BAV AS and BAV AI show partially overlapping mechanisms of regulation. AI (type II) is a disease of valve cusps in which tissue remodeling could be controlled
A murine model for asymptomatic heart valve remodeling

**Aortic valve leaflet thickening**

- Saline
- + Ang II

**Thickness**

- Saline
- + Ang II

**Alizarin Red**

- Saline
- Ang II
A murine model for asymptomatic heart valve remodeling
A murine model for asymptomatic heart valve remodeling

VIC osteogenic-like activation

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<th>OPN</th>
<th>SMA</th>
<th>SOX9</th>
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<td><strong>Saline</strong></td>
<td>![Image]</td>
<td>![Image]</td>
<td>![Image]</td>
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<tr>
<td><strong>Ang II</strong></td>
<td>![Image]</td>
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</table>

ROS

- Saline: ![Image] (10x)  
- Ang II: ![Image] (10x)
A murine model for asymptomatic heart valve remodeling
Ascending Aortic Disease

A
Thoracic Aortic Aneurysm
Abdominal Aortic Aneurysm

B
Diameter (mm)
0  28 days
1  1.5

Saline + Ang II

Leaflet Thickness (µm)
0  100  200  300
Saline + Ang II

C
Nitrotyrosine

Saline Ang II

% Protein Expression
0  20
A murine model for asymptomatic heart valve remodeling


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Ascending Aortic Disease

A

Saline

Ang II

C

Medial Thickness Ascending Aorta

D

miR-143

Vimentin

F

Saline

Ang II

Elk-1

HMGB-1

RAGE

GAPDH

B

Diameter (mm)

0

28 days

* Ang II + Saline

E

RAGE

Vimentin

H

(ROS) Nitrotyrosine

Saline

Ang II
Healthy and end-stage disease human VICs are mostly unresponsive to any treatments in the setting of AS.

Early stage of CAVD represent the optimal temporal opportunity for diagnosis and therapeutic treatments: VIC activation and AV leaflet pathological remodeling are reversible.

Antioxidant strategies, 5HT antagonists, BMP4 inhibitors, and Matricellular proteins are few valuable options as they directly impact AV and ascending aortic remodeling.

We need to better characterize early stage and determine not only the mechanisms of progression but also the molecular mechanisms responsible for the different rate of progression.
Aortic Valve Sclerosis, moderate AI, and Ascending Aortic Disease could be asymptomatic

Heart Valve Working Group
Comparison between echocardiographic evaluation and Osteopontin level in CAVD patients.

- 1 = None
- 2 = Mild
- 3 = Moderate
- 4 = Severe

(A) Absolute OPN levels (in ng/ml of plasma), measured by ELISA, in the plasma of patients with different calcium score (x axis). (B) Comparison between OPN concentration in the plasma of control group (calcium score 1) and patients (calcium score 1 to 4).
In AVSc asymptomatic patients OPN levels are elevated even before Ca++ deposition is detectable on TEE

1 = None
2 = Mild
3 = Moderate
4 = Severe

Comparison between echocardiographic evaluation and Osteopontin level in CAVD patients. (A) Absolute OPN levels (in ng/ml of plasma), measured by ELISA, in the plasma of patients with different calcium score (x axis). (B) Comparison between OPN concentration in the plasma of control group (calcium score 1) and patients (calcium score 1 to 4).
Plasma biomarker profile

Bar graph representing distribution of plasma biomarkers levels with respect to calcium scores determined by echocardiographic evaluation. Groups represented are C1 (Control calcium score 1), ASC1 and ASC2 (aortic sclerosis calcium score 1, 2) and AS3 and AS4 (Aortic Stenosis calcium score 3, 4). Bars represent Mean±SE

- 1 = None
- 2 = Mild
- 3 = Moderate
- 4 = Severe
**Objective:** To create a large case-control study of patients with calcific AS and matched controls and perform a comprehensive biomarker and genetic study
**Objective:** To create a large case-control study of patients with calcific AS and matched controls and perform a comprehensive biomarker and genetic study.
1. A translational approach to complex cardiovascular diseases based on human biospecimens in possible, and requires a multidisciplinary approach

2. Heart Valve Diseases: The Perils of Highly Interconnected Systems: AS, AI, AA. We need a multi-institutional CT surgery biobank

3. Strategies for therapeutic intervention and diagnosis are available but require validation in animal model and clinical trials

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- National Institutes of Health
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  NIH R01HL119297
  NIH R01HL122805

  NIH T32HL007954
  NIH T32HL007915

- The Valley Hospital Foundation
- American Heart Association
  GRNT to 15GRNT24810002
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<td>PhD</td>
<td>Marfan Syndrome and Ascending Aortic Disease</td>
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<td>Katheryn H. Driesbaugh</td>
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