Published in final edited form as:

Curr Cardiol Rep. 2010 March ; 12(2): 99–107. doi:10.1007/s11886-010-0083-z.

The Pathogenesis of Aortopathy in Marfan Syndrome and Related Diseases

Jeffrey A. Jones, Ph.D.^{1,2} and John S. Ikonmidis, M.D., Ph.D.¹

¹Division of Cardiothoracic Surgery, Department of Surgery, Medical University of South Carolina

²Ralph H. Johnson Veterans Affairs Medical Center; Charleston, SC

Abstract

Marfan syndrome is a systemic connective tissue disorder that is inherited in an autosomal dominant pattern with variable penetrance. While there are many clinical manifestations of this disease, the most life threatening are cardiovascular complications including mitral valve prolapse and aortic root aneurysm. When the primary defect was discovered in the fibrillin-1 gene, it was hypothesized that mutations in fibrillin-1 resulted in a weakened and disordered elastic architecture. Recent evidence, however, has suggested that MFS syndrome is caused by more than just a disordered microfibril matrix. Interest was stimulated when it was discovered that fibrillin-1 mutations enhanced the release of sequestered latent transforming growth factor-beta, a well described mediator of vascular remodeling. The present review will focus on the pathophysiology of aortopathy in Marfan syndrome and related diseases, with special emphasis on the role of transforming growth factor-beta in mediating the pathogenesis of this disease.

Introduction

It's been more than 100 years since French pediatrician Antoine Marfan made the original observation of extraordinary musculoskeletal abnormalities in a 5-year old girl, which laid the foundation for the description of Marfan syndrome (MFS). Since that time, many have contributed toward understanding the pathogenesis of this multisystem disorder which affects the skeletal, ocular, and cardiovascular systems. In the past 15 years in particular, research efforts have identified the gene defect responsible, have begun to elucidate the molecular mechanisms driving this disorder, and have begun to devise a therapeutic strategy for treating affected individuals. In spite of these advancements, aortic aneurysm and dissection still remain as the most life-threatening clinical manifestations. The goal of the present review is to provide an overview of the recent advancements in the molecular pathogenesis of MFS, to place them in context to how they relate to aortic disease, and to

Corresponding Author: John S. Ikonmidis, M.D., Ph.D., Medical University of South Carolina, Division of Cardiothoracic Surgery, Strom Thurmond Research Building, 25 Courtney Drive, Suite 7018, MSC 295, Charleston, SC 29425-2950, Phone: (843) 876-4842, FAX: (843) 876-4866, ikonmidj@musc.edu.

Contact Information:

Jeffrey A. Jones, Ph.D., Assistant Professor/Research Health Scientist, Medical University of South Carolina, Ralph H. Johnson VA Medical Center, Cardiothoracic Surgery Research, Strom Thurmond Research Building, 114 Doughty Street • Suite 625, PO Box 250778, Charleston, SC 29425, Phone: (843) 876-5186, FAX: (843) 876-5187, jonesja@musc.edu

discuss how this new information can focus future efforts toward finding better ways to detect, diagnose, and treat this devastating condition.

Clinical Manifestations

Marfan syndrome (MFS; OMIM #154700) is a systemic connective tissue disorder that is inherited in an autosomal dominant pattern with variable penetrance [1]. The incidence approximates 2–3 cases per 10,000 individuals, of which approximately 27% of patients present without a previous family history [2]. Clinical diagnosis is made based on the published international nosology, better known as the Ghent diagnostic criteria [3]. The Ghent nosology is used to assess clinical features in seven major body systems, and to determine whether involvement in each system is classified as a major or minor criterion. The diagnosis of MFS requires the identification of a major criterion in two different body systems, plus a minor criterion in a third body system. The diagnostic criteria have been the focus of several excellent reviews [2, 4, 5]. Briefly, the most common features of MFS include: **A) Skeletal manifestations** [2]. These are predominantly characterized by disproportionate overgrowth of the long bones in the arms and legs, resulting in tall stature, a decreased upper to lower body height ratio, and an arm-span greater than 1.05 times the height. Additionally, overgrowth of the fingers (arachnodactyly) combined with joint laxity, as characterized by the extension of the distal phalanx of the thumb beyond the ulnar border of the hand, and overlap of the distal phalanx of the thumb and fifth finger when wrapped around the wrist, is also a classic feature. **B) Ocular manifestations** [6]. The most common characteristic involves dislocation of the ocular lens of the eye (ectopia lentis), and occurs in about 60% of MFS patients. In addition, early and severe myopia, retinal detachment, and early onset of cataracts or glaucoma have also been observed. **C) Central nervous system manifestations** [7]. One of the most common features of MFS, occurring in 60–90% of patients, is swelling of the dural sac in the lumbar and sacral spine (dural ectasia). While this may remain asymptomatic, it often causes lower back pain, headaches, or lower extremity weakness. **D) Cardiovascular manifestations** [8]. Abnormalities in both the heart and vascular systems have been documented with MFS. Within the heart, the atrioventricular valves are commonly affected by prolapse with a variable degree of regurgitation; mitral valve insufficiency in young children has been associated with dilated cardiomyopathy and pulmonary hypertension, and is the leading cause of MFS-related death in infancy [9]. While in the vascular system, annuloaortic ectasia and dilatation of the aortic root are precedent to ascending aortic aneurysms and dissections, and represent the most life threatening complications of MFS [10]. While these common clinical features appear in most patients, the variable penetrance of this genetic defect leads to inconsistencies in the time of onset, sometimes making a definitive diagnosis difficult. Accordingly, the Ghent diagnostic criteria also consider family history as a major indicator of MFS, if a first-degree relative has independently met the necessary criteria for diagnosis.

Molecular Pathobiology

The primary gene defect in MFS lies on chromosome 15q21.1, within the coding region of the fibrillin-1 gene (FBN1) [1]. Fibrillin-1 is a 350 kDa glycoprotein comprised of multiple epidermal growth factor (EGF)-like motifs that are arranged in tandem. There are 47 motifs

in all, 43 of which contain a calcium binding sequence and are termed calcium-binding EGF (cbEGF) repeats [11]. The cbEGF modules function to: sequester extracellular calcium in order to protect against fibrillin proteolysis, promote interactions between fibrillin monomers and other cellular components such as integrin $\alpha\text{v}\beta\text{3}$, and stabilize the structure of the microfibrils favoring lateral packing [12, 13]. The tandem arrays of cbEGF motifs (1 to 12 repeats) are separated by seven 8-cys/TB modules. These modules have high homology to the latent transforming growth factor binding proteins (LTBPs), and are characterized by eight highly conserved cysteine residues, including a unique cysteine triplet [11, 12]. Fibrillin monomers self-assemble into macroaggregates that form the basic structure on which mature elastin fibers are synthesized from tropoelastin subunits. Thus, it was hypothesized that fibrillin-1 mutations within the aorta result in weakened and disordered elastic fiber formation, as well as disruption of the microfibril network connecting the elastic lamellae to the adjacent interstitial cells [14].

Recent evidence, however, suggests that MFS syndrome is caused by more than just a disordered microfibril matrix. In addition to directing elastogenesis and providing structural integrity to the elastic lamellae, the 8-cys/TB domains of fibrillin are involved in the sequestration of latent transforming growth factor-beta (TGF- β). TGF- β is synthesized as a prepolypeptide that is cleaved in a post-Golgi compartment to yield a mature growth factor molecule and an inactive cleavage fragment, termed the latency-associated peptide (LAP). Homodimers of the mature TGF- β and the LAP then uniquely combine and form a tight biologically inactive complex called the small latent complex (SLC). The SLC then covalently binds to LTBP by forming disulfide bonds between cysteine residues in the LAP and a cysteine rich motif in the LTBP [13, 15, 16]. This large latent complex (LLC) is then secreted from the cell and functions to target latent-TGF- β to the ECM, specifically to fibrillin microfibrils, and the 8-cys/TB motifs they contain [17, 18]. This complex sequestration process may thus serve a dual role in the regulation of TGF- β signaling. First, the LAP sequesters active TGF- β , rendering it inactive thereby preventing unregulated activation of the TGF- β signaling pathway. Second, the interaction of the LLC with microfibrillar proteins localizes and concentrates latent TGF- β at critical signaling sites within the ECM, making it available for release and activation at a moments notice. Interestingly, when Dietz and coworkers identified that multiple mutations within the *FBN1* gene were associated with MFS [19], they hypothesized that abnormalities in fibrillin-1 may lead to impaired sequestration of latent TGF- β complexes and enhanced TGF- β signaling [1].

Several studies provided the basis for support of this hypothesis. First, Neptune *et al.* demonstrated that lung abnormalities, evident in the immediate postnatal period in mice deficient in fibrillin-1 (*Fbn1*^{mg/mg}), were related to enhanced TGF- β activation and signaling, and that the perinatal administration of a TGF- β neutralizing antibody could rescue the defect in alveolar septation [20]. Second, in mice carrying a hypomorphic allele of the fibrillin-1 gene (*Fbn1*C1039G/+), effectively recapitulating human Marfan syndrome in a mouse model, Ng and coworkers similarly demonstrated that treatment with a TGF- β neutralizing antibody was able to reverse myxomatous changes in the mitral valve, rescuing a prolapse defect [21]. Similarly, also using the hypomorphic fibrillin-1 mice

(Fbn1C1039G/+), Habashi and coworkers demonstrated that TGF- β neutralizing antibody treatment was sufficient to attenuate spontaneous aortic root dilatation, elastic fiber fragmentation, and the activation of downstream signaling intermediates (phosphorylation of Smad2) in the TGF- β pathway [22]. Moreover, treatment with an angiotensin-II type-I receptor antagonist (Losartan) or a β -adrenergic receptor antagonist (propranolol), could also mimic the effects of TGF- β receptor antagonism, reversing the aortic pathology. Together, these studies demonstrated a strong link between enhanced TGF- β signaling and the clinical manifestations of MFS.

While evidence did exist that fibrillin-1 and LTBP-1 (the primary component of the LLC) were associated *in vivo* [18], little was known about the mechanism regulating their interaction. Isogai *et al.* suggested that fibrillin-1 and LTBP-1 interacted directly via the cbEGF domains common to both proteins [18]. This was consistent with the Dietz study which localized fibrillin-1 mutations to a cbEGF domain [19]. Recently, Chaudhry and coworkers have identified a peptide of fibrillin-1 (encoded in exons 44–49) that is capable disrupting the interaction between fibrillin-1 and the C-terminal domain of LTBP-1 (the primary component of the LLC) [16]. They demonstrated that the release of the LLC led to enhanced TGF- β signaling as determined by the phosphorylation of downstream signaling mediators. While their work defined a physiological mechanism by which the LLC could be released from fibrillin-1, it did not address how TGF- β was released from the SLC. Recent studies by Ge *et al.* demonstrated that bone morphogenetic protein 1 (BMP1)-like metalloproteinases were capable of cleaving LTBP-1 at two independent sites [23]. This resulted in release of the SLC and the consequent activation of TGF- β via cleavage of the LAP by nonBMP1-like proteinases. Similarly, Tatti and coworkers demonstrated LTBP-1 could be cleaved by membrane-type 1 matrix metalloproteinase in activated endothelial cells [24]. Again, proteolytic processing of LTBP-1 coincided with the release of the SLC, which was shown to require protein kinase C and extracellular signal-regulated protein kinase (ERK 1/2) activity. These studies suggest that the proteolysis of LTBP-1 (the major component of the LLC), was sufficient to release the SLC, which was then subsequently acted upon by other extracellular proteases to release active TGF- β in a non-regulated fashion. In addition to SLC proteolysis, a number of other mechanisms have been shown to release TGF- β or modify its association with the LAP rendering it active; these include: non-proteolytic dissociation by interaction with thrombospondin or integrin $\alpha_v\beta_6$, or release from the LAP by exposure to acidic or oxidative stress (reviewed in [15]).

While the predominant theory for the pathogenesis of MFS originally focused on abnormalities in fibrillin-1 function, several studies began to reveal a direct role for abnormal TGF- β signaling. Thus, the question that remained was how does excessive TGF- β signaling induce the clinical manifestations of MFS? The remainder of this review will focus on TGF- β and its specific role in aortopathy secondary to MFS and related diseases.

TGF- β Signaling Pathway

Transforming growth factor- β is a soluble peptide growth factor that is a member of a superfamily of ligands and receptors that include the TGF- β s, bone morphogenetic proteins, and the activins and inhibins [25]. TGF- β is produced by multiple cells types within the

vascular wall, and has been implicated in numerous divergent cellular processes including proliferation, angiogenesis, differentiation, apoptosis, and wound healing [26]. Moreover, its role in regulating the structure and composition of the vascular ECM is now well described [27].

The classical TGF- β signaling pathway (Figure 1, *classical pathway*), is initiated upon binding of activated TGF- β to a heteromeric receptor complex consisting of two type I receptors and two type II receptors, both of which possess serine/threonine kinase activity. The type II receptor activates the type I receptor through a transphosphorylation event. The activated type I receptor then phosphorylates a receptor-Smad (R-Smad), a class of intracellular signaling intermediates named for their homologues in *C. elegans* (SMA genes) and *Drosophila* (MAD genes; mothers against decapentaplegic). The R-Smad then binds to a co-Smad (Smad4) forming a complex that is shuttled into the nucleus where, upon interaction with transcriptional modifiers (activators or repressors), it forms a competent transcription complex capable of inducing or repressing numerous genes [28].

In addition to the classical signaling pathway, a growing body of evidence now supports the hypothesis that TGF- β signaling can proceed by alternative mechanisms that bypass key mediators in the classical pathway [29]. For example these non-canonical signaling responses include: 1) signals propagated directly by type II receptors without type I receptor involvement; 2) type I receptor signals in the absence of Smad activity; 3) R-Smad signaling to parallel pathways in the absence of co-Smad involvement; and 4) activation of R-Smads by other signaling mediators in response to TGF- β , but not as a result of direct interaction with TGF- β receptors (Figure 1, *non-canonical pathway*). Thus, through combinatorial receptor interactions, multiple regulatory mechanisms, and alternative signaling pathways, the complexity of signaling through this pathway is amplified.

TGF- β Signaling and Vascular Homeostasis

Just as TGF- β was originally identified as a bifunctional regulator of cell growth, TGF- β signaling has been attributed to many other opposing and disparate cellular functions. In the vascular compartment notwithstanding, TGF- β has been implicated not only in matrix deposition, but also in matrix degradation, positioning it as a critical mediator of the structure and composition of the vascular ECM. Studies have shown that TGF- β not only functions to induce genes that code for critical matrix proteins (e.g. collagen, elastin), but it also impacts matrix deposition through other modalities including the induction of other profibrotic genes, the regulation of matrix-protein mRNA stability, and through stimulating the production of endogenous extracellular protease inhibitors (Table 1, *Effects on Matrix Deposition*). Additionally, TGF- β is able to directly repress the gene expression of several matrix metalloproteinases via TIE binding sites (TGF- β inhibitory elements) present in their promoter regions. In direct contrast to these studies, TGF- β has been implicated in regulating pathways that lead to matrix degradation (Table 1, *Effects on Matrix Degradation*). Several studies have demonstrated TGF- β -mediated induction of matrix metalloproteinase abundance and activity. Thus, other factors must participate in determining whether a given TGF- β signal ultimately results in matrix deposition or degradation. Interestingly, while classical TGF- β signaling involving Smad intermediates

often results in enhanced expression of profibrotic genes (CTGF, PAI-1, collagens, etc.), the outcomes of the non-canonical TGF- β signaling pathway are less well understood. Recent work by Carta and coworkers observed the persistent nuclear localization of phosphorylated-Smad2/3 in isolated primary aortic vascular smooth muscle cells from fibrillin-1 deficient or reduced mice (mgN/mgN (null), mgR/mgR (reduced), C1039G/+ (hypomorphic)) [30]. Moreover, they demonstrated *in vitro* and *in vivo* that phosphorylation of Smad2/3 was dependent on p38 mitogen-activated protein kinase (p38MAPK) activity. Treatment of cells or mice with inhibitors of p38MAPK (pharmacologic or siRNA) reduced the phosphorylation of Smad2/3, suggesting that p38MAPK was the upstream activator, and not TGF- β receptor I. Interestingly, two independent groups Sorrentino *et al.* and Yamashita *et al.* have demonstrated that an E3 ubiquitin ligase, called TRAF6, could mediate the TGF- β dependent activation of p38MAPK in a Smad-independent manner [31, 32]. It was demonstrated that TGF- β induced the association of TRAF6 with TGF- β receptor I. This interaction recruited and activated TGF- β -associated kinase-1 (TAK1) which then phosphorylated and activated p38MAPK. These studies suggested that p38MAPK could be activated by TGF- β through a non-canonical signaling pathway independent of interaction with the TGF- β receptors. It is interesting that p38MAPK then retains the ability to subsequently phosphorylate Smad2/3, suggesting that in the absence of TGF- β receptor kinase activity the downstream signaling from Smad2/3 could remain intact. Alternatively, p38MAPK may phosphorylate a unique site on Smad2/3 which could alter its signaling response and result in a different outcome. Thus, the constitutive activation of latent TGF- β resulting from fibrillin-1 mutations may stimulate signaling through classical and non-canonical TGF- β pathways which could alter the typical profibrotic response and result in the enhanced ECM degradation that contributes to aneurysm progression. Furthermore, this response may be independent on TGF- β receptor kinase activity and would likely be modified based on other concurrent signaling inputs.

Other Aortopathies with Abnormalities in TGF- β Signaling

As is evident from the numerous studies highlighted above, TGF- β signaling is likely important in the multiple manifestations of MFS. Because of its widespread role in mediating multiple aspects of vascular remodeling, dysregulation of TGF- β signaling may thus be a common underlying factor in all aortopathies. Accordingly, alterations in normal TGF- β signaling have been implicated in the pathophysiology of several other vascular disorders related to MFS (summarized in Table 2). Importantly, many of the MFS-related diseases are caused by mutations in TGF- β receptor I or II, and are either embryonically lethal or result in enhanced disease manifestations causing aortic dilatation dissection, and rupture at an earlier age [33, 34]. Interestingly, many of these mutations map to the kinase domain of the TGF- β receptors, rendering them kinase deficient. Whether these mutant receptors display gain-of-function properties through non-canonical signaling pathways, or compensate by altering interactions with accessory proteins that regulate normal receptor recycling, resolving the paradox between the excessive TGF- β signaling associated with MFS and the lack of receptor kinase activity/signaling in several MFS-related disorders, will significantly advance our understanding of how TGF- β regulates the structure and composition of the vascular ECM.

Rational Approaches to Treating MFS and Related-Diseases

As early as 1971, it was suggested that MFS patients may benefit from limiting the role of hemodynamic stress and its effects on the proximal aorta during aneurysm development [35]. Accordingly, β -adrenergic blockade was examined as a potential medical therapy due to its well described negative inotropic and chronotropic effects. Several small clinical trials demonstrated the benefit of β -blockade in slowing aortic root dilatation (reviewed in [36]). While this rapidly became the standard of care for MFS patients, a recent meta-analysis concluded that there was insufficient evidence to support the routine use of β -blockers in MFS [37]. This was largely due to the lack of standardized protocols as well as prospective analysis. While one prospective randomized clinical trial did exist, and demonstrated a lower rate of aortic enlargement in the β -blocker group, additional studies are needed to support those results [38].

Because β -blocker usage carried a fair number of difficult to manage side effects for some patients, particularly at higher doses, other treatment options were pursued. One of those strategies was the use of angiotensin converting enzyme (ACE) inhibitors. In a clinical trial comparing ACE inhibitors and β -blockers, it was reported that the ACE inhibitor group had improved aortic distensibility, reduced stiffness, and a smaller root diameter; suggesting that ACE inhibition may provide benefit over the standard of care [39].

ACE inhibitors target two distinct receptors, the type 1 (AT1) and type 2 (AT2), that result in opposite signaling effects following interaction with angiotensin II (AngII). Daugherty *et al.* compared receptor specific inhibitors for their ability to disrupt AngII induced abdominal aortic aneurysm formation [40]. They discovered that treatment with Losartan (an AT1-specific inhibitor) could attenuate aneurysm formation, while treatment with PD123319 (an AT2-specific inhibitor) enhanced aneurysm development. Because Losartan was specific for AT1, AngII was still able to signal through the AT2 receptor. Hence, it was suggested that the AT2-specific outcomes may provide a driving factor for aneurysm development. Moreover, it suggested that AT1 blockade may be preferred over the AT1/AT2 inhibition afforded by ACE inhibitor therapy. Interestingly, it was discovered that AngII signaling through the AT1 receptor enhanced the expression of TGF- β ligands and receptors, as well as stimulated vessel-wall fibrosis [41]. Additionally, in a study by Rodriguez-Vita and coworkers, AngII was shown to activate Smad2 in vascular smooth muscle cells, independent of TGF- β ligands [42]. Furthermore, blocking TGF- β receptors had no effect on Smad activation; however, treatment with Losartan or a p38MAPK inhibitor (SB203580) attenuated Smad2 phosphorylation. These data suggested that AngII could induce Smad2 phosphorylation, in a TGF- β receptor-independent manner, through the activation of p38MAPK via the AT1 receptor pathway. Thus, AT1 antagonism may have the added benefit of interrupting multiple pathways that mediate the vascular remodeling response. As detailed earlier, several investigators from the Dietz group, had treated mice carrying the hypomorphic fibrillin-1 allele (FbnC1039G/+) with either Losartan or propranolol (a β -adrenergic receptor antagonist), and discovered that Losartan was more effective at rescuing several of the severe clinical manifestations of MFS [20–22]. Thus, AT1 antagonism holds much promise for use in patients. Accordingly, in a small proof of principle trial AT1 antagonists (Losartan or Irbesartan) were administered to pediatric patients with MFS who

were then followed during 47 months of therapy [43]. The results clearly demonstrated a significantly slowed rate of aortic root diameter change (3.54 ± 2.87 mm/yr versus 0.46 ± 0.62 mm/yr), and provided the rationale to launch a nationwide prospective randomized trial. Accordingly, the Pediatric Heart Network designed a phase III multi-center randomized clinical trial to compare aortic root growth, and other outcomes, over a 3 year period in MFS patients (ages 6 months to 25 years) receiving Losartan (AT1 inhibitor) or atenolol (β -blocker) [44]. The study began in February 2007 and will target 604 patients with an expected accrual period of 6 years. Thus, this study is a rationale extension of the basic science describing the pathogenesis of MFS, and should make a significant contribution toward understanding the medical management of these patients. Moreover, results from this trial will likely provide the rationale for additional studies aimed at treating patients with other aortopathies that involve dysregulated TGF- β signaling.

Conclusion

Progress in the last 15 years has led to a much greater understanding of the pathogenesis of MFS. Current research has shifted the primary focus of root cause from the consequences of a mutant fibrillin-1 protein, to the excessive signaling through the TGF- β pathway. While much is still to be learned regarding the regulation of TGF- β release, as well as the specific roles of classical versus non-canonical TGF- β signaling, emerging evidence is beginning to define the downstream signaling events that dictate the clinical manifestations of this devastating disorder. In addition, with the current standard of care, β -blocker therapy, MFS patients are living longer and now having to deal with medical issues related to later onset symptoms including joint disease and aneurysm of the descending thoracic aorta. Much hope is being placed on the potential clinical efficacy of angiotensin type-1 receptor antagonism, specifically in relation to its ability to alter TGF- β signaling. Foundational studies in humans and animals suggest the possibility of a significant clinical advancement in the treatment of MFS, which may also be of significant benefit for treating other aortopathies related to TGF- β signaling dysfunction.

References

* Of importance

** Of outstanding importance

1. Dietz HC, Loeys B, Carta L, Ramirez F. Recent progress towards a molecular understanding of Marfan syndrome. *Am J Med Genet C Semin Med Genet.* 2005; 139(1):4–9. [PubMed: 16273535]
2. Judge DP, Dietz HC. Marfan's syndrome. *Lancet.* 2005; 366(9501):1965–1976. [PubMed: 16325700]
3. De Paepe A, Devereux RB, Dietz HC, et al. Revised diagnostic criteria for the Marfan syndrome. *Am J Med Genet.* 1996; 62(4):417–426. [PubMed: 8723076]
4. Ammash NM, Sundt TM, Connolly HM. Marfan syndrome-diagnosis and management. *Curr Probl Cardiol.* 2008; 33(1):7–39. [PubMed: 18155514]
5. Dean JC. Marfan syndrome: clinical diagnosis and management. *Eur J Hum Genet.* 2007; 15(7): 724–733. [PubMed: 17487218]
6. Maumenee IH. The eye in the Marfan syndrome. *Trans Am Ophthalmol Soc.* 1981; 79:684–733. [PubMed: 7043871]

7. Pyeritz RE, Fishman EK, Bernhardt BA, Siegelman SS. Dural ectasia is a common feature of the Marfan syndrome. *Am J Hum Genet.* 1988; 43(5):726–732. [PubMed: 3189335]
8. McKusick VA. The cardiovascular aspects of Marfan's syndrome: a heritable disorder of connective tissue. *Circulation.* 1955; 11(3):321–342. [PubMed: 14352380]
9. Sisk HE, Zahka KG, Pyeritz RE. The Marfan syndrome in early childhood: analysis of 15 patients diagnosed at less than 4 years of age. *Am J Cardiol.* 1983; 52(3):353–358. [PubMed: 6869287]
10. van Karnebeek CD, Naeff MS, Mulder BJ, et al. Natural history of cardiovascular manifestations in Marfan syndrome. *Arch Dis Child.* 2001; 84 (2):129–137. [PubMed: 11159287]
11. Yuan X, Downing AK, Knott V, Handford PA. Solution structure of the transforming growth factor beta-binding protein-like module, a domain associated with matrix fibrils. *EMBO J.* 1997; 16(22):6659–6666. [PubMed: 9362480]
12. Ramirez F, Pereira L. The fibrillins. *Int J Biochem Cell Biol.* 1999; 31(2):255–259. [PubMed: 10216958]
13. Ramirez F, Sakai LY, Dietz HC, Rifkin DB. Fibrillin microfibrils: multipurpose extracellular networks in organismal physiology. *Physiol Genomics.* 2004; 19 (2):151–154. [PubMed: 15466717]
14. Pereira L, Lee SY, Gayraud B, et al. Pathogenetic sequence for aneurysm revealed in mice underexpressing fibrillin-1. *Proc Natl Acad Sci U S A.* 1999; 96 (7):3819–3823. [PubMed: 10097121]
15. Annes JP, Munger JS, Rifkin DB. Making sense of latent TGFbeta activation. *J Cell Sci.* 2003; 116(Pt 2):217–224. [PubMed: 12482908]
16. Chaudhry SS, Cain SA, Morgan A, et al. Fibrillin-1 regulates the bioavailability of TGFbeta1. *J Cell Biol.* 2007; 176(3):355–367. [PubMed: 17242066]
17. Charbonneau NL, Ono RN, Corson GM, et al. Fine tuning of growth factor signals depends on fibrillin microfibril networks. *Birth Defects Res C Embryo Today.* 2004; 72(1):37–50. [PubMed: 15054903]
18. Isogai Z, Ono RN, Ushiro S, et al. Latent transforming growth factor beta-binding protein 1 interacts with fibrillin and is a microfibril-associated protein. *J Biol Chem.* 2003; 278(4):2750–2757. [PubMed: 12429738]
19. Dietz HC, Pyeritz RE. Mutations in the human gene for fibrillin-1 (FBN1) in the Marfan syndrome and related disorders. *Hum Mol Genet.* 1995; 4(Spec No):1799–1809. [PubMed: 8541880]
20. Neptune ER, Frischmeyer PA, Arking DE, et al. Dysregulation of TGF-beta activation contributes to pathogenesis in Marfan syndrome. *Nat Genet.* 2003; 33(3):407–411. [PubMed: 12598898]
21. Ng CM, Cheng A, Myers LA, et al. TGF-beta-dependent pathogenesis of mitral valve prolapse in a mouse model of Marfan syndrome. *J Clin Invest.* 2004; 114(11):1586–1592. [PubMed: 15546004]
- 22*. Habashi JP, Judge DP, Holm TM, et al. Losartan, an AT1 antagonist, prevents aortic aneurysm in a mouse model of Marfan syndrome. *Science.* 2006; 312(5770):117–121. Critical paper describing the use of a TGF- β neutralizing antibody, as well as Losartan and propranolol to reverse the clinical manifestations of MFS in the hypomorphic fibrillin-1 mouse model. [PubMed: 16601194]
23. Ge G, Greenspan DS. BMP1 controls TGFbeta1 activation via cleavage of latent TGFbeta-binding protein. *J Cell Biol.* 2006; 175(1):111–120. [PubMed: 17015622]
- 24*. Tatti O, Vehvilainen P, Lehti K, Keski-Oja J. MT1-MMP releases latent TGF-beta1 from endothelial cell extracellular matrix via proteolytic processing of LTBP-1. *Exp Cell Res.* 2008; 314(13):2501–2514. Study identifying elevated matrix metalloproteinase activity as a mediator of TGF- β release from the large latent complex. [PubMed: 18602101]
25. Feng XH, Derynck R. Specificity and Versatility in TGF-beta signaling through Smads. *Annu Rev Cell Dev Biol.* 2005; 21:659–693. [PubMed: 16212511]
26. Massague J. How cells read TGF-beta signals. *Nat Rev Mol Cell Biol.* 2000; 1 (3):169–178. [PubMed: 11252892]
27. Ruiz-Ortega M, Rodriguez-Vita J, Sanchez-Lopez E, et al. TGF-beta signaling in vascular fibrosis. *Cardiovasc Res.* 2007; 74(2):196–206. [PubMed: 17376414]
28. Shi Y, Massague J. Mechanisms of TGF-beta signaling from cell membrane to the nucleus. *Cell.* 2003; 113(6):685–700. [PubMed: 12809600]

29. Moustakas A, Heldin CH. Non-Smad TGF-beta signals. *J Cell Sci.* 2005; 118(Pt 16):3573–3584. [PubMed: 16105881]
- 30*. Carta L, Smaldone S, Zilberberg L, et al. p38 MAPK is an early determinant of promiscuous Smad2/3 signaling in the aortas of fibrillin-1 (Fbn1)-null mice. *J Biol Chem.* 2009; 284(9):5630–5636. Informative paper describing non-canonical TGF- β signaling in fibrillin-1-null mice. Authors demonstrate p38MAPK can phosphorylate Smad2 independent of TGF- β receptor kinase activity. [PubMed: 19109253]
31. Sorrentino A, Thakur N, Grimsby S, et al. The type I TGF-beta receptor engages TRAF6 to activate TAK1 in a receptor kinase-independent manner. *Nat Cell Biol.* 2008; 10(10):1199–1207. [PubMed: 18758450]
32. Yamashita M, Fatyol K, Jin C, et al. TRAF6 mediates Smad-independent activation of JNK and p38 by TGF-beta. *Mol Cell.* 2008; 31(6):918–924. [PubMed: 18922473]
33. Loeyls BL, Chen J, Neptune ER, et al. A syndrome of altered cardiovascular, craniofacial, neurocognitive and skeletal development caused by mutations in TGFBR1 or TGFBR2. *Nat Genet.* 2005; 37(3):275–281. [PubMed: 15731757]
34. Loeyls BL, Schwarze U, Holm T, et al. Aneurysm syndromes caused by mutations in the TGF-beta receptor. *N Engl J Med.* 2006; 355(8):788–798. [PubMed: 16928994]
35. Halpern BL, Char F, Murdoch JL, et al. A prospectus on the prevention of aortic rupture in the Marfan syndrome with data on survivorship without treatment. *Johns Hopkins Med J.* 1971; 129(3):123–129. [PubMed: 5113220]
36. Judge DP, Dietz HC. Therapy of Marfan syndrome. *Annu Rev Med.* 2008; 59:43–59. [PubMed: 17845137]
37. Gersony DR, McClaughlin MA, Jin Z, Gersony WM. The effect of beta-blocker therapy on clinical outcome in patients with Marfan's syndrome: a meta-analysis. *Int J Cardiol.* 2007; 114(3):303–308. [PubMed: 16831475]
38. Shores J, Berger KR, Murphy EA, Pyeritz RE. Progression of aortic dilatation and the benefit of long-term beta-adrenergic blockade in Marfan's syndrome. *N Engl J Med.* 1994; 330(19):1335–1341. [PubMed: 8152445]
39. Yetman AT, Bornemeier RA, McCrindle BW. Usefulness of enalapril versus propranolol or atenolol for prevention of aortic dilation in patients with the Marfan syndrome. *Am J Cardiol.* 2005; 95(9):1125–1127. [PubMed: 15842990]
- 40*. Daugherty A, Manning MW, Cassis LA. Antagonism of AT2 receptors augments angiotensin II-induced abdominal aortic aneurysms and atherosclerosis. *Br J Pharmacol.* 2001; 134(4):865–870. Authors demonstrate that signaling through the type-1 angiotensin receptor exacerbates aneurysm development in the abdomen. Also suggests that signaling through the the type-2 angiotensin receptor may have protective effects against aneurysm development. [PubMed: 11606327]
41. Kubo A, Fukuda N, Teng J, et al. Angiotensin II regulates the cell cycle of vascular smooth muscle cells from SHR. *Am J Hypertens.* 2000; 13(10):1117–1124. [PubMed: 11041167]
42. Rodriguez-Vita J, Sanchez-Lopez E, Esteban V, et al. Angiotensin II activates the Smad pathway in vascular smooth muscle cells by a transforming growth factor-beta-independent mechanism. *Circulation.* 2005; 111(19):2509–2517. [PubMed: 15883213]
- 43*. Brooke BS, Habashi JP, Judge DP, et al. Angiotensin II blockade and aortic-root dilation in Marfan's syndrome. *N Engl J Med.* 2008; 358(26):2787–2795. Initial clinical study demonstrating attenuation of aortic root dilation in MFS patients treated with Losartan. [PubMed: 18579813]
- 44*. Lacro RV, Dietz HC, Wruck LM, et al. Rationale and design of a randomized clinical trial of beta-blocker therapy (atenolol) versus angiotensin II receptor blocker therapy (losartan) in individuals with Marfan syndrome. *Am Heart J.* 2007; 154(4):624–631. Description of the multicenter randomized clinical trial comparing Losartan and atenolol treatment in Marfan patients. [PubMed: 17892982]
45. Ignatz RA, Massague J. Transforming growth factor-beta stimulates the expression of fibronectin and collagen and their incorporation into the extracellular matrix. *J Biol Chem.* 1986; 261(9):4337–4345. [PubMed: 3456347]

46. Moussad EE, Brigstock DR. Connective tissue growth factor: what's in a name? *Mol Genet Metab*. 2000; 71(1–2):276–292. [PubMed: 11001822]
47. Kucich U, Rosenbloom JC, Abrams WR, Rosenbloom J. Transforming growth factor-beta stabilizes elastin mRNA by a pathway requiring active Smads, protein kinase C-delta, and p38. *Am J Respir Cell Mol Biol*. 2002; 26(2):183–188. [PubMed: 11804868]
48. Keski-Oja J, Raghov R, Sawdey M, et al. Regulation of mRNAs for type-1 plasminogen activator inhibitor, fibronectin, and type I procollagen by transforming growth factor-beta. Divergent responses in lung fibroblasts and carcinoma cells. *J Biol Chem*. 1988; 263(7):3111–3115. [PubMed: 3125175]
49. Kwak HJ, Park MJ, Cho H, et al. Transforming growth factor-beta1 induces tissue inhibitor of metalloproteinase-1 expression via activation of extracellular signal-regulated kinase and Sp1 in human fibrosarcoma cells. *Mol Cancer Res*. 2006; 4(3):209–220. [PubMed: 16547158]
50. Yan C, Boyd DD. Regulation of matrix metalloproteinase gene expression. *J Cell Physiol*. 2007; 211(1):19–26. [PubMed: 17167774]
51. Laiho M, Saksela O, Keski-Oja J. Transforming growth factor beta alters plasminogen activator activity in human skin fibroblasts. *Exp Cell Res*. 1986; 164(2):399–407. [PubMed: 3519251]
52. Kim ES, Kim MS, Moon A. TGF-beta-induced upregulation of MMP-2 and MMP-9 depends on p38 MAPK, but not ERK signaling in MCF10A human breast epithelial cells. *Int J Oncol*. 2004; 25(5):1375–1382. [PubMed: 15492828]
53. Selvamurugan N, Kwok S, Alliston T, et al. Transforming growth factor-beta 1 regulation of collagenase-3 expression in osteoblastic cells by cross-talk between the Smad and MAPK signaling pathways and their components, Smad2 and Runx2. *J Biol Chem*. 2004; 279(18):19327–19334. [PubMed: 14982932]
54. Gordon GM, Ledee DR, Feuer WJ, Fini ME. Cytokines and signaling pathways regulating matrix metalloproteinase-9 (MMP-9) expression in corneal epithelial cells. *J Cell Physiol*. 2009; 221(2):402–411. [PubMed: 19626678]
55. Konrad L, Scheiber JA, Schwarz L, et al. TGF-beta1 and TGF-beta2 strongly enhance the secretion of plasminogen activator inhibitor-1 and matrix metalloproteinase-9 of the human prostate cancer cell line PC-3. *Regul Pept*. 2009; 155(1–3):28–32. [PubMed: 19422858]
56. Safina A, Vandette E, Bakin AV. ALK5 promotes tumor angiogenesis by upregulating matrix metalloproteinase-9 in tumor cells. *Oncogene*. 2007; 26 (17):2407–2422. [PubMed: 17072348]
57. Blaney Davidson EN, Remst DF, Vitters EL, et al. Increase in ALK1/ALK5 ratio as a cause for elevated MMP-13 expression in osteoarthritis in humans and mice. *J Immunol*. 2009; 182(12):7937–7945. [PubMed: 19494318]
58. Cottin V, Dupuis-Girod S, Lesca G, Cordier JF. Pulmonary vascular manifestations of hereditary hemorrhagic telangiectasia (rendu–osler disease). *Respiration*. 2007; 74(4):361–378. [PubMed: 17641482]
59. Mizuguchi T, Matsumoto N. Recent progress in genetics of Marfan syndrome and Marfan-associated disorders. *J Hum Genet*. 2007; 52(1):1–12. [PubMed: 17061023]
60. Pannu H, Tran-Fadulu V, Milewicz DM. Genetic basis of thoracic aortic aneurysms and aortic dissections. *Am J Med Genet C Semin Med Genet*. 2005; 139(1):10–16. [PubMed: 16273536]

Figure 1. TGF-β signaling pathway

TGF-β can signal through both the *classical pathway* and the *non-canonical pathway*. Signaling through the *classical pathway* is initiated upon binding of ligand to the type II TGF-β receptor (TGF-βRII). The type II receptor is autophosphorylated, and recruits and transphosphorylates a type I receptor (TGF-βRI). The activated type I receptor in turn phosphorylates and activates a receptor-Smad (R-Smad). The R-Smad then binds the common co-Smad and translocates to the nucleus. Once in the nucleus, it binds transcriptional co-factors and forms an activated transcriptional complex capable of inducing transcription of profibrotic genes. In the *non-canonical pathway*, TRAF6 associates with the type-I TGF-β receptor (TGF-βRI). It recruits and activates TGF-β associated kinase-1 (TAK1), which in turn activates p38MAPK. The p38MAPK is able to activate an R-Smad independent of TGF-β receptor kinase activity. Alternatively, p38MAK can initiate a signaling cascade resulting in altered transcription through other mediators that lead to enhanced matrix degradation.

Table 1

TGF- β effects on matrix deposition and matrix degradation.

Effects on Matrix Deposition:	References:
Enhanced collagen expression, synthesis, and deposition (<i>COL1A1</i> , <i>COL1A2</i> , <i>COL3A1</i>)	[45]
Increased connective tissue growth factor expression (<i>CTGF</i>)	[46]
Tropoelastin mRNA half-life is stabilized, increasing elastin expression (<i>ELN</i>)	[47]
Increased PAI-1 expression; inhibits activation of uPA and tPA, activators of MMPs (<i>PLAU</i> and <i>PLAT</i> , respectively)	[48]
Induction of TIMP-1, -3 expression, direct inhibitors of MMP activity (<i>TIMP-1</i> , <i>TIMP-3</i>)	[49]
Direct repression of MMP expression mediated by TGF- β Inhibitory Elements (TIE) within the MMP promoter (<i>MMP -1</i> , <i>-7</i> , <i>-13</i>); <i>MMP-3</i> repression by c-fos complexes	[50]

Effects on Matrix degradation:	References:
Elevated plasminogen activator protein, uPA./tPA	[51]
Induced MMP-2 and MMP-9 protein levels, required activation of p38MAPK	[52]
Induced MMP-13 protein levels, dependent on p38MAPK, Smad2, and ERK1/2 activation	[53]
Induced MMP-9 protein levels, dependent on NF- κ B, Smad3, and JNK signaling	[54]
TGF- β 1 and TGF- β 2 induced MMP-9	[55]
Induced MMP-9 protein levels via ALK-5 signaling	[56]
Induced MMP-13 protein levels, dependent on increased ALK-1 signaling and decreased ALK-5 signaling	[57]

Abbreviations: MMP – matrix metalloproteinase; TIMP – tissue inhibitor of metalloproteinase; ALK-1 – activin receptor-like kinase-1; ALK-5 – TGF- β receptor I; JNK – Jun N-terminal kinase

Table 2Aortopathies affected by dysregulated TGF- β signaling.

Disorder (genetic loci):	Manifestations:	References:
<i>Hereditary Hemorrhagic Telangiectasia</i> <ul style="list-style-type: none"> HHT1, OMIM #187300 HHT2, OMIM #600376 	<ul style="list-style-type: none"> HHT1: mutation in TGF-β receptor III, accessory receptor that binds and presents TGF-β to the type-I and type-II TGF-β receptors; results in vascular dysplasia, arteriovenous malformations HHT2: mutation in ALK-1, a type-I TGF-β receptor that binds both TGF-β and activin; results in dilated yolk-sac vasculature and embryonic death 	[58]
<i>Marfan Syndrome 2</i> <ul style="list-style-type: none"> MFS2, OMIM #154705 	<ul style="list-style-type: none"> MFS2: clinical symptoms similar to MFS, but with mild ocular involvement and no <i>FBN1</i> gene mutation; has defect in type-II TGF-β receptor 	[59]
<i>Familial Thoracic Aortic Aneurysm and Dissection</i> <ul style="list-style-type: none"> AAT1, OMIM #607086 AAT2, OMIM #607087 AAT3, OMIM #608967 AAT4, OMIM #132900 AAT5, OMIM #610380 ACTA2, OMIM #102620 	<ul style="list-style-type: none"> TAAD: familial nonsyndromic predisposition to thoracic aortic aneurysms and dissections; linked to six genetic loci AAT3: mutation in type-II TGF-β receptor AAT5: mutation in type-I TGF-β receptor 	[60]
<i>Loeys-Dietz Syndrome</i> <ul style="list-style-type: none"> LDS, OMIM #609192 	<ul style="list-style-type: none"> LDS: clinical symptoms similar to MFS, but also include bifid uvula, cleft palate, hypertelorism, and craniosynostosis; also often associated with arterial tortuosity and early and severe onset; gene are mutations found in both type-I and type-II TGF-β receptors 	[33]