# Editorial

# Insights Into the Role of Regional Proteoglycan Metabolism in Thoracic Aortic Aneurysms

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Thoracic aortic aneurysms (TAAs) are segmental, full-thickness dilations of 50% greater than the width of a healthy aorta. The natural history of a TAA is to asymptomatically enlarge over time until the wall weakens, producing an intimal tear. Consequently, along with a bicuspid aortic valve and hypertension, a transmural TAA is a risk factor for acute aortic dissection or rupture. TAA dissections occur in ≈ 3 to 4 cases per 100 000 person-years.[1] Dissections in TAAs occur in 2 major sites; those dissections occurring between the sinuses and ascending aorta carry substantial morbidity via pericardial tamponade.[2]

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We know that risk for TAA is conferred by a strong heritable component. The genes to date identified map to a common pathway, disrupting mechanosensing between the vascular smooth muscle cell contractile-elastin units.[2,3] Although TAAs involve all layers of the aorta, pathological remodeling of the tunica media, the major structural component of the aorta, is requisite. The media contains fenestrated sheets of elastin-rich lamella organized in a 3-dimensional continuous network between collagen fibers and thin layers of PG (proteoglycan)-rich extracellular matrix (ECM) with embedded smooth muscle cells. These 50 lamellar units found in the normal proximal aorta provide compliance to the pulsatile flow from cardiac systole. In addition to this structural role, matricellular ECM proteins sequester growth factors and provide integrin-receptor signals to maintain vascular smooth muscle cell differentiation, contractility, and viability.

Progressive disease is highly influenced by superimposed mechanical, structural, or inflammatory factors that overwhelm the adaptive changes to the underlying genetically imposed biomechanical stress. Progressive dilation involves elastin fragmentation, releasing ECM fragments, possessing endogenous chemotactic activity, result in infiltration of and amplification of effector leukocyte function.[4] Activated leukocytes release proteases that cooperate with smooth muscle–derived matrix metalloproteinases, resulting in further structural damage, resulting in expansion and rupture. In addition, stretch itself produces endoplasmic reticulum stress in resident vascular smooth muscles, resulting in apoptosis and inflammation.[5] Consequently, progression of a TAA is a multistep process of altered biomechanical sensing, ECM turnover, coupled with inflammation producing focal vascular smooth muscle cell loss, leading to accelerated dilation.

Physicochemical characteristics of the aortic ECM have made dynamic studies of its synthesis and turnover difficult. The ECM is enriched in protease-resistant amino acids, hydroxyproline and proline, with extensive post-translational modifications (glycations, sulfation, hydroxylation, and others) residing in an extensively crosslinked matrix. Recent adaptations of proteomic pipelines have provided tools to address this roadblock. Using a specifically adapted proteomics pipeline of sequential solubilization to remove cellular components, followed by chaotropic solubilization of the ECM, deglycosylation, and quantitative spectral counting, Didangelos et al[6] was able to demonstrate that versican is one of the most abundant PGs in the aorta. Separately, a distinct PG extraction pipeline was developed using native size exclusion of chaotropic extracts followed by ion exchange chromatography PG enrichment, followed by liquid chromatography-mass spectrometry. This approach was applied to a mouse model of ascending thoracic disease ($FBN1^{mgR/mgR}$) and resulted in the observation that versican and aggrecan were 2 of the most abundant PGs found in the aorta.[7] Although this study observed a concomitant decrease in the expression of PGase, ADAMTS (a disintegrin and metalloprotease with thrombospondin motifs)-5, the study was not designed to establish a mechanistic relationship. These data point to a potential important role for abnormal PG synthesis or turnover in TAA and dissection.

In this issue of *ATVB*, Fava et al[8] extend their previous work implicating versican accumulation in models of mechanical and lipid-induced vascular injury. Although ADAMTS1-4 and 5 have been linked to aortic aneurysms, only ADAMTS5 was preferentially expressed in the ascending aorta in the mouse model. Ang II (angiotensin II) infusion produces ascending aortic dissection in mice by activating effector macrophage activity.[9] Using this perturbation, the authors observe that Ang II challenges in mice expressing a catalytically inactive ADAMTS5 ($ADAMTS5^{\Delta CAT}$) have enhanced dilation in the ascending aorta and aortic annulus, despite attenuations in the Ang II–induced pressor response. Reasoning that the catalytic activity of ADAMTS5 is responsible for pathogenesis, the authors then applied proteomics to discover the ADAMTS5 degradosome by SDS-PAGE fractionation of wild-type aortas after their incubation with the active enzyme. In comparison to the proteolytic fragments produced by ADAMTS1, ADAMTS5 produced fragment of versican and MFAP5 (microfibrillar-associated protein 5).

Consistently, versican was elevated in the $ADAMTS5^{\Delta CAT}$ along with a compensatory upregulation of ADAMTS1. These findings were independently validated in discovery proteomics studies using sequential ECM solubilization of aortae from Ang II–infused $ADAMTS5^{\Delta CAT}$ mice, where MFAP5 and

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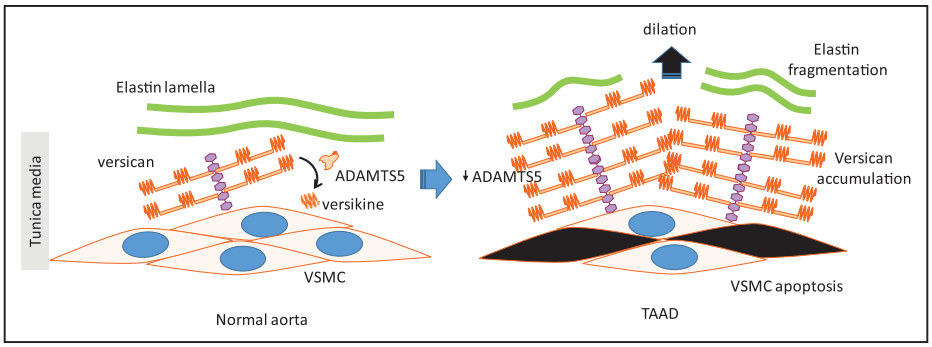


**Figure.** Role of ADAMTS (a disintegrin and metalloprotease with thrombospondin motifs)-5 in accumulation of aggregating proteoglycans in thoracic aneurysms. Schematic view of an elastic lamellar layer in normal aorta. Versican (VCAN) is a chondroitin-rich PG (proteoglycan) component of the normal vascular extracellular matrix that is recycled by ADAMTS5. In pathological conditions, reduction of ADAMTS5 results in accumulation of VCAN, where it may be involved in disrupting elastin fragments, interfering with integrin–VSMC trophic signals and producing focal VSMC apoptosis.

versican were the highest confidence proteins upregulated. Previous work implicated that LRP1 (LDL receptor lipoprotein) mediates ADAMTS5 clearance by an endocytotic pathway. In this study, the authors depleted *LRP1* and observe that *ADAMTS5* is also potently downregulated, along with compensatory upregulation of *ADAMTS1* at the mRNA level. Clinical correlation was established in human TAAD tissues identifying the ADAMTS5-mediated NH2-terminal fragment of versican, versikine, in immunohistochemistry.

This study is an excellent illustration how focused proteomic pipelines coupled with informative genetic mouse models can advance our mechanistic understanding of the critical role of ADAMTS5 metabolism of PG in TAA. These studies show that versican is a major substrate of ADAMTS5; versican is chondroitin sulfate-rich PG that plays important roles in normal cardiovascular development.[10] Although a normal component of vascular ECM, excess versican accumulation seems to be pathogenic (Figure). Modeling studies have suggested that versican accumulation may alter vessel wall hydrodynamics producing enhanced interstitial pressure, thereby disrupting the elastin lamella[11]; this point will need to be directly tested. Other explanations for the pathogenic effects of verican accumulation include effects on vascular smooth muscle cell survival by disrupting trophic adhesion signals or, by affecting versikine signaling, will need to be investigated.

Integrating earlier work that versican plays an important role in normal cardiovascular development with the finding, from this study, that ADAMTS1 partially compensates for ADAMTS5 deficiency undoubtedly suggests that PG turnover in the ECM is tightly regulated. Because of the regional differences in ADAMTS expression, and possible species differences, dysregulation of other ADAMTS isoforms may be linked with other vascular or cardiac valvular disease. The findings that LRP1 upregulates ADAMTS5 mRNA expression may elucidate how *Lrp1*$^{-/-}$ mice are ADAMTS5 deficient and prone to aneurysmal dilation. This pathway may advance understanding the relationship between disorders of lipoprotein metabolism and ECM remodeling. Finally, these studies might have important translational implications. One example may be measurement of circulating components of the ECM degradome to monitor vascular remodeling in high-risk patients for TAA.

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## Disclosures

None.

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