Possible Mechanical Roles of Glycosaminoglycans in Thoracic Aortic Dissection and Associations with Dysregulated Transforming Growth Factor- β

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Key Words

Transforming growth factor- β · Thoracic aneurysm · Glycosaminoglycan · Proteoglycan · Stress concentration · Wall strength

Abstract

Background: Four distinguishing histopathological characteristics of thoracic aortic aneurysms and dissections (TAADs) are the fragmentation or degradation of elastic fibers, loss of smooth muscle, pooling of glycosaminoglycans, and remodeling of fibrillar collagens. Of these, pooling of glycosaminoglycans appears to be unique to these lesions.

Methods: This review acknowledges the importance of dysregulated transforming growth factor- β (TGF- β) in TAADs and offers a complementary hypothesis that increased TGF- β could contribute to the accumulation of glycosaminoglycans in the media of the proximal thoracic aorta. Regardless, observed pools of glycosaminoglycans could decrease tensile strength, cause stress concentrations, and increase intralamellar swelling pressure, all of which could initiate local delaminations that could subsequently propagate as dissections and result in a false lumen or rupture. **Conclusions:** There is a pressing need to investigate potential mechanical as well as biological consequences of accumulated glycosaminoglycans in TAADs and to elucidate responsible signaling pathways, with particular attention to synthetic cells of nonmesodermal lineage. Such research could provide in-

sight into the mechanisms of dissection and the seemingly paradoxical role of the over-expression of a cytokine that is typically associated with fibrosis but is implicated in a degenerative disease of the aorta that can result in a catastrophic mechanical failure.

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Introduction

Aneurysms arise preferentially in three regions of the human vasculature: the thoracic aorta, intracranial circulation, and infrarenal abdominal aorta. Despite differences in etiology, these three classes of aneurysms share three distinguishing structural characteristics: proteolytic degradation of or mechanical damage to elastic fibers, loss of functional smooth muscle cells, and remodeling of fibrillar collagens [1–3]. Of particular note, however, neither intracranial nor abdominal aortic aneurysms typically exhibit localized accumulations of glycosaminoglycans/proteoglycans (GAGs/PGs) and these two classes of aneurysms typically do not dissect. In stark contrast, it has long been known that a unique histopathological characteristic of thoracic aortic aneurysms and dissections (TAADs) is the accumulation of significant pools of GAGs/PGs within the media in regions of compromised elastic fibers and smooth muscle cells [4, 5]. Indeed, this unique feature continues to be

Fig. 1. Sections of the medial layer of the human ascending aorta stained with Alcian blue (which stains GAGs blue) for both a normal (**a**) and an aneurysmal (**b**) wall. In particular, note the marked pooling of GAGs in the latter, indicated by the star, and the lack of smooth muscle or elastic fibers within these pools of GAGs. In contrast, the arrow points to a normal structure in **a**, consisting primarily of smooth muscle, collagen, and modest distributed GAGs. Reprinted from Borges et al. [6], with permission from Elsevier (license No. 2911570557067).

recognized as singularly characteristic of TAADs – see, for example, figure 1, which is reprinted from Borges et al. [6]. See, too, figure 2 in Nataatmadja et al. [7], figure 3 in Collins et al. [8], figure 2 in Milewicz et al. [9], figures 1–3 in Maleszewski et al. [10], figure 1 in Jain et al. [11], figure 2 in Grond-Ginsbach et al. [12], figure 1 in Bode-Janish et al. [13], and figure 2 in Guo et al. [14]. It should be noted that these different histological images come from patients representing a wide range of ages, from children to young and old adults, and presenting with diverse disease origins, including bicuspid aortic valve, Marfan syndrome, familial TAAD, Loeys-Dietz syndrome, and idiopathic TAADs. Nevertheless, there has not been any prior study of possible biomechanical consequences of pooled GAGs/PGs in TAADs even though it is ultimately the mechanics that dictates the fate, that is, possible dissection or rupture, of the compromised aortic wall. Similarly, there has not been any discussion of possible roles of dysregulated transforming growth factor- β (TGF- β), a recognized key player in many cases of TAADs [12, 15, 16], in contributing to the local accumulation of GAGs/PGs [7]. The purposes of this review, therefore, are to synthesize a diverse literature that is seldom considered together and to offer a new hypothesis for testing, namely, that alterations in the intramural mechanical stress field arising from localized accumulations of GAGs/PGs could help initiate aortic dissections and that dysregulated TGF- β signaling could contribute to this perturbation in wall homeostasis.

Aortic Wall Structure

The normal thoracic aorta consists of three well-defined layers: intima, media, and adventitia. Briefly, the intima consists of a monolayer of endothelial cells that adhere to a basement membrane, which in turn consists primarily of type IV collagen and laminin. Albeit extremely important in arterial homeostasis and hemostasis, the intima only occupies about 5% of the normal thoracic aorta [17] and it is generally considered to confer little overall load bearing capability. The media consists of ~ 70 layers of alternating elastic laminae and embedded smooth muscle cells, adhesion molecules, collagen fibers (primarily types I, III, and V), and GAGs/PGs [18]; these separate layers are the building blocks of the media and have been referred to as medial lamellar units (fig. 2). Serving as the parenchymal layer of the aorta, the media occupies nearly 80% of the wall and contributes substantially to overall load bearing, particularly under physiologic hemodynamic conditions. The adventitia consists primarily of type I collagen, but also admixed elastic fibers and fibroblasts [19]; it occupies about 15% of the wall [17]. The adventitia is thought by some to serve, in part, as a strong sheath that mechanically protects the smooth muscle cells of the media from overstretching during acute conditions of extreme loading [20]. Historically, and for good reason, elastic fibers, contractile smooth muscle, and fibrillar collagens have long been considered to be the most important contributors to the structural

integrity of the normal aortic wall, particularly given that the two primary mechanical stresses in the wall – circumferential and axial – remain tensile throughout the cardiac cycle [21]. Quantifying the associated mechanics is widely recognized as important both for understanding the artery as a structure [21] and because all three cell types of the normal aortic wall (endothelial, smooth muscle, and fibroblasts) are mechanobiologically responsive to even small changes in mechanical stimuli [22].

Elastic fibers consist primarily of elastin, but also microfibrils that include the fibulins, fibrillins, Emilin-1, and microfibril-associated glycoproteins [23]. It is thought, for example, that the fibulins and fibrillins contribute to elastogenesis and the long-term stability of elastic fibers. Indeed, in contrast to collagen fibers, which turn over continuously within the arterial wall [24], elastic fibers appear to be deposited primarily during the perinatal period and, because of their normally long half-life, they must withstand the millions of cycles of loading throughout one's lifetime [25]. Proteolytic degradation of, or mechanical damage to, elastic fibers thus results in irreversible effects on wall structure, biology, and mechanical properties and, as noted above, clearly plays a role in all three major classes of aneurysms (thoracic aortic, intracranial, and abdominal aortic).

Aortic dissections tend to propagate much easier within elastic lamellae than across the lamellae [26, 27], which is consistent with clinical observations of extensive dissections prior to rupture [e.g. 28]. An unresolved question, however, is: what initiates the initial defect, or delamination, that renders the wall vulnerable to subsequent dissection and possible rupture? That is, whereas it is often tacitly assumed that dissections, and the associated false lumens, arise following an intimal tear that propagates into the media under the action of blood pressure [29, 30], it has been noted correctly that it is actually 'uncertain whether the initiating event is a primary rupture of the intima with secondary dissection of the media' [31]. As discussed below, it is suggested herein that pools of GAGs/PGs may represent initiation sites within the media that contribute to the development and propagation of a dissection that could subsequently lead to an intimal tear or rupture.

Fundamental Role of Genetics

Dissections of the thoracic aorta occur most often in the ascending portion (Stanford type A), but in the descending portion as well (Stanford type B). Although

Fig. 2. Schematic drawing of a normal aortic medial lamellar unit [18] consisting of paired elastic laminae (at the top and bottom) with an embedded smooth muscle cell (SMC) as well as collagen fibers, adhesion molecules (e.g. fibronectin), and GAGs (not to scale). Shown, too, are thin 'radially oriented' elastic fibers (i.e. elastin and associated microfibrils, predominately fibrillin-1) that may provide direct mechanical connections between the elastic laminae and smooth muscle cell and thus a mechanosensory capability beyond the typical cytoskeletal (CSK)-integrin-extracellular matrix (fibronectin/collagen) axis. It is possible that negatively charged GAGs sequester water and may thereby contribute a normal intralamellar pressure that could help maintain the thin elastic fibers in tension. The primary directions of tension are, however, the circumferential and axial directions, with normal lamellar tensions on the order of 2 N/m. Artwork by Carolyn Valentin.

these dissections can result from blunt trauma or extreme physical exertion [12], they frequently associate with heritable disorders and aneurysmal dilatations [3, 9, 12, 16]. In particular, syndromic TAADs include those resulting from mutations in genes coding fibrillin-1 (Marfan syndrome), TGF- β receptor II (Loeys-Dietz syndrome), and collagen III (Ehlers-Danlos IV syndrome) – see, for example, Jain et al. [11]. Similarly, mutations in genes coding for α -smooth muscle actin and smooth muscle-specific β -myosin are responsible for a significant percentage of familial TAADs [9].

With regard to Marfan syndrome, loss of the structural role of fibrillin-1 in stabilizing elastic fibers, which would be expected to hasten mechanical fatigue and thus the fragmentation or degradation of these fibers, must contribute to the compromised structural integrity of the aortic wall [32, 33] and subsequent mechanobiological responses [9, 34]. Nevertheless, another important finding was that normal fibrillin-1 also contributes to the seques-

tration of latent TGF- β within the extracellular matrix, which attenuates the activity of this important cytokine [35, 36]. Hence, loss of fibrillin-1 can increase TGF- β activity and thereby play another important role in the natural history of TAADs in Marfan syndrome [15, 16, 37]. Similarly, albeit seemingly paradoxical, mutations in the gene that codes for TGF- β receptor II also lead to increased TGF- β activity and play an important role in Loeys-Dietz syndrome [15, 16]. Among other effects, altered TGF- β receptor II decreases contractile protein expression in thoracic aortic smooth muscle cells, which has been suggested to play a role in decreased fibronectin/fibrillin-1 assembly within the extracellular matrix and thus decreased sequestration of latent TGF- β [38]. Indeed, altered α -smooth muscle actin and β -myosin, which similarly compromise smooth muscle contractility, have also been shown to increase TGF- β activity [39]. It is thus clear that, despite very different reasons, an increased bioavailability of TGF- β is involved in diverse types of TAADs, which in many cases likely exacerbates the diminished structural integrity of the wall that results directly from mutations in the genes that code important structural proteins (e.g. collagen III, actin, or myosin) and glycoproteins (e.g. fibrillin-1, which co-localizes with elastin to form elastic fibers).

Transforming Growth Factor- β

TGF- β is a pleiotropic cytokine that belongs to a superfamily of extracellular polypeptides that regulate diverse cellular activities, including adhesion, migration, division, differentiation, and the production of diverse extracellular matrix constituents [40, 41]. Notwithstanding its important roles in normal arterial development and homeostasis, TGF- β is perhaps best known as a pro-fibrotic cytokine. In particular, TGF- β can stimulate vascular smooth muscle cells and fibroblasts to increase their synthesis of collagen (a hallmark of TAADs [42]), downregulate the activity of matrix metalloproteinases (MMPs, which play important roles in TAADs [43]), upregulate the activity of tissue inhibitors of MMPs (TIMPs), and upregulate the activity of plasminogen activator inhibitor-1 (PAI-1), all of which should contribute to a pro-fibrotic phenotype [40, 41]. Indeed, over-expression of TGF- β can stabilize pre-existing abdominal aortic aneurysms [44] and similarly prevent aortic dilatation and atherosclerotic plaque rupture in apolipoprotein-null mice [45], each consistent with the notion that increased collagen synthesis, decreased MMP activity, and increased TIMP activity should strengthen the wall of a

lesion. Also consistent with these findings, it was recently shown that increased TGF- β activity can stimulate adventitial fibroblasts to downregulate miR-29b (a micro-RNA that regulates genes that mediate the turnover of extracellular matrix), which in turn led to an increased deposition of collagen; when miR-29b was inhibited further, there was a marked attenuation of abdominal aortic aneurysm development [46, 47]. For obvious reasons, then, the precise roles of increased TGF- β activity in syndromic and familial TAADs remain perplexing. In other words: how does a cytokine best known for its pro-fibrotic activity contribute significantly to degenerative thoracic aortic diseases characterized by aneurysmal dilatation or dissection?

Possible reasons for the unexpected deleterious consequence of increased TGF- β activity in TAADs include the existence of both canonical (via Smad2/3 [48]) and non-canonical (via MAPK [49]) TGF- β signaling pathways. Noncanonical signaling has been suggested, for example, to increase MMP activity [50], which could compromise the aortic wall. Another suggested reason is that TGF- β activity could exert differential effects on different cell types and arteries. For example, the aforementioned miR-29b study shows differential activity by smooth muscle cells and fibroblasts in mouse models of abdominal aortic aneurysms [46]. Moreover, smooth muscle cells and fibroblasts in the proximal thoracic and infrarenal abdominal aorta have different embryonic lineages [16, 51, 52], and it has long been known that TGF- β differentially stimulates cells from the neural crest (proximal thoracic) and mesoderm (abdominal) – see, for example, Thieszen et al. [53] and Gadson et al. [54]. There is clearly a need to determine whether this regional heterogeneity could explain, at least in part, a possibly protective role of TGF- β in abdominal aortic aneurysms [44] and a detrimental role in TAADs, at least during particular periods of lesion development [e.g. 55]. In summary, however, as recognized by many and noted in a recent *Nature* review, we must conclude at present that ‘little is known about the precise pathogenetic sequence downstream of TGF- β that is involved in aneurysm progression’ [16].

Glycosaminoglycans/Proteoglycans

According to Alberts et al. [56], GAGs consist of linear chains of repeating disaccharide units; they are highly negatively charged and thus sequester water and contribute directly to the compressive, rather than tensile, stiffness of a soft tissue. The four primary classes of GAGs are

hyaluronan, chondroitin sulfate/dermatan sulfate, heparan sulfate, and keratan sulfate. Of these, chondroitin sulfate, dermatan sulfate, and hyaluronan are commonly found in the aortic wall [57]. Hyaluronan is unique in that it does not associate directly with a protein core. It expands significantly when hydrated and occupies large volumes of the extracellular space, thus allowing it both to resist compressive loads and to facilitate cell migration; it is particularly abundant in morphogenesis and early wound healing. When GAGs associate with a protein core, the composite molecules are called PGs. In addition to serving as space-filling, negatively charged gels within the extracellular matrix, PGs also control the activity of many cytokines, chemokines, and proteases. The primary PGs in arteries are versican, which is rich in chondroitin sulfate, and the small leucine-rich PGs biglycan and decorin, which are rich in dermatan sulfate or chondroitin sulfate [18, 57]. Under normal conditions, at least in heart valves, biglycan and decorin tend to exist in regions of cyclic tension whereas hyaluronan and versican tend to aggregate in regions of cyclic compression [58]. The former is consistent with biglycan and decorin contributing to collagen fibrillogenesis [59] and the latter is consistent with the ability of versican to bind hyaluronan and form large multi-molecular hydrophilic complexes within the aortic wall that support compressive loads [57]. It is interesting that decorin, like fibrillin-1, can also sequester TGF- β in the extracellular matrix.

GAGs/PGs are fundamental contributors to the development and maintenance of the structure and function of the aortic wall [18, 57, 60, 61]; hence it is unfortunate that their contributions to wall mechanics have not received the extensive consideration given to other structural constituents, namely, the elastic fibers, smooth muscle, and fibrillar collagens. Reasons for this prior lack of attention likely include the normally low mass fraction of GAGs/PGs (they occupy only ~ 1 –5% of the normal arterial wall) and that they are thought to be most important mechanically in resisting compressive stresses. As noted earlier, the two primary stresses in the arterial wall are the circumferential and axial components, both of which are tensile and on the order of 100 kPa in magnitude *in vivo*; in contrast, the radial stress is compressive and on the order of only ~ 10 kPa [21]. Nevertheless, recent studies using mutant mice reveal that, albeit indirectly, PGs can contribute significantly to the tensile strength of arteries. For example, recalling that biglycan aids in collagen fibrillogenesis, biglycan deficiency has been shown to lead to aortic rupture [62]. This study reminds us that whereas locally compromised elastic fibers or diminished

smooth muscle contractility can allow an initial dilatation of the arterial wall [33, 63], collagen fibers must ultimately be compromised for the wall to rupture [63, 64].

Many studies report increased accumulations of GAGs/PGs in diverse vascular diseases and injuries, including atherosclerosis, hypertension, and diabetes [65–68]. Of particular note herein, accumulation of GAGs in mucopolysaccharidosis has been associated with aortic aneurysms [69, 70]. There have also been reports of significant increases in hyaluronan and dermatan sulfate GAGs in dissecting aortas [5] and significant increases in versican in ascending aortic aneurysms, with no significant increases in biglycan [71]. Further reports that MMP-3 and particularly MMP-7 co-localize with accumulated GAGs/PGs in TAADs suggest that some of the mucoid material could be fragmented [6]. MMP-3 and MMP-7 can also degrade elastin and collagen III [72, 73], which could contribute further to medial degeneration. Of more importance herein, however, many reports also reveal that increased TGF- β activity leads to an increased production of GAGs/PGs within the arterial wall [57, 66–68, 74, 75], similar to that in other tissues [e.g. 76–78]. In particular, Nataatmadja et al. [7] reported a significant increase in hyaluronan in aortic aneurysms in Marfan syndrome and specifically implicated the decreased fibrillin-1 and increased TGF- β as causative. This finding is supported by an earlier observation that cultured fibroblasts from Marfan patients exhibit significant increases in both hyaluronan (11-fold) and chondroitin sulfate (3-fold) production [79]. It should also be noted that angiotensin II has long been known to promote TGF- β activity [80], and angiotensin II and TGF- β have overlapping signaling pathways (e.g. Smad and MAPK [41]). Indeed, there are reports that angiotensin II promotes the accumulation of PGs in atherosclerotic lesions [81] and that an angiotensin II type 1 receptor antagonist inhibits the accumulation of PGs in atherosclerosis [82]. These observations are particularly relevant here given that it was the effectiveness of the angiotensin II type 1 receptor antagonist losartan in preventing thoracic aneurysms in mouse models of Marfan syndrome that helped identify important roles of dysregulated TGF- β activity in TAADs [83].

Finally, it is interesting that there are reports of decreased GAGs/PGs in abdominal aortic aneurysms [84–86], which tend not to dissect. Again, this is in stark contrast to the aforementioned significant increase, indeed pooling, of GAGs/PGs in TAADs [6–14], which often dissect. This comparison again points to a possible difference in response between synthetic cells of nonmesodermal and mesodermal origin [16, 51, 52]. Also possibly related to this

Fig. 3. Similar to figure 2, but this schematic drawing depicts the central hypothesis herein. A localized accumulation of GAGs, on the right side of the medial lamellar unit, results in an increased Donnan swelling pressure, which in turn helps to separate the elastic laminae and possibly disrupt connections between the smooth muscle cells (SMCs) and either thin elastic fibers or the collagenous matrix. Such effects could initiate a local delamination and/or altered mechanosensitive cellular response leading to dysregulated wall homeostasis. Artwork by Carolyn Valentin.

differential response, it was reported many years ago that, in chickens, the normal fixed charge density (resulting from the negatively charged GAGs/PGs) is 25% greater in the thoracic than the abdominal aorta [87], thus suggesting that GAG/PG production may vary regionally even under normal conditions. It should be noted further that, regardless of arterial segment, most of GAGs/PGs appear to reside primarily in the media in a healthy artery [88], which is also where most dissections tend to originate.

Possible Mechanical Effects of Pooled GAGs/PGs

Whereas little is known about mechanical roles of GAGs/PGs in arteries [88], much has been learned regarding their importance in GAG-rich tissues such as cartilage. For example, it was recently suggested that central fissures could develop within tissue engineered cartilage due to the high Donnan swelling pressures that resulted from the negatively charged GAGs [89]. Briefly, based on physicochemical principles [90], the so-called Donnan effect explains thermodynamically how equilibrium can be maintained while gradients or jumps exist in mobile ions

(e.g. Na^+ in an aqueous solution) due to a region of the system containing fixed molecules that are charged (e.g. negative charges due to SO_3^- or COO^- groups within GAGs). These ionic gradients or jumps, in turn, alter the regional distribution of interstitial water and, as in the case of GAGs, cause a swelling pressure within the region containing the fixed charges. Indeed, it was shown many years ago that the high concentrations of GAGs in cartilage can cause significant interstitial Donnan swelling pressures, with values ranging from 16 kPa for a fixed charge density of 0.05 mEq per gram of wet tissue to 170 kPa for a fixed charge density of 0.18 mEq per gram [91]. This range of swelling pressures was confirmed more recently for solutions of chondroitin sulfate having fixed charge densities of up to 0.35 mEq/ml [92], noting that tissue mass density is typically ~ 1.05 g/ml. Recall that chondroitin sulfate is similarly important in aortic mechanics (with a fixed charge density in the normal thoracic aorta on the order of 0.05 mEq/ml [87, 88]), particularly given the reported 2.6-fold increase in the chondroitin sulfate-rich PG versican in human thoracic aortic aneurysms [71].

Given that the blood pressure-induced compressive radial stress within the normal aortic wall is on the order of -10 to -16 kPa (where 10 kPa = 75 mm Hg), we submit that pooled GAGs/PGs could produce intralamellar Donnan swelling pressures having magnitudes comparable to or well greater than normal compressive stresses. Such swelling [93] could contribute to separating the elastic lamellae (fig. 3), as reported for both experimental models of thoracic aneurysms that dissect [94] and human data; for example, greater separation distances have been reported between elastic lamellae that contain accumulated GAGs/PGs [14], and dissections have been associated with marked pooling of GAGs/PGs [95]. Clearly, therefore, there is a need to examine rigorously if GAG-associated Donnan swelling pressures could directly cause delaminations within the aortic media that, in turn, could initiate dissections. Moreover, perhaps both prior to and after local dissection, swelling-induced disruptions of smooth muscle interactions with ‘radially’ oriented elastic fibers (fig. 3) could negatively impact cell mechanotransduction [96], perhaps leading to altered cell signaling pathways with deleterious effects on matrix turnover and thus wall homeostasis [97]. Similar concerns have been raised regarding perturbed mechanotransduction in cases of dysregulated smooth muscle contractility [9]. In addition, generation of elastin fragments or ‘elastin degradation products’ due to swelling-induced damage to inter-lamellar elastic fibers could also alter wall homeostasis [98] and thereby exacerbate the loss of structural integrity of the wall.

With the exception of PGs that promote collagen fibrillogenesis, such as biglycan, GAGs contribute primarily to the compressive, not tensile, stiffness of a soft tissue. It is intuitive, therefore, that replacing tensile-bearing elastic fibers, contractile smooth muscle, and collagen fibers with pools of GAGs/PGs (fig. 1), particularly hyaluronan and versican, within localized regions of the media would also necessarily reduce the tensile carrying capability of that portion of the wall. That is, one should expect a local weakening of the wall in response to the dominant cyclic circumferential and axial stresses within regions containing pools of GAGs/PGs. Moreover, it is well known in engineering that material or geometric discontinuities can give rise to 'stress concentrations', which for holes or cavities can typically raise the local stress by 2- to 3-fold. Possibly exacerbating this increase in stress is the often increased aortic pulse pressure [99] that results from stiffening of the aorta and the earlier return of reflected pressure waves in cases wherein elastic fibers are fragmented and collagen fibers remodeled, as, for example, in Marfan syndrome [33, 100]. Recalling that MMP activity may also be heightened within the pools of GAGs/PGs [6], or the compromised aortic wall in general [50], the combination of local decreases in wall strength and increases in wall stress could further increase the likelihood of dissection or rupture [101].

In summary then, pooling of GAGs/PGs within the aortic wall could contribute to the delamination of elastic lamellae via one of three means: Donnan swelling pressures, loss of tensile stiffness, and development of stress concentrations. Clearly, however, such concerns need to be investigated via rigorous calculations of the wall mechanics, which should account for the complex hemodynamic loads that act on the aortic wall and the nonlinear, regionally anisotropic, materially nonuniform properties of the residually stressed wall under cyclic finite deformations [21, 102]. In contrast, recent finite element models of TAADs have inappropriately been based on isotropic linearized elasticity under the assumption of uniform homogenized wall properties [103–105], which cannot account for the characteristic histopathological features that are unique to TAADs.

Finally, given the apparently different degrees of structural defects among the different types of TAADs – for example, Maleszewski et al. [10] reported that Marfan syndrome is characterized by larger cystic spaces ($220\text{--}500 \times 200\text{--}1,000 \mu\text{m}$) and inter-lamellar fragmentation whereas Loeys-Dietz syndrome is characterized by smaller cystic spaces ($20\text{--}50 \times 100\text{--}200 \mu\text{m}$) and intra-lamellar fragmentation – there is a need for disease-specific,

microstructurally motivated, biomechanical analyses. For example, notwithstanding structural implications associated with the accumulation of GAGs/PGs within the media in TAADs, Guo et al. [14] reported a novel genetic locus that appears to result in slowly expanding ascending aortic aneurysms having a low risk of dissection. The associated histopathology revealed a 'focal loss of SMCs and increased PG deposition but only minimal degradation of the elastic fibers' which resulted in a 'widening between elastic fibers'. These findings, like those from the biglycan-null mouse [62], remind us of the multi-factorial biological nature of TAADs and associated structural consequences, and thus the need to consider mechanical consequences of varying the degrees of all four characteristic histopathologic features – fragmentation of elastic fibers, loss of smooth muscle, pooling of GAGs within the media, and remodeling of fibrillar collagens – within the different types of TAADs.

Conclusions

TAADs are responsible for significant morbidity and mortality across a wide range of ages. Due in large part to advances in genetics and medical imaging, the number of diagnosed TAADs has increased 2- to 4-fold over the past three decades [13, 43] and there is a correspondingly greater need for a better understanding of this disease [3, 9, 16, 106]. Many of the best reviews on TAADs have not considered the potential structural implications of GAGs/PGs [3, 16, 107, 108] despite the pooling of GAGs/PGs consistently being recognized as one of the four primary histological features of TAADs [e.g. 9, 12, 13]. Similarly, many of the best reviews on TGF- β in vascular health and disease [37, 40, 41, 50] have not emphasized the well-documented effects of TGF- β in promoting GAG/PGs synthesis [57, 66, 68]. Given that the natural history of TAADs depends strongly on the mechanics and underlying mechanobiology [3, 9, 51, 71], even to the point that past clinical treatment has focused on minimizing wall stress that results from hypertension or extreme physical exertion [108, 109], and that 'the powerful techniques of mechanical analysis have not been widely applied to the study of the thoracic aorta' [110], it is suggested here that there is a pressing need for a rigorous analysis of the possible mechanical consequences of the singularly important histopathological characteristic of pooled GAGs/PGs in TAADs. Moreover, given significant and diverse evidence that increased availability of active TGF- β is both a hallmark of many different types of TAADs and a

promoter of GAG/PGs synthesis, there is also a pressing need for increased attention to this possible downstream consequence of dysregulated TGF- β . Taken together, mechanical and biological research along these lines may help explain why the thoracic aortic wall becomes increasingly susceptible to the mechanical processes of dissection and rupture despite an otherwise expected profibrotic and potentially protective response.

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