Mechanism of aortic medial matrix remodeling is distinct in patients with bicuspid aortic valve

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Objectives: Patients with bicuspid aortic valves (BAV) are predisposed to developing ascending thoracic aortic aneurysms (TAA) at an earlier age than patients who develop degenerative TAAs and have a tricuspid aortic valve (TAV). The hypothesis tested is that BAV-associated aortopathy is mediated by a mechanism of matrix remodeling that is distinct from that seen in TAAs of patients with tricuspid aortic valves.

Methods: Aortic specimens were collected during ascending aortic replacement, aortic valve replacement, and heart transplants from nonaneurysmal (NA) donors and recipients. Matrix architecture of the aortic media was assessed qualitatively using multiphoton microscopy followed by quantification of collagen and elastin fiber orientation. α-Elastin was determined and matrix maturity was assessed by quantifying immature and mature collagen and lysyl oxidase (Lox) expression and activity in aortic specimens. Matrix metalloproteinase-2/9 activity was quantified in aortic smooth muscle cells.

Results: Elastin and collagen fibers were more highly aligned in BAV-NA and BAV-TAA cases than in TAV-TAA cases, whereas TAV-TAA cases were more disorganized than TAV-NA cases. α-Elastin content was unchanged. Immature collagen was reduced in BAV-NA and BAV-TAA cases when compared with TAV-NA and TAV-TAA cases. Mature collagen was elevated in TAV-TAA cases compared with TAV-NA and BAV-TAA cases. There was a trend toward elevated *Lox* gene expression and activity and matrix metalloproteinase-2/9 activity for TAV-TAA, BAV-NA, and BAV-TAA specimens.

Conclusions: The highly aligned matrix architecture in patients with BAVs indicates that wall remodeling is distinct from TAV-TAA. Altered matrix architecture and reduced collagen maturity suggest that the effector molecules mediating the remodeling of TAAs are different in BAV and TAV cases. (J Thorac Cardiovasc Surg 2014;147:1056-64)

Congenital bicuspid aortic valve (BAV) is associated with an aortopathy manifesting as ascending thoracic aortic aneurysm (TAA) or aortic dissection. BAV is the most common congenital heart anomaly and affects 1% to 2% of the population.[1,2] Data from large surgical centers, including our own, report a BAV occurrence rate of approximately 40% for patients undergoing aortic replacement as the result of TAA. BAV is heritable,[1,3] but the cause is unknown. The mechanism mediating the aortopathy associated with BAV also is not defined, but the pathology is localized distinctly to the ascending thoracic aorta.

A final common pathway of matrix degeneration is shared between aneurysms of the ascending thoracic and abdominal aorta. Increased expression of the degrading matrix metalloproteinases (MMPs) and reduced expression of their counterparts, the tissue inhibitors of matrix metalloproteinases (TIMPs), result in fragmentation of collagen and elastin fibers. Histopathologic observations of cystic medial degeneration (CMD), smooth muscle cell (SMC) loss, lack of inflammatory cells, and proteoglycan accumulation are unique to TAAs and suggest that the inciting mechanism of MMP/TIMP imbalance and matrix degeneration differs for ascending thoracic and abdominal aortic aneurysms.

Patients with BAV are at an increased risk of developing TAA at least 10 to 15 years earlier than patients with TAV. Although the mechanism of action mediating matrix degeneration in BAV-associated aortopathy is unknown, we postulate that SMCs of the BAV aorta possess an inherent defect that causes a material property change in the vessel wall leading to TAA formation. We previously demonstrated that the oxidative stress response is compromised in SMCs in patients with BAV.[4] We recently reported altered biomechanical strength of the vessel wall between BAV and TAV

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Abbreviations and Acronyms

BAV = bicuspid aortic valve
CMD = cystic medial degeneration
HYP = hydroxyproline
MMP = matrix metalloproteinase
MPM = multiphoton microscopy
NA = nonaneurysmal
NOI = normalized orientation index
OI = orientation indice
SMC = smooth muscle cell
TAA = thoracic aortic aneurysm
TAV = tricuspid aortic valve
TGF = transforming growth factor
TIMP = tissue inhibitors of matrix metalloproteinase

(J Pichamuthu et al, unpublished data).[5] These data led us to hypothesize that TAAs arise in patients with BAV and TAV by unique mechanisms of matrix remodeling. To test this hypothesis, collagen and elastin matrix architecture and maturity were qualitatively and quantitatively studied in human thoracic aorta from patients with and without aortic aneurysm. We found that patients with BAV and patients with TAV exhibit distinctly different aortic matrix architecture, suggesting distinct mechanisms of aortic wall remodeling in the 2 populations.

MATERIALS AND METHODS

Patient Selection and Specimen Acquisition

Ascending thoracic aortic specimens were collected during elective surgery for ascending aortic replacement or aortic valve replacement from patients with BAV or tricuspid aortic valve (TAV) with institutional review board approval and informed patient consent. Specimens were also collected from heart transplant donors and recipients with TAV and absence of aortic disease with approval from the Center for Organ Recovery and Education or institutional review board approval and informed patient consent, respectively. Patient demographics of sex, age, maximal orthogonal aortic diameter, degree of aortic insufficiency and stenosis, and history of hypertension and cigarette smoking were documented (Table 1). Patients with TAA enrolled in our tissue bank with BAV (median age, 53 years; n = 142) presenting for elective surgery at the University of Pittsburgh Medical Center for ascending aortic replacement do so at least 10 years earlier than patients with TAV (median age 63 years; n = 99). Specimens chosen for each experiment were diameter-matched (median diameters within 2 mm) for patients with TAV-TAA and patients with BAV-TAA, and ages span the distribution given above. Specimens of both sexes were included in the study.

On excision, the specimens were placed in ice-cold saline and transported to the laboratory. A portion of tissue was processed for isolation of primary aortic SMC cultures as previously described.[4,6]

Multiphoton Microscopy of Aortic Matrix Architecture

A 1 cm^2 portion of the specimen was dissected, noting proximal/distal orientation. The adventitia was left intact to avoid potential deformation in the native matrix architecture. The specimen was kept in phosphate-buffered saline on ice and imaged immediately (within 2 hours of harvest) using multiphoton microscopy (MPM) or fixed in 2% paraformaldehyde for 1 hour, followed by storage in phosphate-buffered saline at 4°C until MPM was performed. Specimens were imaged using an Olympus FV1000 MPE (Tokyo, Japan) equipped with a Spectra-Physics DeepSee Mai Tai Ti-Sapphire laser (Newport, Mountain View, Calif) using an excitation wavelength of 830 nm and 1.12 numerical aperture 25× MPE water immersion objective. Epi-detectors were used to collect the second harmonic generation at 400 ± 50 nm for collagen fibers and autofluorescence of elastin at 525 ± 25 nm. Images were captured using a dwell time of 4 μs/pixel and a Kalman filter of 2. Images of the media were captured as 2-μm slices starting just beyond the intimal surface sequentially moving toward the adventitia. An attempt to capture images spanning the full thickness of the medial layer was made with each specimen; however, the number of images collected varied with each sample because of thickness and geometry of the specimen and ranged from 14 to 108 μm.

Determination of Collagen and Elastin Fiber Alignment

After MPM analysis, projected image stacks were assembled for each channel (collagen and elastin separately) using ImageJ (National Institutes of Health, Bethesda, Md). To quantify collagen and elastin fiber orientation on superimposed 2-dimensional image stacks, the gradient of image intensity at each pixel taken above a threshold was determined for an edge detection algorithm using a custom MATLAB program (The MathWorks Inc, Natick, Mass) previously described.[7] Briefly, these values were entered into an accumulator bin defined over a subregion, and for each angle the summed gradient-weighted contribution of each pixel was calculated. Histograms of dominant orientation, defined as the angle associated with the maximum accumulator bin value were plotted, and orientation indices (OIs) were determined on the basis of variance of fiber angle ($\Delta\theta$) of 50% of the total number of fibers as one half the area under the curve of fiber angle distribution histogram. For images with a high degree of fiber alignment, it was necessary to rotate the image clockwise 90 degrees to generate half-area calculations from fiber-count angle histograms. The normalized orientation index (NOI) was then calculated from Equation 1 as described previously[8] where OI = $\Delta\theta$.

$$\text{NOI} = 90\text{-OI}/90\times 100 \text{ so that NOI } \in [0,\ 100] \qquad \text{(Equation 1)}$$

RNA Isolation and Quantitative Real-Time Polymerase Chain Reaction

A portion of each specimen was placed in RNA*later* (Life Technologies, Carlsbad, Calif) and stored at −20°C until use. Total RNA was isolated using the RNeasy Plus kit (Qiagen, Valencia, Calif). Gene expression of *Lysyl oxidase* (*Lox*) was quantified by real-time polymerase chain reaction as previously described[6] using the One-Step Taqman PCR kit and custom-designed Taqman gene expression assay for human *Lox* (Assay ID# Hs00184700_m1) (Life Technologies).

Histology

Specimens (~0.5 × 1 cm^2) were fixed in 10% buffered formalin and paraffin embedded. Four-micron sections were stained using Masson's trichrome and Verhoeff–Van Gieson (Research Histology Services, University of Pittsburgh Thomas E. Starzl Transplantation Institute, Pittsburgh, Pa) to ascertain collagen and elastin composition respectively. Slides were stained using picrosirius red stain kit (Polysciences Inc, Warrington, Pa) according to the manufacturer's instructions to assess collagen fiber thickness. Slides were visualized using a Nikon TE-2000-E inverted microscope under polarized light and captured using