Published in final edited form as:

Annu Rev Med. 2017 January 14; 68: 51–67. doi:10.1146/annurev-med-100415-022956.

Therapeutics Targeting Drivers of Thoracic Aortic Aneurysms and Acute Aortic Dissections: Insights from Predisposing Genes and Mouse Models

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Abstract

Thoracic aortic diseases, including aneurysms and dissections of the thoracic aorta, are a major cause of morbidity and mortality. Risk factors for thoracic aortic disease include increased hemodynamic forces on the ascending aorta, typically due to poorly controlled hypertension, and heritable genetic variants. The altered genes predisposing to thoracic aortic disease either disrupt smooth muscle cell (SMC) contraction or adherence to an impaired extracellular matrix, or decrease canonical transforming growth factor beta (TGF-β) signaling. Paradoxically, TGF-β hyperactivity has been postulated to be the primary driver for the disease. More recently, it has been proposed that the response of aortic SMCs to the hemodynamic load on a structurally defective aorta is the primary driver of thoracic aortic disease, and that TGF-β over-activity in diseased aortas is a secondary, unproductive response to restore tissue function. The engineering of mouse models of inherited aortopathies has identified potential therapeutic agents to prevent thoracic aortic disease.

Keywords

thoracic aortic disease; aortopathy; mutation; phenotype; Marfan syndrome; ACTA2; TGF-β; angiotensin receptor; losartan

INTRODUCTION

Aneurysms can occur in any region of the aorta, but they are commonly located in the first section above the heart and termed thoracic aortic aneurysms. These aneurysms can involve the aortic root at the level of the sinuses of Valsalva; spare the root and involve the ascending, tubular section of the aorta; or involve both the root and ascending aorta, in which case they are termed fusiform aneurysms (Figure 1). The natural history of a thoracic

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DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

aortic aneurysm is to asymptomatically enlarge over time until the wall weakens and an acute tear in the intimal layer occurs at the junction between the sinuses and the tubular ascending aorta, leading to an ascending aortic dissection (designated Stanford type A dissection). With dissection, blood penetrates the inner layer of the aorta, then separates the layers within the thick middle layer, either retrograde or antegrade up the ascending aorta. Type A aortic dissections cause sudden death in up to 50% of individuals, and survivors of the acute event continue to have a high mortality rate despite emergency surgery to repair the dissected ascending aorta (1, 2). The majority of the deaths outside the hospital are due to blood entering the aortic wall and the dissection progressing retrograde and rupturing into the pericardial sac, causing pericardial tamponade (3). Survivors of the acute event usually have a dissection that progresses through the ascending aorta and in most cases continues to dissect down the descending aorta. Less deadly aortic dissections termed Stanford type B dissections originate in the descending thoracic aorta, just distal to the origin of the subclavian artery, and progress to involve the descending aorta. These dissections are also part of the spectrum of thoracic aortic disease and typically occur with little to no enlargement of the descending aorta.

Treatment with β-adrenergic antagonists (β-blockers) can slow the rate of enlargement of a thoracic aortic aneurysm, but the mainstay of treatment to prevent premature deaths due to type A dissections is surgical repair of the thoracic aortic aneurysm. Surgery is typically recommended when the aneurysm's diameter reaches approximately twice the normal diameter. This recommendation is based on data from patients with thoracic aneurysms who were medically managed, and the optimal timing of surgery, when the risk of an adverse event (dissection, rupture, death) exceeds the risk of an elective operation, usually occurred at diameters greater than 5.5–6.0 cm in these case series (4, 5). However, studies on patients with acute type A dissections indicate that up to 60% present with aneurysms under 5.5 cm in diameter, including individuals who dissect with little to no aortic enlargement (6). This sharp contrast between patients with apparently stable aneurysms who were followed in clinics and patients who presented with acute ascending dissections suggests that there may be distinct pathologic processes mediating the development and progression of chronic aneurysms versus acute dissections.

The major risk factors for thoracic aortic aneurysms and acute aortic dissections are genetic alterations, hypertension, and the presence of a bicuspid aortic valve (2, 7). Pregnancy and weight lifting both increase biomechanical forces on the ascending aorta and similarly increase the risk for dissection. Consistent with the hypothesis that the architecture of the smooth muscle cell (SMC) contractile-elastin unit protects the aorta from mechanical forces, many of the altered genes that predispose to thoracic aortic disease disrupt components of this unit (7–9). Sex also plays a role, as aneurysms and dissections are more frequent in men (10). Just as diabetes mellitus may protect against the development of abdominal aortic aneurysms (11–13), the presence and severity of diabetic complications have been inversely correlated with thoracic aortic disease in multiple studies (14–16).

The aorta is an elastic artery that is uniquely designed to withstand a lifetime of biomechanical forces due to pulsatile blood flow from the heart. Its three layers are the intima, media, and adventitia. The thick medial layer, composed of >50 alternating layers of

elastic laminae and SMCs in humans, provides the structural support for the aorta to withstand these biomechanical forces (Figure 2) (8, 9). Microfibril extensions from the elastic lamellae are anchored obliquely to the surface of SMCs through focal adhesions (also termed dense plaques) and thus link the SMCs to the elastin fibers, resulting in the propagation of mechanical forces between elastin and SMCs via integrin receptors (17, 18). The oblique orientation of the elastin-contractile units reverses direction in successive SMC layers in a herringbone-like pattern, a unique design that minimizes the biomechanical forces on individual aortic SMCs (reviewed in 8, 9). This elastin-contractile unit is uniquely designed to coordinate SMC contractile and elastic tensions in response to mechanical stresses imposed on the vessel wall.

The aortic pathology associated with thoracic aortic disease, whether genetically triggered or due to other factors like hypertension, is characterized by disruption of the structure of the medial layer of the aorta (Figure 2). Although the pathology was initially described as cystic medial necrosis, neither cyst formation nor necrosis is present. The pathology, more accurately termed medial degeneration, is characterized by loss and fragmentation of the elastin layers and increased deposition of proteoglycans. Although there is debate as to whether there is overall loss or gain of SMCs as aneurysms grow, there are regions with clear focal loss of medial SMCs. Levels and activity of proteases that degrade elastin, matrix metalloproteinases 2 and 9 (MMP-2 and MMP-9), are increased in the aortic media of patients and mouse models of Marfan syndrome (MFS) (19, 20).

This review summarizes what has been learned from the altered genes that predispose to thoracic aortic disease, collectively termed heritable thoracic aortic disease (HTAD) genes. How altered genes drive molecular pathways to induce aortic disease in mouse models is also discussed. Clinical trials testing novel therapies to prevent thoracic aortic disease, along with other therapeutic options to be further explored, are presented.

HERITABLE THORACIC AORTIC DISEASE GENES: IMPORTANCE OF TGF-β SIGNALING AND THE AORTIC ELASTIN-CONTRACTILE UNIT

For almost 60 years, MFS has illustrated that alteration of a single gene in the human genome can confer a high risk for thoracic aortic disease (21). MFS is a highly penetrant autosomal dominant condition that displays significant intra- and interfamilial variability. The cardinal features of MFS are thoracic aortic aneurysms and dissections, ocular complications, and skeletal abnormalities characterized by overgrowth of long bones. The syndrome is caused by mutations in *FBN1*, which encodes fibrillin-1, the major protein in the microfibrils that link the SMCs to the elastic lamellae.

Once *FBN1* was identified as the defective gene underlying MFS, it was evident that there were individuals and families with Marfanoid skeletal features and thoracic aortic disease who did not have *FBN1* mutations (22). Mutations in the gene that encodes transforming growth factor beta (TGF-β) receptor type II (*TGFBR2*) were identified in these individuals, and the condition was named Marfan syndrome 2 (23). Subsequently, mutations in genes encoding other proteins in the canonical TGF-β signaling pathway have been identified that predispose to thoracic aortic disease. The proteins include TGF-β receptor type I (*TGFBR1*),

SMAD3 (*SMAD3*), SMAD4 (*SMAD4*), and two of the three TGF-β ligands, TGF-β2 (*TGFB2*) and TGF-β3 (*TGFB3*). In addition to Marfanoid skeletal features and thoracic aortic disease, mutations in these genes predispose to aneurysms and dissections beyond the aorta, including arterial branches of the aorta and intracranial arteries. Additional systemic features can also be present, including bifid or wide uvula, as well as thin or translucent skin with delayed wound healing similar to that observed in individuals with vascular Ehlers-Danlos syndrome. *TGFBR1* and *TGFBR2* mutations also cause Loeys-Dietz syndrome, characterized by craniosynostosis, cleft palate/bifid uvula, developmental delay, congenital heart disease, and aggressive and early onset of both thoracic aortic disease and aneurysms and dissections involving other arteries (24). The causative mutations identified in these genes are predicted or have been shown to decrease canonical TGF-β signaling (25–27).

Altered genes can also lead to an inherited predisposition to thoracic aortic disease in the absence of syndromic features, termed familial thoracic aortic aneurysms and dissections (familial TAAD). Nonsyndromic familial TAAD was first described in a family with nine affected members over two generations, who did not have phenotypic features of MFS, vascular Ehlers-Danlos syndrome, or systemic hypertension (28). An underlying *TGFBR1* mutation was later identified as the cause of disease in this family (29). Subsequent studies found that up to 20% of patients presenting with thoracic aneurysms or dissections who do not have a syndrome have an affected first-degree relative (30, 31). Familial TAAD occurs primarily as an autosomal dominant condition, with decreased penetrance and variable expression with respect to age of presentation, location of aneurysms, and aortic diameter prior to dissection (32). It is important to emphasize that the distinction between syndromic and nonsyndromic causes of TAAD is a continuum for most of the genes that cause thoracic aortopathy. That is, genes that cause TAAD associated with syndromic features, such as *FBN1* (MFS) or *TGFBR2* (Loeys-Dietz syndrome), can also lead to autosomal dominant inheritance of TAAD in the absence of syndromic features.

Additional familial TAAD genes have been identified that encode proteins involved in the structural elements and kinases controlling SMC contraction (33). We determined that the most frequently altered familial TAAD gene is *ACTA2*, which encodes the SMC-specific isoform of α-actin (SM α-actin) (34). SM α-actin oligomerizes to form thin filaments of the contractile unit in SMCs. Specific *ACTA2* mutations predispose to early-onset coronary artery disease, Moyamoya-like cerebrovascular disease, and multisystemic smooth muscle dysfunction syndrome (35, 36). The thick filaments are composed of a smooth muscle–specific isoform of myosin heavy chain dimer (encoded by *MYH11*), and four light chains: two regulatory light chains and two essential light chains. Mutations in *MYH11* are also a cause of familial TAAD (37, 38). We identified loss-of-function mutations in the gene encoding the stretch-activated kinase that controls SMC contraction, myosin light chain kinase (*MYLK*), as a cause of familial TAAD (39). Myosin light chain kinase is the dedicated kinase that drives SMC contraction through phosphorylation of the regulatory light chain on the thick filaments, thus initiating movement of the motor head of the myosin heavy chain. Additionally, a rare *PRKG1* variant (p.R177Q) was also identified as a cause of FTAAD in multiple unrelated families worldwide (40). *PRKG1* encodes a type I cGMP-dependent protein kinase (PKG I) that is activated upon binding of cGMP and controls SMC relaxation. We showed that although *PRKG1* p.R177Q disrupts cGMP binding to its binding

site within the regulatory domain, the mutant PKG I protein is constitutively active even in the absence of cGMP, leading to increased activation of the enzyme that dephosphorylates the regulatory light chain. Thus, mutations in these genes are all predicted or have been shown to decrease aortic SMC force generation.

Two recently identified HTAD genes, *MFAP5* and *LOX*, further emphasize the structural importance of elastin fiber and fibrillin-1-containing microfibrils in the aortic wall. Because *MFAP5* encodes another microfibril protein (microfibril-associated protein 5), mutations in this gene are expected to interfere with microfibril formation and/or integrity (41). *LOX* mutations, in contrast, decrease the activity of lysyl oxidase and consequently are predicted to disrupt the formation of lysine-derived crosslinks in elastin that are required to maintain aortic integrity (42). Mutations in the genes encoding elastin (*ELN*) or fibulin-4 (*EFEMP2*), which are respectively a driver and a regulator of elastogenesis, are rare causes of TAAD associated with systemic features of cutis laxa (43, 44).

Other HTAD genes alter SMC metabolism or survival. *MAT2A*, which encodes methionine adenosyltransferase II alpha, is a cause of familial TAAD, but how disruption of this enzyme predisposes to disease is unknown. *FOXE3* mutations also lead to FTAAD, and the molecular pathogenesis was assessed in a mouse model (*Foxe3*$^{-/-}$ mice). These mutant mice had fewer SMCs in the aortic media and increased SMC apoptosis in response to increased biomechanical forces, thus defining an additional molecular alteration leading to FTAAD (45).

The genetic factors contributing to thoracic aortic disease in the larger population of affected individuals who do not have a single-gene disorder have shown that these variants disrupt the same genes and pathways as the genes causing Mendelian inheritance. The strongest association for common variants with thoracic aortic disease identified through genome-wide association studies is with single-nucleotide polymorphisms in *FBN1* (46). A recurrent copy-number variant present in the general population, with a greater-than-tenfold enrichment in aortic dissection patients, involves duplication of *MYH11* (47). In fact, genes altered by rare copy-number variants identified in thoracic aortic disease patients are predicted by pathway analyses to disrupt the SMC contractile complex or the extracellular matrix, again similar to functions that the Mendelian disease genes disrupt (48).

MANAGEMENT OF THORACIC AORTIC DISEASE IN INDIVIDUALS WITH MARFAN SYNDROME TO PREVENT ACUTE AORTIC DISSECTIONS

Current management of HTAD has been informed extensively by studies of individuals with MFS. The majority of MFS patients have evidence of aortic disease at the time of diagnosis, typically enlargement of the aortic root at the sinuses of Valsalva. The distal ascending aorta and arch typically do not enlarge in the absence of a type A dissection. At diagnosis, the entire aorta is routinely imaged to assess the extent of disease. Subsequent surveillance imaging focuses on the aortic root, if other regions of the aorta are not affected. Consistent measurements at anatomical landmarks are critical to accurately assess potential changes in aortic diameter at the annulus, root, sinotubular junction, and ascending aorta (49). Because most aneurysms in MFS patients are located immediately above the aortic valve,

transthoracic echocardiography is adequate to assess the aorta in the majority of cases. As aortic root aneurysms progressively enlarge over time, the risk for acute type A dissections increases, and aortic valve regurgitation may develop due to distortion of the valve by the aneurysm. This slow and progressive disease course may provide opportunities to intervene in order to retard or prevent aortic enlargement in patients with MFS. The primary goals of these interventions are to prevent acute dissections or aortic regurgitation, and to delay or potentially avoid surgical repair of the aorta. The longstanding recommended therapy to decrease the rate of enlargement of the aorta is treatment with β-blockers, which reduce cardiac inotropy and the number of contractions per minute, thus decreasing forces on the aorta. Beta-blockers were first successfully used in thoracic aortic disease to prevent spontaneous aortic dissections and improve survival in a breed of hypertensive turkeys (50, 51). Later, β-blockers were shown to reduce both growth rates of the aortic root and aortic complications in MFS patients in an open-label randomized trial (52). Individuals with MFS are frequently advised to avoid contact sports and isometric exercises in order to limit hemodynamic stresses on the aorta.

No therapeutic agent has been shown to prevent aortic growth and the associated risk for acute dissection, so the mainstay of treatment to prevent ascending aortic dissection in individuals with MFS remains surgical repair of the aortic aneurysm (1). Surgical repair is recommended when the aortic root enlarges to 5.0 cm in diameter because current data indicate that the risk of dissection is low until then (1, 53). Prior to the advent of surgical repair of the aortic root aneurysm, patients with MFS died on average in their mid-forties due to dissections or congestive heart failure from aortic insufficiency (54). In 1968, Bentall & De Bono (55) reported a method of replacing the aortic valve and aortic root simultaneously by using a composite valve graft (CVG) and reimplanting the coronary ostia into the graft. CVG repair replaces the entire root and aortic valve, even if the valve is normal, because of the technical difficulty of removing the entire aortic root, leading to the need for anticoagulation if a mechanical valve is used. The safety, reproducibility, and long-term durability of the CVG procedure have been clearly documented (56). Valve-sparing procedures to preserve the native aortic valve and replace the entire aortic root with a conduit have been popularized as an alternative to mitigate the need for chronic anticoagulation (57). A multi-institutional prospective outcome study is in progress to define the clinical outcomes of valve-sparing versus CVG procedures in MFS (58).

The life expectancy of patients with MFS has increased by an estimated 30 years because of earlier diagnosis, as well as proper medical and surgical management (59, 60). Current research is directed toward using the knowledge of the aortopathy genes and animal models harboring mutations in these genes to understand the molecular pathogenesis of MFS, so that targeted therapies that prevent aneurysms and dissections may be developed, negating the need for aortic surgical repair.

TGF-β AND ANGIOTENSIN II SIGNALING IN THORACIC AORTIC DISEASE

Genetically engineered mouse models of MFS have been instrumental in providing insight into the molecular pathogenesis of thoracic aortic disease. These experimental models include mice that make no fibrillin-1 (*Fbn1*$^{-/-}$ mice), 20% of normal fibrillin-1

(*Fbn1*$^{mgR/mgR}$ mice), and equal amounts of wild-type and mutant fibrillin-1 harboring a disease-causing missense mutation (*Fbn1*$^{C1039G/+}$ mice). In these mice, the severity of disruption of fibrillin-1 production correlates with the severity of the aortic disease. MFS mice that make no or little fibrillin-1 die early from thoracic aorta rupture (*Fbn1*$^{-/-}$ mice within the first two weeks of postnatal life and *Fbn1*$^{mgR/mgR}$ within a year). In contrast, *Fbn1*$^{C1039G/+}$ mice exhibit slowly enlarging aortic root aneurysms but seldom progress to dissection or rupture. Fetal development progresses normally in *Fbn1*$^{-/-}$ mice with no detectable morphologic abnormalities in aortic anatomy or histology, which suggests that fibrillin-1 may be essential for postnatal aortic function, rather than for aortic development. Despite the normal macroscopic development of the aorta, electron microscopic analyses have revealed loss of connections between the SMCs and elastin fibers in *Fbn1*$^{mgR/mgR}$ aortas (61). The absence of any gross abnormalities of aortic development in these MFS mice reiterates the potential feasibility of preventing thoracic aortic disease in adult MFS patients by using drugs that target pathologic molecular pathways.

Investigations of *Fbn1*$^{C1039G/+}$ and *Fbn1*$^{mgR/mgR}$ mice identify abnormally high TGF-β and angiotensin II (Ang II) type I receptor (AT1R) signaling. However, these investigations have reached different conclusions about the underlying molecular mechanisms of aortic disease and corresponding potential therapeutic opportunities for MFS patients. Initial studies characterizing thoracic aortic enlargement in *Fbn1*$^{C1039G/+}$ mice identified excessive TGF-β signaling as the driver of aortic disease (62). This conclusion was based on the finding that TGF-β inhibition by a pan-TGF-β neutralizing antibody (TGF-β-NAb) prevented aneurysm formation and normalized phosphorylation of the TGF-β targets Smad2 and Erk1/2 in medial SMCs. Because the AT1R antagonist losartan had been shown to inhibit TGF-β signaling in animal models of chronic renal insufficiency and cardiomyopathy (63, 64), this drug was used to blunt TGF-β hyperactivity in the medial layer of *Fbn1*$^{C1039G/+}$ mice. Losartan was shown to be more effective than β-blockers, and as effective as TGF-β-NAb, in preventing aortic root enlargement and pathologic changes (medial thickening and elastin fragmentation).

Fibrillin-containing microfibrils not only provide a structural scaffold, which stabilizes elastin fibers and links SMCs to the elastic lamellae, but also regulate the bioavailability of latent TGF-β complexes. Different TGF-β molecules are all secreted as part of a trimeric latent complex (the large latent complex, LLC) consisting of TGF-β, the cleaved TGF-β pro-peptide dimer (the latency-associated protein, LAP), and a latent TGF-β-binding protein (LTBP-1, -3, or -4) (66). TGF-β and LAP form a noncovalent complex (the small latent complex, SLC) that precludes ligand interaction with its receptor. Covalent association of the SLC with LTBPs results in tethering of the LLC to ECM components, including fibrillin-1 microfibrils. Activation of ECM-bound TGF-β thus requires release from its association with LAP. There are multiple latent TGF-β activators, including integrins, proteases, thrombospondin, and reactive oxygen species (ROS) (67–69). Integrin-mediated TGF-β activation is driven by receptor binding to LAP, adhesion-generated cell forces, and ECM stiffness. Metalloproteinase-2 and -9 (MMP2 and MMP9), which are highly expressed in aneurysmal tissues, can also activate ECM-bound TGF-β (19, 70). The combination of impaired ECM storage and AT1R-dependent overactivation of LLCs was originally

proposed to account for TGF-β hyperactivity in the aorta of *Fbn1*$^{C1039G/+}$ mice (65). However, subsequent studies have called into question this disease model.

Recent observations have led to questions as to whether the excessive TGF-β is coming from microfibrils, whether TGF-β activation is truly the primary driver of aortic disease, and whether losartan effectiveness is exclusively the result of TGF-β inhibition. First, the molecular assays that were used to compare the outcomes of losartan- and TGF-β-NAb-treated *Fbn1*$^{C1039G/+}$ mice do not discriminate between the pathogenic contributions of TGF-β and AT1R activation. Smad2 and Erk1/2 may be activated by either TGF-β or Ang II signaling, and AT1R activation can increase TGF-β synthesis and activate Smad2 in the absence of TGF-β ligands (71–76). Second, losartan treatment delayed, but did not prevent, aneurysm formation and aortic ruptures in *Fbn1*$^{mgR/mgR}$ mice, suggesting that additional, AT1R-independent signaling pathways may contribute to aortic disease progression. Third, genetic disruption of TGF-β signaling, by deleting one allele of the TGF-β type II receptor (*Tgfbr2*) in SMCs of newborn *Fbn1*$^{C1039G/+}$ mice or by crossing *Fbn1*$^{C1039G/+}$ mice with mice deficient in Tgf-β2 (*Tgfb2*$^{+/-}$), exacerbates aortic pathology (27). It is important to note that TGF-β activity reduced aortic disease that was induced by Ang II infusions (77). Finally, as described previously, loss-of-function mutations in genes in the canonical TGF-β signaling pathway, including mutations in the TGF-β receptors, downstream signaling molecules, and TGF-β ligands, actually cause rather than prevent thoracic aortic disease.

Recent studies have addressed these apparent discrepancies by directly comparing TGF-β-NAb and losartan therapies in *Fbn1*$^{mgR/mgR}$ mice (78). In these mice, losartan delayed aneurysm formation and reduced aortic dissections but did not prevent these complications in *Fbn1*$^{mgR/mgR}$ mice. Blocking TGF-β signaling in young animals at an early stage of disease worsened aortic outcomes, whereas treatment at later stages attenuated disease. Furthermore, this study also identified associations between AT1R hyperactivity and ERK-driven aortic enlargement, as well as between TGF-β hyperactivity and Smad-driven medial pathology. Based on these observations, the treatment strategy was altered so that AT1R antagonists were administered early and continuously, followed by TGF-β-NAb administration at later timepoints. This combined strategy was effective to prevent aortic complications, and the results linked aberrant AT1R and TGF-β signaling with distinct stages of the disease processes. The relatively late onset of excessive TGF-β signaling, which was extrapolated from the accumulation of phosphorylated Smad2 in *Fbn1*$^{mgR/mgR}$ mice, supports the notion that TGF-β hyperactivity is a secondary determinant of disease progression. In contrast to the previously suggested losartan monotherapy in MFS, these comparative analyses argue for preserving TGF-β signaling during the early stages of thoracic aortic disease, followed by combinatorial therapies that blunt both TGF-β and AT1R signaling during later stages.

Several key aspects of TAAD pathogenesis in MFS remain unresolved, including the nature of the late-stage stimulation of TGF-β activity and the cause of aberrant AT1R activity. The conclusion that TGF-β hyperactivity is solely due to inappropriate release of TGF-β from LTBPs is based on a speculative interpretation of in vitro data, and alternative explanations for the source of excessive TGF-β have not been explored. Ang II and ROS were also shown to stimulate TGF-β synthesis in SMCs (71). Alternatively, SMCs and inflammatory cells

may be additional sources of TGF-β and contribute to the activation of ECM-bound LLCs through the secretion of MMPs and other proteases.

Another critical unknown aspect of the molecular pathogenesis of aneurysm progression is the source of excessive AT1R activity in the MFS mouse models. Recent biomechanical analyses of *Fbn1*$^{mgR/mgR}$ mice have raised the possibility that a structurally impaired aortic matrix may perturb AT1R mechanosignaling (72, 73). Characterization of cardiomyopathy in *Fbn1*$^{mgR/mgR}$ mice has in dependently supported this notion (74). Mice deficient in *Tgfbr1* or *Tgfbr2* also form aneurysms, and losartan prevents both the aneurysms and excessive TGF-β signaling in the aortic media. These observations provide evidence that TGF-β may be activated downstream of AT1R, but they fail to identify the source of the increased AT1R signaling. TGF-β hyperactivity and aneurysm formation are also blocked by losartan in Fbln4-deficient mice (79, 80). Aneurysm formation in these models is associated with increases in ERK1/2 activation, angiotensin-converting enzyme, and Ang II, thus implicating excessive production of Ang II in the aorta as the source of AT1R activation.

It is also important to note that thoracic aortic disease in either *Fbn1*$^{C1039G/+}$ or *Fbn1*$^{mgR/mgR}$ mice can be mitigated by pharmacologic inhibition of MMP activity or caspase-driven SMC apoptosis (81, 82). How these targeted pathways may be integrated with the AT1R and TGF-β pathways has not been determined.

ROLE OF THE ELASTIN-CONTRACTILE UNIT AND SMOOTH MUSCLE CELL MECHANOSENSING IN THORACIC AORTIC DISEASE

Several lines of correlative evidence suggest that progressive hemodynamic load on a structurally defective aorta may be the primary driver of thoracic aortic disease, and that TGF-β overactivation in diseased aortas is a secondary unproductive response to restore tissue function (8, 9). This hypothesis is supported by the observation that comparable thoracic aortic disease and aortic pathology occur in individuals with mutations in genes encoding components and kinases for SMC contractile function, including *ACTA2*, *MYH11*, *MYLK*, and *PRKG1* (34, 37, 39, 40). Mutations in these genes have been shown or are predicted to decrease the ability of SMCs to contract in response to agonists, thus impairing the cellular response to hemodynamic load (37, 83, 84). It is important to note that HTAD genes encoding proteins in the TGF-β pathway decrease TGF-β signaling and thus are expected to perturb the proper development of fully differentiated SMCs in a mechanically compliant aortic wall (85, 86).

Studies of the Acta2-null mouse model have implicated a molecular pathway that may be responsible for increased AT1R signaling in aneurysm progression, in the absence of elevated Ang II levels. The lack of SM α-actin in *Acta2*$^{-/-}$ mice leads to decreased contraction of aortic rings, decreased arterial resistance, and hypotension (J. Chen, A. Peters, C. Papke, et al., manuscript submitted). Similar to the *Fbn1*$^{C1039G/+}$ mice, *Acta2*$^{-/-}$ mice have slowly progressive aortic root dilation that can be attenuated by treatment with losartan, but Ang II levels are not increased in either the aortas or kidneys. Instead, explanted SMCs and aortic tissue from *Acta2*$^{-/-}$ aortas were associated with increased ROS levels, which in turn increase basal NF-κB signaling and AT1R (*At1a* and *At1b*) expression. This escalation

of AT1R in the *Acta2*$^{-/-}$ SMC leads to >100-fold increased sensitivity to exogenous Ang II. The *Acta2*$^{-/-}$ elastin-contractile complexes in the aorta lack contractile filaments in SMCs, and focal adhesion links from the SMCs to elastin fibers are greatly diminished. Thus, disruption of the elastin-contractile unit in *Acta2*$^{-/-}$ mice leads to cellular stress, ROS production, increased AT1R signaling, and aneurysm formation without altering exogenous levels of Ang II.

The hypothesis that progressive hemodynamic load on a structurally defective aorta is the primary driver of thoracic aortic disease is further supported by the critical role of fibrillin-1 in the elastin-contractile unit. *FBN1* mutations in MFS patients have been shown to decrease the amount of fibrillin-1 synthesized by SMCs or to disrupt the polymerization of fibrillin-1 into microfibrils, thus diminishing microfibril formation in the matrix (88–90). As mentioned above, the connections between SMCs and elastin fibers are completely lost in *Fbn1*$^{mgR/mgR}$ mice (61). Studies of the *Fbn1*$^{C1039G/+}$ mice have revealed decreased contraction of isolated aortic rings, suggesting that the force generated by SMCs is diminished when connections to elastic lamellae are lost (91). Importantly, contraction of aortic segments is also diminished in Fbnl4-deficient mice (80). Interestingly, the cellular pathways driving increased ROS levels in *Acta2*$^{-/-}$ SMCs are similarly activated in aortic SMCs explanted from MFS patients, and are also associated with overexpression of contractile proteins, increased focal adhesion signaling, and increased cell stiffness (92, 93). Furthermore, cellular ROS levels are increased in *Fbn1*$^{mgR/mgR}$ aortic SMCs (94). These data suggest that cellular ROS levels can be elevated with disruption of either the contractile unit within the SMCs or the extracellular matrix. Elevated ROS levels may activate common downstream signaling pathways, resulting in increased AT1R levels and heightened sensitivity to exogenous Ang II.

The above studies strongly implicate disruption of mechanosensing through the elastin-contractile complex as a cause of thoracic aortic aneurysms and have identified pathways that lead to AT1R activation. The importance of maintaining SMC force generation for proper mechanosensing to prevent thoracic aortic disease is further illustrated by the fact that antihypertensive drugs that are direct-acting smooth muscle relaxants, including hydralazine and calcium channel blockers, are associated with more frequent elective aortic surgery in patients and accelerate disease in animal models (95, 96). Furthermore, risk factors for thoracic aortic disease, such as hypertension, cocaine/methamphetamine abuse, and pregnancy, all increase hemodynamic forces on the ascending aorta. In these cases, minor or no disruption of the elastin-contractile unit may occur, but rather the aortic SMCs' mechanosensing is stressed by the increased forces on the wall.

LOSARTAN CLINICAL TRIALS IN MARFAN SYNDROME

The success of losartan in preventing aortic enlargement in the *Fbn1*$^{C1039G/+}$ mice, along with a retrospective study's finding that losartan prevented aortic root growth in young children with severe MFS, prompted physicians to begin off-label losartan use in thoracic aortic disease patients with and without MFS, justified in part by the drug's excellent safety profile. The success of using losartan to prevent aneurysm formation in the *Fbn1*$^{C1039G/+}$

mice also led to the initiation of randomized clinical trials of losartan in patients with MFS worldwide (Table 1).

The first prospective trial to be completed was the Dutch COMPARE trial, which assessed 233 MFS patients over the age of 18 years who met diagnostic criteria for MFS and were assigned to receive losartan or no additional medications (97). Losartan significantly and specifically reduced dilation of the aortic root when compared with usual therapies, including β-blockers. More patients in the losartan group than in the usual-care group took β-blockers, leading to speculation that losartan and β-blockers may interact to reduce aortic growth. Subsequent analyses of the data indicated that losartan was primarily effective in younger patients and in patients with *FBN1* mutations that were predicted to cause haploinsufficiency of fibrillin-1, i.e., predicted to produce decreased amounts of protein, but not in patients with missense mutations that were predicted to produce equal amounts of the normal and mutant fibrillin-1 (98).

The US Pediatric Heart Network trial was the largest losartan trial. It enrolled 608 patients with MFS between the ages of 6 months and 25 years who met Ghent criteria and had significant aortic root enlargement (defined by a Z-score >3.0) (99). The two arms of the randomized trial were atenolol (started at 0.5 mg/kg·day and increased to a maximum of 4 mg/kg·day) and losartan (started at 0.4 mg/kg·day and increased to a maximum of 1.4 mg/kg·day). Both groups showed a comparable decrease in the rate of aortic root growth with no significant differences between the groups. Two additional trials comparing losartan to β-blockers or placebo reached similar conclusions (100, 101).

Therefore, the current evidence shows that losartan and β-blockers may be equivalent to slow aortic disease progression in most patients with MFS and aortic dilation. The choice of therapies primarily depends on patient tolerance or preference and may be influenced by the type of genetic mutation.

CONCLUSION

Accumulating evidence suggests that progressive hemodynamic load on a structurally defective aorta is the primary trigger of thoracic aortic disease, rather than TGF-β hyperactivity in the aortic media. Given that β-blockers decrease the force and number of heartbeats and thus decrease the hemodynamic load, these drugs will continue to have a role in preventing thoracic aortic disease. Limiting hemodynamic forces, and in particular controlling hypertension, is particularly important given the hypothesis that the primary driver of thoracic aortic disease is aberrant SMC mechanosensing (8, 9). Mouse and human genetic data indicate that loss of canonical TGF-β signaling causes or worsens thoracic aortic disease. However, targeting TGF-β hyperactivity late in disease may be beneficial. Therefore, development of therapeutics that directly block TGF-β signaling should be pursued with caution. Blocking AT1R signaling using losartan has been beneficial in both mouse models and individuals with MFS and can be used as an alternative to or in combination with β-blockers. Further data are needed to determine if losartan is beneficial only in a subset of individuals with particular mutations or with specific underlying genes altered. Finally, accumulating evidence indicates that hypertension therapies that directly

decrease SMC contraction, such as hydralazine and calcium channel blockers, should be used with caution in patients with thoracic aortic disease (95, 102).

Other potential therapeutic targets have been identified to prevent thoracic aortic disease. Increased ROS in aortic SMCs have been identified in *Fbn1*$^{C1039G/+}$ and *Acta2*$^{-/-}$ mice, but attempts at treating diseases with antioxidants have been largely ineffective (91, 92). Inhibition of MMP activity by doxycycline and MMP2 deletion attenuate disease in both *Fbn1*$^{C1039G/+}$ and *Fbn1*$^{mgR/mgR}$ mice (81, 103). Aortic dilation was attenuated in *Fbn1*$^{C1039G/+}$ mice and thoracic aortic ruptures were prevented in *Foxe3*$^{-/-}$ mice using drugs or genetic ablation to prevent SMC apoptosis (45, 82). Most likely a multidrug regimen targeting various molecular pathways will be required to prevent thoracic aortic aneurysms and aortic dissections in individuals with an underlying genetic predisposition.

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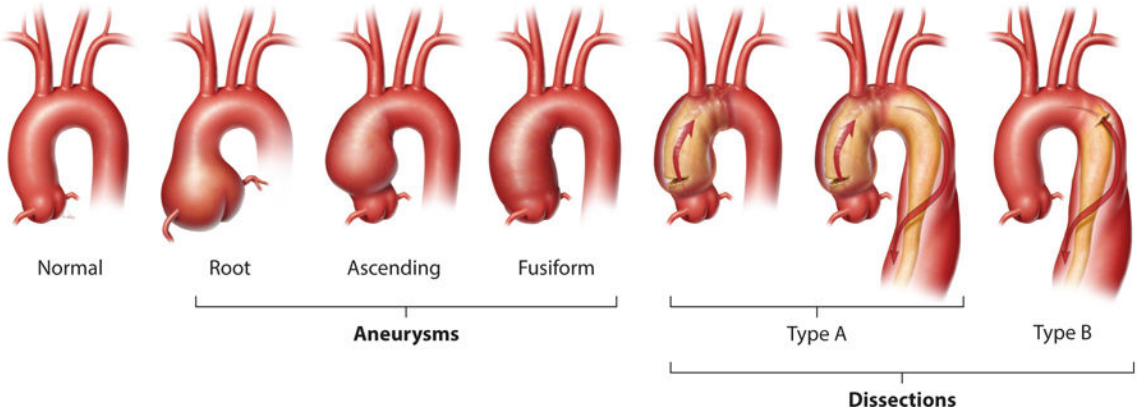

Figure 1.
Schematic representation of thoracic aortic aneurysms and aortic dissections.

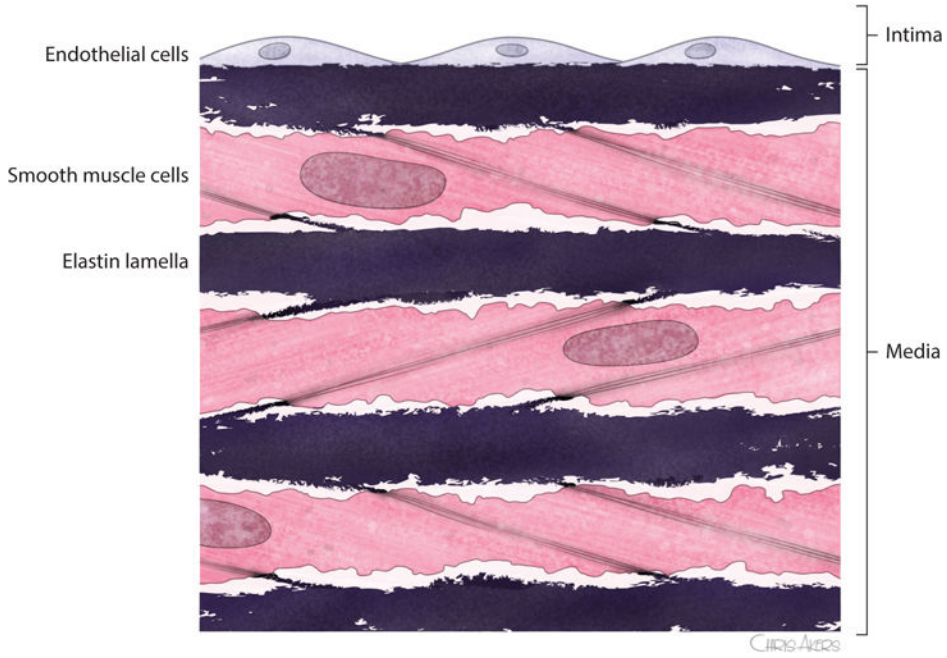

Figure 2.

Schematic representation of a cross section of the aorta illustrating the intimal layer of endothelial cells and the first three out of >50 layers of elastin lamellae and smooth muscle cells (SMCs). The elastic lamellae (*black*) and SMCs (*pink*) are connected through oblique extensions of elastin to the surface of the SMCs. The microfibrils at the end of the elastin extensions link to integrin receptors in the focal adhesions on the SMC surface. The contractile units, composed of SM α-actin (the SMC-specific isoform of α-actin) and myosin filaments, attach to the focal adhesions and extend obliquely across the cells. The direction of these oblique extensions changes from layer to layer.

Table 1

Summary of randomized prospective clinical trials involving losartan in Marfan syndrome[a]

Trial name	Trial type	Drugs tested	Dose range	Number enrolled	Enroll age (y)	Follow-up	Primary outcome	Results	Notes
Pediatric Heart Network	Double-blind, stratified	Atenolol Losartan	0.5–4.0 mg/kg/d 0.4–1.4 mg/kg/d	608	0.5–25	3.0 ± 0.1 y	Rate of change in root Z-score/y	Primary outcome NS (−0.139 vs –0.107 Z/y, p = 0.08)	Pharmacogenomics and *FBN1* sequencing sub-studies pending
COMPARE	Open-label	Losartan No additional drug	50 or 100 mg/d	233	>18	3.1 ± 0.4 y	Change in absolute root diameter	Primary outcome NS (0.77 vs 1.35 mm, p = 0.014)	Significant only for *FBN1* haploinsufficiency
Spanish	Double-blind	Atenolol Losartan	25–100 mg/d	140	5–60	3.0 y	Change in absolute diameter or Z-score of root and ascending aorta	Primary outcome NS (–0.04 vs −0.01 Z, *P* = 0.193)	Ambulatory blood pressures not different betweeen groups
Marfan Sartan	Double-blind	Losartan Placebo	50 or 100 mg/d	299	>10	3.5 y	Rate of change in root Z-score/y	Primary outcome NS (–0.01 vs –0.03 Z/y, p = 0.68)	100% sequenced: 78% *FBN1* mutations
Taiwanese	Open-label	Losartan + BB BB	Losartan: 25–100 mg/d or 0.7 mg/kg/d–50 mg/d BB: 50 mg atenolol/20 mg propranolol or 1 mg/kg/d Any dose <150 mg/d or <2 mg/kg/d	29	None specified	~3 y	Rate of change in absolute root diameter/y	Primary outcome significant (0.10 vs 0.89 mm/y, p = 0.02)	
Aortic Irbesartan Marfan Study[b]	Double-blind	Irbesartan Placebo	150–300 mg/d	490	6–40	5 y	Rate of change in absolute root diameter/y		Pharmacogenomics sub-study
Ghent Marfan Trial[b]	Double-blind, stratified	Losartan + BB Placebo + BB	Losartan: 50 or 100 mg/d BB: NA	174	>10	3 y	Rate of change in absolute root diameter or Z-score/y		Genetic modifier study of 160 candidate TGF-β pathway single-nucleotide polymorphisms
Italian[b]	Open-label	Losartan Nebivolol Losartan + nebivolol	0.2–1.4 mg/kg/d or 25–100 mg/d 0.02–0.16 mg/kg/d or 1.25–10 mg/d	291	1–55	4 y	Rate of change in absolute root diameter or Z-score/y		

[a] All studies included the modified Ghent criteria as inclusion criteria.

[b] Unpublished study.

Abbreviations: BB, β-blocker; d, day; m, month; y, year; NA, not available; NS, not significant.