8 Connective Tissue Disorders and Cardiovascular Complications: The Indomitable Role of Transforming Growth Factor-Beta Signaling

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Abstract

Marfan Syndrome (MFS) and Loeys-Dietz Syndrome (LDS) represent heritable connective tissue disorders that cosegregate with a similar pattern of cardiovascular defects (thoracic aortic aneurysm, mitral valve prolapse/regurgitation, and aortic root dilatation with regurgitation). This pattern of cardiovascular defects appears to be expressed along a spectrum of severity in many heritable connective tissue disorders and raises suspicion of a relationship between the normal development of connective tissues and the cardiovascular system. Given the evidence of increased transforming growth factor-beta (TGF-β) signaling in MFS and LDS, this signaling pathway may represent the common link in this relationship. To further explore this hypothetical link, this chapter will review the TGF-β signaling pathway, heritable connective tissue syndromes related to TGF-β receptor (TGFBR) mutations, and discuss the pathogenic contribution of TGF-β to these syndromes with a primary focus on the cardiovascular system.

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Keywords

Shprintzen-Goldberg syndrome, hereditary hemorrhagic telangiectasia (HHT) • Marfan syndrome (MFS) • Loeys-Dietz syndrome (LDS) • Primary pulmonary hypertension • Fibrodysplasia ossificans progressiva (FOP) • Familial thoracic aortic aneurysm and dissection syndrome (FTAAD) • Smad • TGF-β receptor • Curacao diagnostic criteria

Abbreviations

AAT	Aortic Aneurysm Thoracic
ACE	Angiotensin Converting Enzyme
ALK-1	Activin Receptor-like Kinase 1
ALK-3	Activin Receptor-like Kinase 3
ALK-5	Activin Receptor-like Kinase 5
AOS	Aneurysm-Osteoarthritis Syndrome
ATI	Angiotensin II Receptor Type I
ATII	Angiotensin II Receptor Type II
ATS	Arterial Tortuosity Syndrome
AVM	Arteriovenous Malformation
BAV	Bicuspid Aortic Valve
BMP	Bone Morphogenetic Protein
BMPR1A	Bone Morphogenetic Protein Receptor 1A
BMPR2	Bone Morphogenetic Protein Receptor 2
Co-Smad	Common Smad
CTGF	Connective Tissue Growth Factor
ECM	Extracellular Matrix
ERK1/2	Extracellular Signal-Regulated Kinase 1/2
EVAR	Endovascular Aortic Repair
FBN1	Fibrillin-1
FOP	Fibrodysplasia Ossificans Progressiva
FTAAD	Familial Thoracic Aortic Aneurysm and Dissection Syndrome
GI	Gastrointestinal
HHT1	Hereditary Hemorrhagic Telangiectasia, Type 1
HHT2	Hereditary Hemorrhagic Telangiectasia, Type 2
JNK	c-Jun N-terminal Kinase
LAP	Latent Associated Protein
LDS	Loeys-Dietz Syndrome
LLC	Large Latent Complex
LTBP	Latent Transforming Growth Factor-Beta Binding Protein
MAPK	Mitogen-Activated Protein Kinase
MFS	Marfan Syndrome
MMPs	Matrix Metalloproteinases
MVP	Mitral Valve Prolapse
OMIM	Online Mendelian Inheritance in Man
PAH	Pulmonary Artery Hypertension
PDA	Patent Ductus Arteriosus
PI3K	Phosphoinositide 3-Kinase
RA	Rheumatoid Arthritis
R-Smad	Receptor Smad
SARA	Smad Anchor for Receptor Activation
SGS	Shprintzen-Goldberg Syndrome
SLC2A10	Solute Carrier Family 2, Facilitated Glucose Transporter Member 10
SLE	Systemic Lupus Erythematosus
Smurf	Smad Ubiquitination Regulatory Factor
TAA	Thoracic Aortic Aneurysm
TAK1	Transforming Growth Factor-Beta Associated Kinase 1
TFs	Transcription Factors
TGF-β	Transforming Growth Factor-Beta
TGFBRI	Transforming Growth Factor-Beta Receptor, Type-I
TGFBRII	Transforming Growth Factor-Beta Receptor, Type-II
TIMPs	Tissue Inhibitors of Matrix Metalloproteinases
TRAF6	Tumor Necrosis Factor Receptor Associated Factor 6

8.1 Introduction

Marfan syndrome (MFS) is a well described connective tissue disorder characterized by musculoskeletal, ocular, and cardiovascular defects including: ascending aortic aneurysm with dissection, mitral valve prolapse (MVP)/regurgitation,

and aortic root dilatation with regurgitation [1] and it is discussed to considerable detail in Chap. 6 by Cook and Ramirez. A mutation in fibrillin-1 (FBN1), a protein component of microfibrils, accounts for more than 90 % of MFS [2]. Fibrillin-1 was demonstrated through multiple studies to interact with and sequester latent transforming growth factor-beta (TGF-β) within the extracellular matrix (ECM) [3–6]. In 2003, Neptune et al. hypothesized that the loss of microfibrils may have an effect on the sequestration of TGF-β within the ECM and demonstrated that TGF-β signaling was markedly activated within lung tissue of a mouse MFS model [7]. Furthermore, the emphysematous lung phenotype of the MFS mice was restored to wild type with anti-TGF-β antibody, strongly suggesting that TGF-β signaling dysregulation contributed to the pathogenesis of MFS [7].

Subsequently in 2005, Loeys and Dietz described a cohort of patients with a connective tissue disorder that significantly overlapped with the phenotype of MFS [8] (see also Chap. 7). Both disorders exhibit a marfanoid habitus (pectus deformity, arachnodactyly-elongated fingers, scoliosis, and dolichostenomelia-elongated limbs), valvular prolapse/regurgitation, and an arterial aneurysm with dissection phenotype [8]. Additionally, Loeys and Dietz identified mutations within type-I (TGFBRI) or II (TGFBRII) TGF-β receptors in these patients [8]. Interestingly, despite mutated receptors incapable of propagating signal, patients with Loeys-Dietz syndrome (LDS) paradoxically exhibited indications of increased TGF-β signaling: increased expression of collagen and connective tissue growth factor (CTGF), much like MFS patients [8].

Taken together, MFS and LDS represent connective tissue disorders that cosegregate with a similar pattern of cardiovascular defects. This pattern of cardiovascular defects appears to be expressed along a spectrum of severity in many heritable connective tissue disorders and raises suspicion of a relationship between the normal development of connective tissues and the cardiovascular system. Given the evidence of increased TGF-β signaling in MFS and LDS, this signaling pathway may represent the common link in this relationship. To further explore this hypothetical link, this chapter will review the TGF-β signaling pathway, heritable connective tissue syndromes related to TGF-β signaling-particularly TGFBR mutations, and discuss the pathogenic contribution of TGF-β to these syndromes with a primary focus on the cardiovascular system.

8.2 TGF-β, Signaling Pathways, and Physiological Effects

Transforming growth factor-β is a soluble cytokine secreted by cells in the form of a large latent complex (LLC) composed of a homodimer of mature TGF-β peptide, a homodimer of TGF-β's inactive cleaved peptide fragment (latent associated protein, LAP), and latent transforming growth factor binding protein (LTBP) [9]. Motifs within fibrillin-1 interact with LTBP and target the LLC to the ECM [6]. Thus, the ECM serves to sequester and concentrate TGF-β in locations where it may be rapidly activated when needed [10]. Indeed, the ECM is no longer thought to be a passive structural support but rather a dynamic regulator of growth factor bioavailability and signaling [11, 12].

8.2.1 "Canonical" TGF-β Signaling Pathway

Mature TGF-β (types 1–3) is activated by release from the LAP through multiple mechanisms including: direct proteolysis, by non-proteolytic dissociation mediated by thrombospondin-1 or integrin $\alpha_v\beta_6$, as well as exposure to reactive oxygen species, or low pH [13]. Once activated, TGF-β is free to bind a TGF-β receptor in the first step of the signaling cascade [9]. Transforming growth factor-β receptors have been subdivided into three types. Type-I (also known as Activin receptor-like kinase 5/ALK-5 or TGFBRI) and type-II are the primary receptors of the classical - or "canonical"-pathway and both possess serine/threonine kinase activity. Type-III (also known as betaglycan) is an accessory receptor that binds TGF-β and presents it to the type-I and II receptor complex [9]. Other receptors that bind and signal in response to TGF-β include endoglin

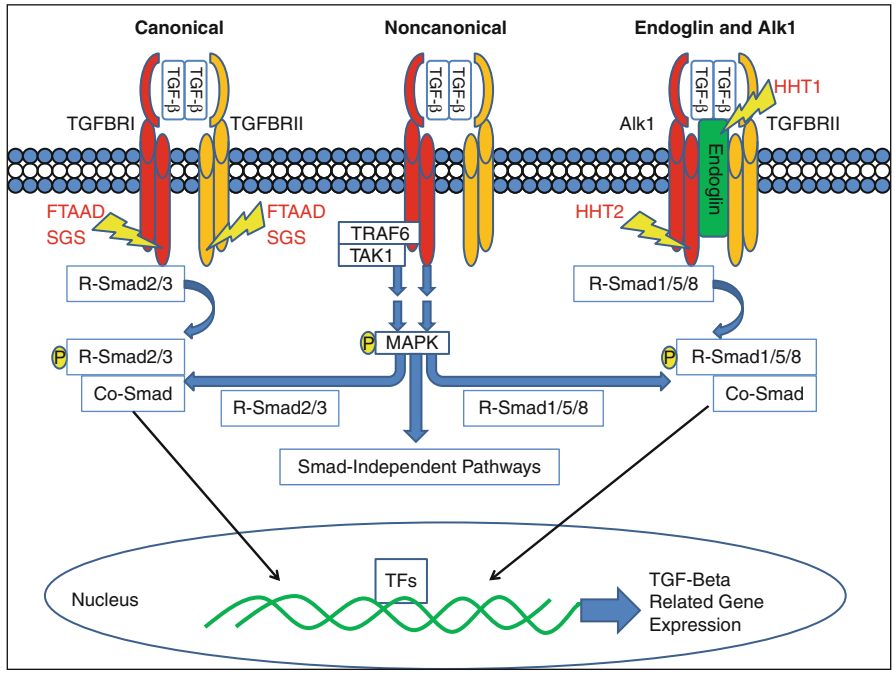

Fig. 8.1 Canonical, Noncanonical, and Endoglin/ALK-1 signaling pathways. Both Canonical and Endoglin/ALK-1 TGF-Beta signaling is mediated by the phosphorylation of distinct receptor Smad proteins. Nuclear translocation requires Co-Smad binding in both pathways. Inside the nucleus, R- and Co-Smads form a complex with transcription factors (TFs) to either repress or activate TGF-Beta related gene expression. The non-canonical pathway is mediated by TGFBRI, TRAF6, and TAK1 and results in the phosphorylation of MAPKs such as Erk1/2, JNK, and p38. These MAPKs can re-enter the Smad-dependent pathway through phosphorylation or mediate downstream signaling through other Smad-independent pathways. The lightning bolts represent mutations to the indicated proteins causative of the syndromes listed in *red*

(type-III receptor) and the Activin receptor-like kinase 1(ALK-1), a type-I receptor family member.

After release from the ECM, mature TGF-β first binds a homodimer of the type-II receptor inducing an autophosphorylation event. This, in turn, recruits a homodimer of the type-I receptor forming the complete ligand-receptor complex. The type-II receptor then activates the type-I receptor via transphosphorylation [14]. The kinase domain of the activated type-I receptor propagates the intracellular signal through the phosphorylation of specific receptor-regulated Smad proteins (R-Smads; Smad 1, 2, 3, 5, and 8), which are the second messengers of the canonical TGF-β signaling pathway. For example, activation of the type-I receptor TGFBRI, results in the phosphorylation of Smad 2 or 3; while activation of the type-I receptor ALK-1 results in the phosphorylation of Smad 1, 5 or 8. The choice of Smad is likely tissue-specific and context-dependent. The phosphorylated R-Smad then interacts with a common Smad or "co-Smad" (Smad4), which induces translocation of the complex to the nucleus. The nuclear Smad complex along with multiple co-regulatory factors form a transcription regulating complex capable of activating or repressing TGF-β associated genes [15, 16] (Fig. 8.1).

Activation of the TGF-β system stimulates a number of diverse cellular processes, such as cell growth, proliferation and apoptosis and therefore requires strict regulation at multiple levels. An example of this regulation, is the negative feedback of inhibitory Smads (I-Smads; Smad6 and 7) induced by TGF-β stimulation [17]. Smad6 exerts its effects by binding directly to type-I receptors and blunting R-Smad phosphorylation [18].

Smad6 also inhibits signaling by competing with Smad4 for receptor Smad binding sites, reducing nuclear translocation [19]. Smad7 inhibits TGF-β signaling by targeting TGFBRI and II for ubiquitination and subsequent degradation, through the recruitment of Smurfs 1 and 2 (Smad ubiquitination regulatory factor 1 and 2) [20–22]. Additionally, many regulatory proteins influence the bioavailability of TGF- β, such as the structurally related scavenging proteoglycans decorin and biglycan, which bind and reduce its availability for signaling [23–25].

8.2.2 Alternate "Noncanonical" TGF-β Signaling Pathways

Recent studies have expanded upon the hypothesis that TGF-β signaling can occur independently of Smad mediators, through alternative pathways. Several alternative signaling pathways exist: (1) type-I receptors signaling in the absence of Smads [26–30]; (2) type-II receptors signaling without type-I receptors [31, 32]; (3) R-Smad signaling to parallel pathways [33–35]; and (4) activation of R-Smads independent of TGFBRs [36, 37]. However, downstream intracellular mediators of these alternative pathways are not as well understood as the Smad proteins. Studies of noncanonical signaling in FBN1 deficient mice have proved helpful in this regard. Carta et al. demonstrated in vivo that p38 mitogen-activated protein kinase (p38 MAPK) mediated phosphorylation of Smad2/3, which was attenuated with p38 MAPK inhibitors suggesting independence from TGFBRI [38]. Further studies performed by independent groups elucidated the TGF-β dependent activation of p38 MAPK independently of Smad proteins. Tumor necrosis factor receptor-associated factor 6 (TRAF6), an E3 ubiquitin ligase, was demonstrated to associate with TGFBRI in a TGF-β dependent manner [39, 40]. This newly formed complex recruits and activates TGF-β associated kinase 1 (TAK1), which activates p38 MAPK via phosphorylation [39, 40] (Fig. 8.1). In addition to p38 MAPK, TGF-β can activate many other signal pathways not directly involving Smads: extracellular-signal regulated kinase 1 and 2 (ERK1/2) [41], c-Jun N-terminal kinase (JNK) [39, 40], and phosphoinositide 3-kinase-Akt (PI3K-Akt) [42]. Importantly, the canonical and noncanonical pathways appear to exert differential effects on the connective tissues within the ECM.

Transforming growth factor-β signaling is known to contribute to a number of disparate and opposing physiologic processes including angiogenesis, proliferation, differentiation, apoptosis, and wound healing, and is an established modulator of ECM structure and composition [43, 44]. Within the vascular ECM, TGF-β demonstrates opposing effects by its involvement in both matrix deposition and degradation. Recent evidence suggests that stimulation of a particular pathway determines whether deposition or degradation will predominate. Stimulation via the canonical pathway induces profibrotic effects including increased ECM protein deposition (collagen and elastin) [45], decreased expression of proteolytic enzymes (matrix metalloproteinases, MMPs) [46], and increased proteolytic inhibition (tissue inhibitors of MMPs, TIMPs) [47]. Alternatively, stimulation via the noncanonical pathway degrades matrix proteins through increased proteolysis via MMPs (2, 9, and 13) [48] and increased MMP activation via plasminogen activators [49]. Thus, matrix degradation appears to occur through Smad-independent pathways, though Smad activation may also be involved [50]. Noncanonical p38 MAPK mediated signaling has been associated with MMP-2 and -9 production and release in breast cancer cells [48]. In vitro expression of MMP-13 by rat osteoblasts was dependent on p38 MAPK, Smad2 (classical pathway), and extracellular signal-regulated kinase (ERK) 1/2 signaling [51]. This dual regulation underscores the importance of TGF-β as a key regulatory factor in maintaining homeostatic balance within the structure and composition of the ECM and implicates the dysregulation of the noncanonical signaling pathways in disorders associated with elevated TGF-β signaling and ECM degradation.

It must be recognized that the TGF-β family of ligands is part of a larger "superfamily" of growth factors and receptors, which includes bone morphogenetic proteins (BMPs), activins, and inhibins [16]. These superfamily members

also occupy roles in normal connective tissue development and repair. Like the TGF-β family, dysregulation of BMP signaling has been implicated in heritable connective tissue disorders. Mutations within activin receptor-like kinase 2, a type-I BMP receptor, are associated with fibrodysplasia ossificans progressive (FOP), a skeletal dysplasia characterized by progressive heterotopic bone formation [52]. Activin and inhibin signaling dysregulation has not been linked to heritable connective tissue disorders. However, activin signaling has been implicated in the regulation of wound healing and scar formation, processes dependent upon normal connective tissue remodeling [53]. While the TGF-β family is an established modulator of ECM remodeling, it may also occupy a similar role in vascular development. Evidence for this may be seen in the range of vascular abnormalities characteristic of TGFBR-mutation-related connective tissue disorders.

8.3 TGF-β Receptor Related Connective Tissue Disorders

To date several heritable connective tissue disorders have been associated with mutations in TGFBRs (and therefore disturbances in TGF-β signaling) including LDS, familial thoracic aortic aneurysm and dissection syndrome (FTAAD), Shprintzen-Goldberg syndrome (SGS), and hereditary hemorrhagic telangiectasia (HHT) (Table 8.1). Interestingly, each of these disorders also display unique cardiovascular manifestations, resulting in a spectrum of disorders ranging from heart valve defects to thoracic aortic aneurysms, characteristic of MFS and LDS, the prototypical disorders of aberrant enhanced TGF-β signaling. These disorders have been placed in context below.

8.3.1 Familial Thoracic Aortic Aneurysm and Dissection Syndrome (FTAAD)

Marfan syndrome, Loeys-Dietz syndrome, and Ehlers-Danlos (type-IV) syndrome are the primary inherited connective tissue disorders associated with thoracic aortic aneurysms (TAA). However, many patients presenting with a history of familial TAAs and dissections cannot be classified into any of these syndromes. These TAAs and dissections have a heterogeneous etiology, and at least 7 associated gene mutations have been identified in the Online Mendelian Inheritance in Man (OMIM) database: Aortic Aneurysm Thoracic (AAT) 1–7. Two of these mutations, AAT5 (OMIM #610380) and AAT3 (OMIM #608967) are located within the genes for TGFBRI and TGFBRII, respectively [54]. As with most aneurysm patients, the initial presentation event is often incidentally discovered as aortic dilatation, dissection or sudden death [55–57]. Subsequently, an aortopathy syndrome is suspected when a family history of early aortic disease or sudden death is revealed or the constellation of unique connective tissue symptoms (marfanoid habitus like MFS and LDS and/or the FTAAD specific iris flocculi) provokes suspicion. While these seemingly non-syndromic TAAs and dissections may be the result of contributing risk factors (e.g., hypertension, atherosclerosis), it has been reported that almost 20 % of these patients have a first degree relative with a similar presentation, suggesting a genetic predisposition [58]. Diagnosing this group of patients is complicated due to the autosomal dominant inheritance with variable penetrance and expression [59]. Accordingly, these cases have been grouped as familial thoracic aortic aneurysm and dissection syndrome (FTAAD) and encompass all familial cases not captured by defined syndromes.

Several vascular and cardiac pathologies have been associated with FTAAD, signs and symptoms of which may be detected during a physical exam. The primary vascular disturbance in FTAAD involves aneurysmal dilatation (>1.5× the normal diameter) and dissection (an intimal tear that initiates progressive medial separation) of the thoracic aorta, most commonly in the ascending aorta [60]. Symptoms of aortic rupture and dissection include tearing chest pain, hypotension, differential pulse pressures, and rapid clinical decompensation [61]. Like LDS, FTAAD patients can also present with abdominal aortic aneurysm or cerebral aneurysms [54]. Arterial tortuosity (twisted, corkscrew arteries), which is

Table 8.1 TGF-β related heritable connective tissue disorders

Connective tissue syndrome	Associated mutations	Connective tissue manifestations	Cardiovascular manifestations	References
Shprintzen-Goldberg syndrome (SGS)	Reported FBN1, TGFBR I & II OMIM#182212 (FBN1)	Marfanoid habitus: dolichostenomelia, reduced upper:lower body ratio, scoliosis, pectus excavatum or carinatum; club foot; flat foot; hernias; scaphocephaly; craniosynostosis; digital contractures; Chiari-I; osteopenia	Mitral valve prolapse and regurgitation; aortic valve insufficiency; occasional aortic root dilatation	Greally et al. 1998 [75], Van Steensel et al. 2008 [146]
Loeys-Dietz syndrome (LDS)	TGFBRI & II OMIM#609192	Bifid uvula; cleft palate; clubfoot; hypertelorism; thin/velvety skin; blue sclera; cervical anomaly/instability; craniosynostosis; scoliosis; dural ectasia; protrusion acetabuli; lax joints	Ascending aortic aneurysm and dissection; diffuse arterial tortuosity and aneurysms; easy bruising; mitral valve prolapse and regurgitation	Loeys et al. 2005, 2006 [8, 59]
Familial thoracic aortic aneurysm and dissection syndrome (FTAAD)	AAT1-7 AAT5 TGFBRI OMIM#610380 AAT3 TGFBRII OMIM#608967	Marfanoid Habitus: dolichostenomelia, reduced upper:lower body ratio, scoliosis, pectus excavatum or carinatum; livedo reticularis; iris flocculi	Ascending and aortic root aneurysm and dissection; mitral valve prolapse and regurgitation	Gleason, TG. 2005 [155], Pannu et al. 2005 [156]
Marfan syndrome (MFS)	FBN1 OMIM#154700	Marfanoid habitus: dolichostenomelia, reduced upper:lower body ratio, scoliosis, pectus excavatum or carinatum; protrusio acetabuli; ectopia lentis; high arched palate; dural ectasia; lax joints	Ascending aortic aneurysm involving sinuses of Valsalva and dissection; aortic root dilatation with possible valve insufficiency; mitral valve prolapse and regurgitation	Judge et al. 2005 [1], Dietz et al. 1993 [2]
Aneurysm-osteoarthritis syndrome (AOS)	Smad3	Early onset osteoarthritis; osteochondritis dissecans; mild hypertelorism; abnormal uvula	Aortic aneurysms and dissection; tortuosity of large and medium sized vessels, even intracranial	Van de Laar et al. 2011, 2012 [71, 158]
Arterial tortuosity syndrome (ATS)	SLC2A10 OMIM#208050	High palate; skin and joint laxity; hernias; keratoconus; facies; micrognathia; contractures; arachnodactyly	Large and medium vessel tortuosity; diffuse aneurysms; aortic regurgitation; telangiectases; pulmonary artery stenoses and aneurysms	Callewaert et al. 2008 [157], Coucke et al. 2006 [73]
Hereditary hemorrhagic telangiectasia (HHT)	HHT1 TGFBR Type-III (Endoglin; ENG) OMIM#187300 HHT2 TGFBR Type-I (Activin receptor-like kinase-1/ALK-1) OMIM#600376	Specialized connective tissues: blood-thrombophilia; lymphatic tissue-immunodeficiency	Diffuse GI and mucocutaneous telangiectasais; arteriovenous malformations in lungs, brain and liver; nosebleeds; easy bleeding and bruising; iron deficiency anemia; pulmonary artery hypertension	Govani et al. 2009 [97], Fernandez et al. 2006 [154]

also a key feature of LDS, may also be seen in medium to large arteries (e.g. carotid arteries, aorta, etc.) [54]. Interestingly, some FTAAD patients exhibit Moyamoya disease, characterized by occlusive intimal thickening of the primary cerebral vessels progressing to transient ischemic events and stroke [62, 63].

Cardiac manifestations of FTAAD include patent ductus arteriosus (PDA), aneurysm of the aortic root, and bicuspid aortic valve (BAV) [64]. Bicuspid aortic valves occur in 1–2 % of the population and are the most common cardiac malformation. With age, BAVs calcify prematurely and may result in aortic stenosis or regurgitation, both of which produce distinctive murmurs and progressive symptoms of heart failure [64]. A PDA produces a continuous murmur and with time increased pulmonary blood flow will induce pulmonary hypertension [65].

8.3.1.1 Paradoxical Signaling by Mutated TGFBRs in FTAAD

Thoracic aortic aneurysms are characterized by progressive ECM degradation, elastin fragmentation, smooth muscle apoptosis and dilatation with or without dissection, likely due to an imbalance between matrix production and proteolysis [66]. The balance between ECM deposition and degradation is tightly regulated, and the mechanism of the loss of balance in TAAs is not clearly understood. For example, recent evidence uncovered paradoxical effects in the TGF-β signaling pathway, specifically identifying mutations in TGFBRs that resulted in elevated TGF-β signaling [8, 67]. Several mechanisms, however, have been proposed.

First, that heterozygous mutation of TGFBRs may enhance TGF-β signaling in functional TGFBR complexes by facilitating ligand interaction much like the role of type-III TGFBRs (endoglin and betaglycan). This was hypothesized after authors of a study using transgenic mice with a fibroblast-specific heterozygous TGFBRII mutation noticed increased pulmonary and dermal collagen deposition rather than a dominant negative phenotype which was expected to reduce deposition [67, 68]. Second, alternate pathways of receptor recycling could also explain the enhanced signaling seen. Internalization of TGFBRs is mediated by either clathrin- or caveolin- dependent endocytosis [69]. A plasma membrane protein, Smad anchor for receptor activation (SARA) binds TGFBRII and mediates interactions with Smad2 and the clathrin endocytosis pathway leading to receptor recycling [69]. A mutation that enhanced interaction of TGFBRII with SARA could increase signaling and favor recycling over degradation. Alternatively, the Smurfs (Smad ubiquitination regulatory factors) mediate TGF-β receptor interactions with the caveolin pathway [69, 70]. The interaction of the TGFBR complex with Smad7 recruits Smurf1 and 2, activating the caveolin pathway and leading to proteasomal degradation of the receptors. In this case, a TGFBR mutation that decreased interaction with either Smad7 or the Smurf proteins could decrease receptor degradation and prolong signaling. Finally, mutated TGFBRs may enhance signaling because their ability to form functional signaling platforms is unaffected by mutation. These signaling platforms are sites where multiple signaling intermediates aggregate. This was demonstrated when Smad3 was found to be phosphorylated by PI3K at residues not within TGFBRI's target site after TGF-β stimulation and increased collagen expression [36]. This suggests that those mutated TGFBRs with intact TGF-β binding but deficient kinase domains may still induce TGF-β dependent signaling through alternate pathways. Thus, enhanced TGF-β signaling may drive vascular ECM destruction through non-Smad signaling pathways alone, as a result of imbalanced homeostasis having direct implications for the formation and progression of thoracic aortic aneurysms and dissections.

Lending support to the pivotal role of TGF-β in connective tissues and the cardiovascular system, heritable mutations in downstream and upstream mediators of TGF-β signaling also display symptoms overlapping with TGFBR mutation syndromes. A mutation in Smad3 was recently linked to a heritable syndrome of vascular aneurysms, arterial tortuosity (twisted, corkscrew like arteries), skeletal/craniofacial abnormalities, and osteoarthritis that is referred to as Aneurysm-Osteoarthritis syndrome (AOS) [71]. Similarly, arterial tortuosity

syndrome (ATS), characterized by tortuosity of medium to large vessels and aneurysms, has associated with loss-of-function mutations in SLC2A10 (Solute Carrier Family 2, Facilitated Glucose Transporter Member 10) [72, 73]. Increased TGF-β signaling is also believed to be associated with this syndrome, as ATS vascular smooth muscle cells exhibit decreased production of decorin, a large extracellular leucine rich proteoglycan that is known to bind and sequester TGF-β in the ECM [73]. The decorin promoter contains a glucose response element which is less active with fewer functional glucose transporters, creating a decorin deficiency that results in increased TGF-β abundance and signaling [73]. While these two syndromes further implicate TGF-β signaling through similar connective tissue and cardiovascular phenotypes, syndromes such as Shprintzen-Goldberg syndrome and hereditary hemorrhagic telangiectasia illustrate the wide variation in connective tissue and cardiovascular involvement possible with mutations in TGFBRs.

8.3.2 Shprintzen-Goldberg Syndrome (SGS)

In 1982, Shprintzen and Goldberg first described their eponymous heritable connective tissue syndrome in two patients [74]. Shprintzen-Goldberg syndrome is characterized by anomalies of the head/face, skeleton, brain, and cardiovascular system [75]. Shprintzen-Goldberg syndrome has since been recognized as part of a group of phenotypically overlapping syndromes associated with TGFBR mutations (LDS and FTAAD) affecting connective tissues and the cardiovascular system [76]. However, SGS has been linked to mutations in TGFBRI and II, as well as fibrillin-1 (OMIM #182212). Thus, unlike LDS and MFS, it is not yet known whether the connective tissue and cardiovascular manifestations of SGS are due to a defect in a TGFBR (causing dysregulated TGF-β signaling that results in tissue defects) or a connective tissue component like fibrillin-1 (a structural defect causing dysregulated TGF-β signaling). Independent of the initiating event, the defect lies somewhere in the TGF-β pathway creating a heterogeneous range of symptoms, making a definite genotype-phenotype correlation difficult. Consequently, the clinical presentation of SGS is not well defined and still developing. Intellectual impairment may be the only regularly occurring symptom, with all documented patients presenting with a range from moderate retardation to learning disabilities [77]. These impairments are known to occur simultaneously with brain abnormalities: communicating hydrocephalus, dilated lateral ventricles, and Arnold-Chiari formation type-I [78]. Ocular defects may also be present in SGS patients. Lens dislocation, while seen in MFS, does not appear to be a typical feature of SGS [8]. Hypertelorism (seen in LDS), myopia and exophthalmos, however, are characteristic of SGS [75]. Several skeletal anomalies are identified in early childhood [79]. The major characteristic skeletal finding is scaphocephaly (boat shaped skull) with craniosynostosis (premature fusion of skull) [79]. In fact, SGS has been referred to as marfanoid habitus with craniosynostosis [80].

Many of the skeletal findings associated with MFS and LDS are observed in SGS: dolichostenomelia (long limbs), arachnodactyly (long fingers), scoliosis, pectus excavatum or carinatum (hollowed or pigeon chest), joint hypermobility, and contracture of the proximal joints of the hand [59]. Regarding facial dysmorphic features, SGS may produce micrognathia, midface hypoplasia, low-set ears, and palatal soft tissue hyperplasia (pseudocleft palate) that may be noted as early as the first year of life and become more pronounced with time [77]. Additional characteristic findings include: minimal subcutaneous fat, hypotonia, obstructive apnea, defects in the abdominal wall musculature with hernias, hyperelastic skin, and cryptorchidism [77].

Cardiovascular defects are mostly limited to the heart valves. Mitral and/or aortic valve regurgitation is commonly observed [75]. Mitral valve prolapse, often seen in MFS, occurs commonly as well [77]. Mitral valve prolapse (MVP) and regurgitation are commonly found in many but not all patients with syndromes related to TGFBR mutations. Given that FBN1 mutations have been

associated with an increase in TGF-β release and signaling, Ng et al. examined the association of TGF-β pathway signaling with the pathogenesis of MVP using a mouse model of MFS. Changes in mitral architecture were observed to be temporally and spatially linked with increases in TGF-β activation, signaling and cell processes within the mitral valve (increased proliferation/growth and decreased apoptosis) [81]. Furthermore, normal valve phenotype was restored with the administration of a TGF-β neutralizing antibody. This study provided a potential pathogenic mechanism for MVP in MFS/LDS and perhaps SGS, as mutations in TGFBRs have been linked to increased markers of TGF-β signaling.

Aortic root dilatation and aneurysm has been previously described in SGS, but is not present in most affected individuals, though it is common in MFS, LDS, and FTAAD [8]. The presence of aortic dilatation may suggest overlap with one of these phenotypically similar syndromes. Aortic valve pathology has also been linked to TGFBRII mutations. An SGS patient with a BAV and an ascending aortic aneurysm that later dissected was found to have a mutation in TGFBRII [82]. Thus, both the mitral and aortic valvular manifestations of SGS may be due to mutations in TGFBRs that result in increased TGF-β pathway signaling. While SGS displays a high ratio of connective tissue to cardiovascular symptoms, hereditary hemorrhagic telangiectasia (HHT), another hereditary disorder associated with TGFBR mutations, displays primarily diffuse vascular symptoms.

8.3.3 Hereditary Hemorrhagic Telangiectasia (HHT)

Originally described in the nineteenth century by Osler, Weber, Rendu and Hanes, HHT is an autosomal dominant disorder characterized by vascular malformations and dilated small blood vessels which are fragile due to thin supporting connective tissue [83–85]. HHT is most commonly caused by mutations within TGFBRs that disrupt normal TGF-β signaling, which induces the characteristic vascular and connective tissue defects. Epidemiologic reports estimate the prevalence of HHT between 1 in 5,000 and 1 in 8,000, though some reports believe HHT may be underreported due to many patients being unaware of their diagnosis [86–88]. The diagnosis is often difficult due to its variable penetrance and severity, as well as its relatively slow progression. Manifestations of HHT typically are not present at birth and develop with time. Clinical signs and symptoms may be present in childhood though generally are noted after puberty with an estimated 7 in 10 HHT patients developing at least one clinical symptom or sign by age 16 and almost 100 % by 40 years of age [89–91].

Initially, HHT patients will develop telangiectasias, small blood vessels that dilate near the surface of the skin, mucous membranes and gastrointestinal tract. These telangiectasias increase in number and size with age [92, 93]. Nosebleeds (also known as epistaxis), the most common clinical manifestation of HHT, result from ruptured telangiectasias of the nasal mucosa. Epistaxis and telangiectasias within the gastrointestinal tract, commonly in the duodenum, are the two major mechanisms of iron deficiency anemia secondary to hemorrhage in this population. Most HHT patients experience only these three symptoms: nosebleeds, mucocutaneous telangiectasias, and iron deficiency anemia. These symptoms are relatively minor, in terms of their contribution to the morbidity and mortality associated with HHT, while the primary concern results from vascular abnormalities resulting from malformed connections between arteries and veins in the visceral organs [86].

While arteriovenous malformations (AVMs) may occur sporadically in the general population, AVMs occur in high numbers in multiple organs in HHT patients. The most clinically relevant locations are distributed among the lungs (50 %), the liver (30 %) and the brain (10 %) [94–96]. A further pulmonary manifestation of HHT is severe pulmonary artery hypertension (PAH) arising mainly from two sources in HHT: (1) high output heart failure secondary to hepatic AVM shunting and (2) primary PAH without signs of heart failure [97].

Additionally, HHT patients may also exhibit pathologic defects within specialized connective tissues such as the blood and the immune system/lymphoid tissue. Elevated clotting factor VIII and von Willebrand factor were measured in the blood of HHT patients versus normal controls and associated with venous thromboembolism [98]. Reports of defects in adaptive immunity and a mononuclear cell infiltrate around telangiectases spawned a suspicion of immune system involvement in HHT. These reports were further supported by an analysis of the oxidative burst activity of HHT monocytes and polymorphonuclear cells, which found single or multiple deficits in both cell groups in 20 of 22 HHT patients [99]. Thus, the connective tissue component of HHT has only recently been demonstrated.

The Curacao diagnostic criteria are based on international consensus and used to diagnose HHT with a score that gauges the likelihood of its presence [100, 101]. The criteria include a first degree relative with HHT, the presence of several telangiectasias on the skin and mucous membranes, recurrent and spontaneous epistaxis, and visceral AVMs. One point is scored for each of the criteria present. If only 1 of the criteria is noted, HHT is "unlikely." Two criteria indicate "suspected" HHT. More than two criteria present is evidence of "definite" HHT disease. The diagnosis of HHT is made clinically, without requiring genetic testing to identify a potentially causative mutation. If desired, genetic testing may be employed to confirm the diagnosis.

At least five genes have been identified in which mutations will cause HHT. These are subdivided based on the gene loci involved [97]. HHT1 and 2 are the major subtypes linked to mutations within endoglin (a type-III TGFBR, OMIM #187300) [102] and activin receptor-like kinase 1/ALK-1 (a type-I TGFBR, OMIM #600367) [103, 104]. Additionally, mutations in Smad4 produce HHT with juvenile polyposis. Interestingly, juvenile polyposis in the general population results from mutation in activin receptor-like kinase 3/ALK-3 (also known as BMP Receptor 1A), which signals through Smad4 [97]. HHT producing mutations within downstream proteins involved in TGF- β signaling further confirms the role of TGF-β dysregulation in the pathogenesis of HHT. Of note, a family with juvenile polyposis, aortopathy and mitral valve dysfunction co-segregating with a Smad4 mutation has recently been described [105]. Aortopathy and mitral valve defects are typical features of MFS and LDS, not HHT (though case reports of large vessel aneurysms in HHT exist) [105]. The presence of these features associated with a Smad4 mutation supports the role of TGF-β signaling in the common pathogenesis of all of these features, particularly noncanonical signaling given the role of Smad4 in the canonical pathway. Furthermore, it provides a spectral link between the vascular features (aortopathy, aneurysm, and mitral valve defects) of MFS and LDS and those seen in HHT (AVMs, small vessel dilatation, and juvenile polyposis).

Activin receptor like kinase 1 and endoglin are expressed on the surface of vascular endothelial cells, suggesting that dysregulated TGF-β signaling in endothelial cell plays a major role in inducing telangiectasia/dilatation and AVM formation [106]. Interestingly, homozygous ALK-1 mutations in zebrafish and mice produce embryonic lethality and exhibit severely dilated vessels (including the aorta) and abnormal vessel fusion [107, 108]. These vascular defects were associated with increased endothelial cell number, enhanced expression of angiogenic factors and proteases, and deficient differentiation and recruitment of smooth muscle cells. Thus, the small vessel dilatation in HHT represents a phenotypic microcosm of the aortic and extra-aortic dilatation seen in MFS and LDS. Furthermore, Seki et al. demonstrated in mice, that ALK-1 is predominantly expressed in the developing endothelium of arteries [109]. Taken together, these observations support the role of TGF-β signaling in early vascular development and dilatation.

Transforming growth factor-β can signal through two distinct type-I receptors (ALK-1 and TGFBRI) in endothelial cells [43]. Signaling through TGFBRI activates Smads2/3, while ALK-1 is unique among type-I receptors in that it activates Smads 1, 5 or 8 (Fig. 8.1). Endoglin interacts with ALK-1 and is required for TGF-β-dependent ALK-1 signaling by facilitating the

binding of TGF-β to the ALK-1 receptor [43]. TGF-β stimulation of the endoglin/ALK-1 pathway via Smads 1, 5, or 8 is associated with endothelial proliferation and migration-essential to angiogenesis, while signaling through the ALK-5 pathway via Smad2/3 produces opposite results [110]. This would seem to suggest that mutated ALK-1 or endoglin would result in a quiescent endothelium, opposite to that seen in HHT. However, the ALK-1 pathway regulates the expression of ALK-5 such that decreases in ALK-1 signaling produce a reduction in ALK-5 signaling as evidenced by an 80 % decrease in ALK-5 RNA transcripts in HHT 1 and 2 endothelial cells [111]. This adaptive compensation may produce an imbalance favoring dysregulated angiogenesis and the formation of AVMs. Evidence suggests that the ratio of ALK-1 to ALK-5 determines whether or not endothelial cells will become quiescent or actively proliferate and migrate [112]. Alternatively, similar to LDS in which increased TGF-β signaling was described despite TGFBRI and II mutations, endoglin and ALK-1 mutations may paradoxically increase signaling through angiogenic pathways thus resulting in AVMs.

In comparison to what is known about the mechanisms of AVMs and dilatation, little was known about the mechanism of primary PAH in HHT until recently. The primary PAH phenotype (without heart failure) observed in <2 % of HHT patients is identical to inherited primary PAH due to a loss of function mutation in Bone Morphogenetic Protein receptor 2 (BMPR2), another receptor of the TGF-β superfamily [97]. The mechanism is thought to involve a loss of pulmonary artery endothelial and smooth muscle cell apoptosis mediated by BMPR2 that results in abnormally elevated growth and proliferation [113–115]. Accordingly, the linkage between BMPR2 and ALK-1 was studied in HHT families with PAH and suggestive linkage was found [116]. However, the HHT patients only exhibited mutations in ALK-1, not BMPR2. This suggests a common signaling pathway downstream of BMPR2 and ALK-1 is involved in the pathogenesis of primary PAH. Endoglin, as a type-III TGFBR, facilitates signaling through ALK-1, and endoglin mutations could also result in PAH. Transforming growth factor-β receptor III, also known as betaglycan, has been implicated in the regulation of muscle cell proliferation and was demonstrated to inhibit myoblast proliferation through increasing Smad3 and p38 MAPK signals [107, 117]. Thus, interrupted TGF-β signaling via a mutated ALK-1 or endoglin gene may remove a TGF-β mediated growth inhibitory effect on vascular endothelial or smooth muscle cells and contribute to the development of PAH and other cardinal manifestations of HHT-telangiectasias and AVMs.

8.3.4 Other Connective Tissue Disorders with TGF-β Involvement

TGF-β is also implicated in several other connective tissue disorders which are not commonly defined by gene abnormalities. Most include a hyperactive immune system as a component and are referred to as autoimmune connective tissue diseases. These diseases include systemic sclerosis/scleroderma, rheumatoid arthritis (RA), and systemic lupus erythematosus (SLE). Scleroderma (a disease of excessive fibrosis of vessels, organs and particularly the skin) severity has been correlated with levels of TGF-β, a potent regulator of ECM deposition [118]. Rheumatoid arthritis patients have elevated plasma thrombospondin-1 and TGF-β; the former activates TGF-β and the latter induces the expression of connective tissue growth factor (CTGF), which has been associated with atherosclerosis, a process occurring prematurely in RA [119, 120]. Recent evidence has identified a role for TGF-β in suppressing immune function directly and stimulating T cell conversion to a suppression phenotype [121]. Interestingly, TGF-β production is decreased in SLE [122]. Thus, a lack of TGF-β may contribute to SLE through diminished ability to suppress the immune system [123]. Due to its relationship with fibrosis and immune modulation, TGF-β may plausibly be involved in all autoimmune connective tissue disorders though its exact roles remain to be clarified.

8.4 Current Standard of Care for TAAs and Genetic Testing

8.4.1 Current Standard of Care for TAAs

The prognosis of aortic aneurysms has improved substantially with evolving surveillance, medical and invasive management. Consensus guidelines developed by several medical profession organizations for aortic disease with specific syndrome recommendations were published in 2010 [124]. Routine vascular imaging is the mainstay of prognosis (predicting rupture) with all aortic aneurysms. Patients should undergo aortic diameter screening immediately upon diagnosis of a TAA or gene mutation associated with TAA (TGFBRI or II, FBN1, Ehlers-Danlos and FTAAD genes) [124]. Follow-up imaging should be done 6 months later to determine the rate of progression of the aneurysm, with increasing diameter associated with increasing rupture risk [124]. The aortic diameter is followed with at least annual imaging until the rupture risk outweighs the risks of aortic replacement surgery, which is the definitive treatment for TAA. Special consideration is also given if valve or cardiac function is impaired. Rapid progression (defined as > or = 1 cm/year) or a diameter between 5 and 6 cm or 6 and 7 cm in the ascending and descending aorta, respectively, indicates the need for surgery in normal patients [125–128]. For patients with aortic aneurysm and a mutation in TGFBRI or II, guidelines recommend surgery at an external diameter of the ascending aorta >4.4 cm given that rupture and/or dissection is known to occur at smaller diameters in this population [59, 124]. Due to the tendency of patients with these mutations to develop cerebral and abdominal aneurysms, annual MRIs from the head to pelvis are also recommended.

The primary surgical treatment of those with TAA and aortic aneurysms/dissections in general is aortic replacement with artificial grafts. The approach, techniques, and risks involved depend greatly on the location of the aneurysm along the aorta. The major risks involved with an open surgical procedure include death, stroke, paresis/paralysis and renal failure [129, 130]. Endovascular aortic repair (EVAR) is a recent advancement employing stent-grafts to isolate aneurysms from blood flow and pressure/tension that has reduced early post-operative morbidity and mortality compared to open surgery. Long-term observation has revealed late complications related to the EVAR procedure and stent-grafts, provoking doubts about the durability of EVAR [131, 132]. Additionally, this new technique is limited to only 20 % of TAA patients due to its applicability only at certain anatomical sites [133, 134]. Thus, replacement surgery remains the gold standard of care [135, 136].

To delay TAA progression, medical treatment focuses on reducing blood pressure and therefore aortic wall tension, with beta blocker therapy as the current standard of care. However, a clinical trial has suggested that angiotensin converting enzyme (ACE) inhibitors are superior to beta blockers, demonstrating reduced aortic stiffness, improved distensibility, and reduced increase in aortic root diameter [137]. Angiotensin II, a circulating protein that regulates vascular smooth muscle tone, is synthesized by ACE and exerts its effects via two receptor types, ATI and II. Interestingly, ATI stimulation has been linked to enhanced expression of TGF-β and its receptors as well as the promotion of vascular fibrosis [138]. In mice, treatment with losartan, an ATI-specific inhibitor, prevented aneurysm development [139]. Additionally, Habashi et al. noted that mice heterozygous for FBN1 mutation and ATII deficient displayed worse aortic disease and decreased survival compared to mice with only FBN1 mutation, suggesting stimulation through ATI is deleterious and stimulation through ATII is beneficial [140]. When the ATII deficient/heterozygous FBN1 mice were compared to mice with only FBN1 mutation, enhanced phosphorylation of extracellular-signal regulated kinase (ERK) 1 and 2 was noted in the ATII receptor deficient/heterozygous FBN1 mice [140]. After noting increased TGF-β and increased pERK1/2 in FBN1 mutation only mice, this group demonstrated that aortic disease was improved by

stimulation of ATII receptors to decrease ERK1/2 phosphorylation, partially explaining the mechanism of benefit of losartan treatment in early clinical trials [140]. Furthermore, losartan blockade was also shown to rescue the MFS-dependent skeletal muscle defects (impaired regeneration phenotype) and improve alveolar septation defects in murine models [139, 141]. Besides ERK1/2, other noncanonical pathways have also been implicated in MFS mouse models of aneurysm. In Smad4 deficient MFS mice, like the ATII deficient MFS mice, aortic disease was exacerbated and accelerated death associated with elevated c-Jun N-terminal Kinase-1 (JNK1) activation was observed [142]. Interestingly, antagonism of JNK1 attenuated aortic growth in MFS mice with and without Smad4 [142]. As enhanced noncanonical TGF-β signaling is increasingly associated with MFS aortic pathology, ATI, ERK1/2 and JNK1 blockade represent potentially new standards of care for MFS and possibly other TGFBR mutation syndromes. As new pathway-specific treatment modalities are developed, identification of the causative mutation by genetic testing will be increasingly important in cases of inherited aortopathies.

8.4.2 Genetic Testing

8.4.2.1 Genetic Testing for FTAAD

Due to its phenotypic overlap with other inherited aortopathies, FTAAD should be confirmed with genetic testing. Genetic testing in cases of inherited aortopathy can be beneficial in several ways. Identification of an associated mutation can change follow up and medical management of the affected patient. Furthermore, identification of the mutation present in the proband will narrow and facilitate testing in potentially affected relatives as well as with prenatal testing. Beginning with identification of the first family member (proband) with an FTAAD mutation, guidelines recommend all first degree relatives be genetically counseled and screened [143]. Those relatives found to have the genetic mutation should obtain baseline aortic imaging immediately, and second degree relatives could reasonably be notified. If aortic disease is found in any first degree relatives, imaging of second degree relatives would be warranted [143]. If a patient with aneurysm/dissection does not have any of the major gene mutations associated with heritable aortic disease, first degree family members are recommended to seek aortic imaging rather than genetic testing [143]. This recommendation is particularly relevant because only ~25 % percent of FTAAD patients will have one of the seven known associated mutations [54].

Prenatal testing is possible, but it cannot be used to determine the severity of disease in a child inheriting any of the mutations described in this chapter. In addition, limited outcome data on the benefits of genetic testing in patients with heritable aortopathies is available. Given the good longevity of patients with heritable aortic disease with medical treatment and surgery, prenatal testing could reasonably be delayed until childhood, though this issue ideally should be discussed by the parents prior to conception.

8.4.2.2 Genetic Testing for SGS

SGS is clinically suspected when an individual presents with a combination of the major characteristics: marfanoid skeletal features, craniosynostosis, craniofacial dysmorphism, left sided heart valve prolapse or regurgitation, intellectual disability with delayed milestones, and brain abnormalities [75]. No specific diagnostic criteria or scoring rubric exists for SGS as for MFS with the Ghent criteria and HHT with the Curacao criteria. Genetic diagnosis of SGS is difficult due to the limited number of SGS patients, the range of mutations associated, and phenotypic overlap of related syndromes –MFS and LDS- known for their variability in presentation. Fibrillin-1 mutations were initially reported in three clinically diagnosed SGS patients, two of whom had a mutation atypical of MFS and exhibited an overlapping phenotype between SGS and MFS [144, 145]. A later genetic study of multiple SGS patients found no FBN1 mutations [80]. These observations suggest that a similar signaling pathway is involved in both SGS and MFS even though more than one gene may be affected. A patient described by van Steensel et al. with a

TGFBR2 mutation displayed a phenotype overlap between SGS and LDS [146]. In a study describing TGFBR mutation phenotypes, a TGFBR1 mutation was identified in a patient with clinically diagnosed SGS [147]. Currently, no specific mutations have to be identified to diagnose SGS, though identification of an FBN1 or TGFBR mutation may suggest it.

8.4.2.3 Treatment and Genetic Testing for HHT

In those cases where HHT is symptomatic, the management is the typical treatment of each of the individual conditions as though they occurred in a patient without HHT. AVMs are treated based on their location by clinicians with expertise pertaining to the organ involved, HHT or both. Embolotherapy is the preferred and hopefully definitive therapy for AVMs [88], though surgical resection or arterial ligation are further options [101]. If treatment of hepatic AVMs is not successful, the only definitive therapy remaining that will prevent the dire complications of hepatic AVMs (e.g., heart failure, pseudocirrhosis, and portal hypertension) is hepatic transplantation [148, 149].

HHT presents typically in childhood with a progression of subtle clinical signs increasing with age. Therefore, whether a newborn has inherited HHT cannot possibly be determined clinically, and as result genetic testing becomes essential for this purpose [150]. Today, genetic tests examine endoglin, ALK-1 and Smad4 whose mutations are responsible for more than 80 % of HHT cases [97]. However, this means the diagnosis for a substantial portion (~20 %) of individuals with HHT symptoms cannot be confirmed or excluded by molecular genetic testing. With regard to prenatal genetic testing for HHT, little impetus exists for it because of HHT's longevity and relatively asymptomatic course of most patients. In most instances, a positive genetic test does not alter the recommended treatment and screening course of HHT. However, if a Smad4 mutation (associated with juvenile polyposis) is detected in a HHT patient with a family history of gastrointestinal (GI) polyps and/or malignancy, more rigorous GI screening is recommended to reduce the risk of GI cancers [151]. As noted previously, few people diagnosed with HHT know they have it and genetic counseling offers the opportunity to address this communication deficiency directly with patients. For these reasons, genetic testing and counseling are of great potential benefit to HHT suspected and affected families.

8.5 Summary

We have reviewed several heritable connective tissue syndromes associated with mutations in TGF-β receptors I and II as well as accessory receptors and related proteins. Significant progress has been made in understanding their underlying mechanisms, and with their refinement, the probability for insights yielding treatment strategies increases. In particular, the study of TGFBR mutation syndromes holds great promise in this regard for the treatment of both connective tissue and cardiovascular disorders. Mutations in FBN1 or TGFBRs appear to result in a number of phenotypically overlapping connective tissue/cardiovascular syndromes involving dysregulation of the TGF-β signaling pathway. These TGF-β dysregulation syndromes (MFS, LDS, FTAAD, HHT, SGS, AOS and ATS) exhibit a spectrum of cardiovascular defects including arterial aneurysm/dilatation, dissection, mitral valve disease, arterial tortuosity, and primary PAH (Fig. 8.2). Their pathogeneses emphasize a common theme, that normal TGF-β family signaling is integral to the normal development and homeostasis of connective tissues and the cardiovascular system. This family contains potent regulators of many cell types within mesoderm-derived tissues. Thus, perturbations within their signaling pathways are uniquely situated to produce defects in these tissue types.

Clearly, normal TGF-β signaling is essential in angiogenesis. This is certainly evident in hereditary hemorrhagic telangiectasia (HHT), a small vessel phenocopy of the dilatation seen in large vessels of MFS, LDS, FTAAD, ATS and AOS. Angiogenesis, though, not only involves the proliferation and migration of endothelial cells to form new vessels but also smooth muscle cells

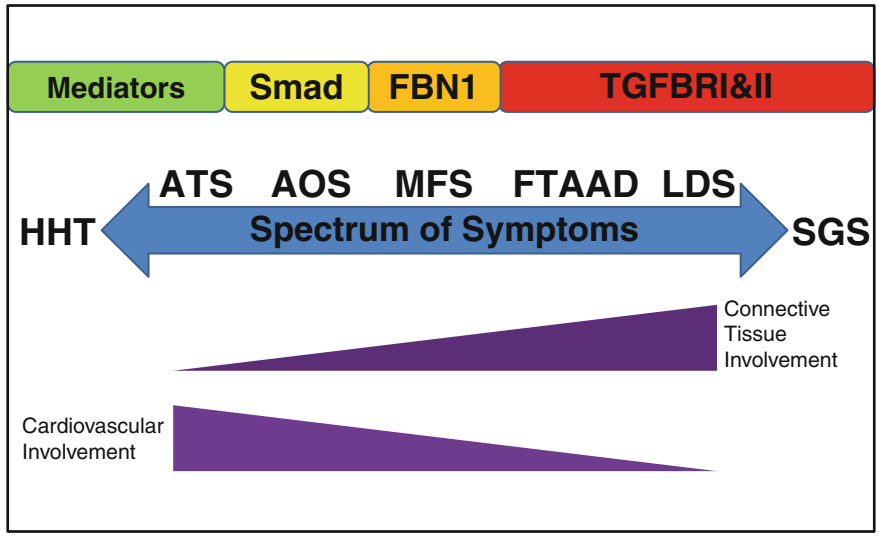

Fig. 8.2 Known heritable connective tissue disorders with cardiovascular involvement that associate with gene mutations related to TGF-Beta signaling. These disorders are arranged based on their increasing level of either connective tissue or cardiovascular involvement and notably share a spectrum of common symptoms, which supports their related pathophysiology

and fibroblasts to strengthen and support these new vessels. Fibroblasts in particular are major cellular regulators of connective tissue homeostasis. Angiogenesis is certainly a process of coordinating endothelial cell behavior in concert with the behavior of fibroblasts and smooth muscle cells. Although many of the mechanisms by which this coordination occurs have yet to be delineated, the TGF-β superfamily is highly implicated in regulating the normal behavior of all of the cells involved.

Many major connective tissue syndromes including Marfan syndrome, Loeys-Dietz syndrome, and Ehlers-Danlos syndrome exhibit concomitant cardiovascular manifestations. In Marfan syndrome and its related disorders, characteristic abnormalities of these syndromes that were once thought to result from purely structural deficiencies (e.g. FBN1) are now attributed to disruptions of normal TGF-β signaling. The undeniable overlap in connective tissue and cardiovascular phenotypes of fibrillinopathies and mutated TGF-β receptor syndromes supports this notion of a common signaling pathway [8]. Indeed, these mutated receptor phenotypes are even recapitulated by mutations of downstream TGF-β pathway components (e.g. Smad4 in HHT and Smad3 in LDS and FTAAD) [71, 151].

Our new understanding of causal signaling disturbances in these disorders significantly improves the treatment prospects for highly morbid cardiovascular and debilitating connective tissue defects beyond the difficult prospect of restoring structural integrity to weakened tissues. Recently, additional heritable connective tissue disorders-cutis laxa and congenital contractural arachnodactyly-have been linked to dysregulated TGF-β and BMP signaling.[152, 153] Perhaps, those heritable connective tissue syndromes currently attributed to structural ECM weakness (e.g., osteogenesis imperfecta) will also be revealed to involve disturbances in signaling pathways, boding well for the development of future medical treatments.

References

1. Judge DP, Dietz HC (2005) Marfan's syndrome. Lancet 366(9501):1965–1976
2. Dietz HC (1993) Marfan syndrome. In: Pagon RA (ed) Gene reviews. University of Washington, Seattle

3. Unsold C et al (2001) Latent TGF-beta binding protein LTBP-1 contains three potential extracellular matrix interacting domains. J Cell Sci 114(Pt 1):187–197
4. Taipale J et al (1996) Latent transforming growth factor-beta 1 and its binding protein are components of extracellular matrix microfibrils. J Histochem Cytochem 44(8):875–889
5. Dallas SL et al (1995) Dual role for the latent transforming growth factor-beta binding protein in storage of latent TGF-beta in the extracellular matrix and as a structural matrix protein. J Cell Biol 131(2):539–549
6. Isogai Z et al (2003) Latent transforming growth factor beta-binding protein 1 interacts with fibrillin and is a microfibril-associated protein. J Biol Chem 278(4):2750–2757
7. Neptune ER et al (2003) Dysregulation of TGF-beta activation contributes to pathogenesis in Marfan syndrome. Nat Genet 33(3):407–411
8. Loeys BL et al (2005) A syndrome of altered cardiovascular, craniofacial, neurocognitive and skeletal development caused by mutations in TGFBR1 or TGFBR2. Nat Genet 37(3):275–281
9. Wrana JL et al (1992) TGF beta signals through a heteromeric protein kinase receptor complex. Cell 71(6):1003–1014
10. Ramirez F, Rifkin DB (2003) Cell signaling events: a view from the matrix. Matrix Biol 22(2):101–107
11. Hynes RO (2009) The extracellular matrix: not just pretty fibrils. Science 326(5957):1216–1219
12. Brekken RA, Sage EH (2001) SPARC, a matricellular protein: at the crossroads of cell-matrix communication. Matrix Biol 19(8):816–827
13. Annes JP, Munger JS, Rifkin DB (2003) Making sense of latent TGFbeta activation. J Cell Sci 116(Pt 2):217–224
14. Wrana JL et al (1994) Mechanism of activation of the TGF-beta receptor. Nature 370(6488):341–347
15. Moustakas A, Souchelnytskyi S, Heldin CH (2001) Smad regulation in TGF-beta signal transduction. J Cell Sci 114(Pt 24):4359–4369
16. Feng XH, Derynck R (2005) Specificity and versatility in tgf-beta signaling through Smads. Annu Rev Cell Dev Biol 21:659–693
17. Park SH (2005) Fine tuning and cross-talking of TGF-beta signal by inhibitory Smads. J Biochem Mol Biol 38(1):9–16
18. Imamura T et al (1997) Smad6 inhibits signalling by the TGF-beta superfamily. Nature 389(6651):622–626
19. Hata A et al (1998) Smad6 inhibits BMP/Smad1 signaling by specifically competing with the Smad4 tumor suppressor. Genes Dev 12(2):186–197
20. Wicks SJ et al (2006) Reversible ubiquitination regulates the Smad/TGF-beta signalling pathway. Biochem Soc Trans 34(Pt 5):761–763
21. Ebisawa T et al (2001) Smurf1 interacts with transforming growth factor-beta type-I receptor through Smad7 and induces receptor degradation. J Biol Chem 276(16):12477–12480
22. Kavsak P et al (2000) Smad7 binds to Smurf2 to form an E3 ubiquitin ligase that targets the TGF beta receptor for degradation. Mol Cell 6(6):1365–1375
23. Droguett R et al (2006) Extracellular proteoglycans modify TGF-beta bio-availability attenuating its signaling during skeletal muscle differentiation. Matrix Biol 25(6):332–341
24. Stander M et al (1999) Transforming growth factor-beta and p-21: multiple molecular targets of decorin-mediated suppression of neoplastic growth. Cell Tissue Res 296(2):221–227
25. Lopez-Casillas F et al (1994) Betaglycan can act as a dual modulator of TGF-beta access to signaling receptors: mapping of ligand binding and GAG attachment sites. J Cell Biol 124(4):557–568
26. Yu L, Hebert MC, Zhang YE (2002) TGF-beta receptor-activated p38 MAP kinase mediates Smad-independent TGF-beta responses. EMBO J 21(14):3749–3759
27. Dumont N, Bakin AV, Arteaga CL (2003) Autocrine transforming growth factor-beta signaling mediates Smad-independent motility in human cancer cells. J Biol Chem 278(5):3275–3285
28. Griswold-Prenner I et al (1998) Physical and functional interactions between type-I transforming growth factor beta receptors and Balpha, a WD-40 repeat subunit of phosphatase 2A. Mol Cell Biol 18(11):6595–6604
29. Itoh S et al (2003) Elucidation of Smad requirement in transforming growth factor-beta type-I receptor-induced responses. J Biol Chem 278(6):3751–3761
30. Wilkes MC et al (2003) Cell-type-specific activation of PAK2 by transforming growth factor beta independent of Smad2 and Smad3. Mol Cell Biol 23(23):8878–8889
31. Choy L, Derynck R (1998) The type-II transforming growth factor (TGF)-beta receptor-interacting protein TRIP-1 acts as a modulator of the TGF-beta response. J Biol Chem 273(47):31455–31462
32. Ozdamar B et al (2005) Regulation of the polarity protein Par6 by TGFbeta receptors controls epithelial cell plasticity. Science 307(5715):1603–1609
33. Hocevar BA, Brown TL, Howe PH (1999) TGF-beta induces fibronectin synthesis through a c-Jun N-terminal kinase-dependent, Smad4-independent pathway. EMBO J 18(5):1345–1356
34. Imamichi Y et al (2005) TGF beta-induced focal complex formation in epithelial cells is mediated by activated ERK and JNK MAP kinases and is independent of Smad4. Biol Chem 386(3):225–236
35. Remy I, Montmarquette A, Michnick SW (2004) PKB/Akt modulates TGF-beta signalling through a direct interaction with Smad3. Nat Cell Biol 6(4):358–365
36. Runyan CE, Schnaper HW, Poncelet AC (2004) The phosphatidylinositol 3-kinase/Akt pathway enhances Smad3-stimulated mesangial cell collagen I expression in response to transforming growth factor-beta1. J Biol Chem 279(4):2632–2639

37. Yakymovych I et al (2001) Regulation of Smad signaling by protein kinase C. FASEB J 15(3): 553–555
38. Carta L et al (2009) p38 MAPK is an early determinant of promiscuous Smad2/3 signaling in the aortas of fibrillin-1 (Fbn1)-null mice. J Biol Chem 284(9):5630–5636
39. Yamashita M et al (2008) TRAF6 mediates Smad-independent activation of JNK and p38 by TGF-beta. Mol Cell 31(6):918–924
40. Sorrentino A et al (2008) The type-I TGF-beta receptor engages TRAF6 to activate TAK1 in a receptor kinase-independent manner. Nat Cell Biol 10(10):1199–1207
41. Lee MK et al (2007) TGF-beta activates Erk MAP kinase signalling through direct phosphorylation of ShcA. EMBO J 26(17):3957–3967
42. Wilkes MC et al (2005) Transforming growth factor-beta activation of phosphatidylinositol 3-kinase is independent of Smad2 and Smad3 and regulates fibroblast responses via p21-activated kinase-2. Cancer Res 65(22):10431–10440
43. Bertolino P et al (2005) Transforming growth factor-beta signal transduction in angiogenesis and vascular disorders. Chest 128(6 Suppl):585S–590S
44. Massague J (2000) How cells read TGF-beta signals. Nat Rev Mol Cell Biol 1(3):169–178
45. Ignotz RA, Massague J (1986) Transforming growth factor-beta stimulates the expression of fibronectin and collagen and their incorporation into the extracellular matrix. J Biol Chem 261(9):4337–4345
46. Yan C, Boyd DD (2007) Regulation of matrix metalloproteinase gene expression. J Cell Physiol 211(1):19–26
47. Kwak HJ et al (2006) 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 MCR 4(3):209–220
48. Kim ES, Kim MS, Moon A (2004) 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 25(5):1375–1382
49. Laiho M, Saksela O, Keski-Oja J (1986) Transforming growth factor beta alters plasminogen activator activity in human skin fibroblasts. Exp Cell Res 164(2): 399–407
50. Jones JA, Spinale FG, Ikonomidis JS (2009) Transforming growth factor-beta signaling in thoracic aortic aneurysm development: a paradox in pathogenesis. J Vasc Res 46(2):119–137
51. Selvamurugan N et al (2004) 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 279(18):19327–19334
52. Pignolo RJ, Shore EM, Kaplan FS (2011) Fibrodysplasia ossificans progressiva: clinical and genetic aspects. Orphanet J Rare Dis 6:80
53. Werner S, Alzheimer C (2006) Roles of activin in tissue repair, fibrosis, and inflammatory disease. Cytokine Growth Factor Rev 17(3):157–171
54. Milewicz DM, Regalado E (1993) Thoracic aortic aneurysms and aortic dissections. In: Pagon RA (ed) Gene reviews. University of Washington, Seattle
55. Attias D et al (2009) Comparison of clinical presentations and outcomes between patients with TGFBR2 and FBN1 mutations in Marfan syndrome and related disorders. Circulation 120(25):2541–2549
56. von Kodolitsch Y et al (2004) Chest radiography for the diagnosis of acute aortic syndrome. Am J Med 116(2):73–77
57. Bruno L et al (1984) Cardiac, skeletal, and ocular abnormalities in patients with Marfan's syndrome and in their relatives. Comparison with the cardiac abnormalities in patients with kyphoscoliosis. Br Heart J 51(2):220–230
58. Pannu H, Tran-Fadulu V, Milewicz DM (2005) Genetic basis of thoracic aortic aneurysms and aortic dissections. Am J Med Genet C Semin Med Genet 139C(1):10–16
59. Loeys BL et al (2006) Aneurysm syndromes caused by mutations in the TGF-beta receptor. New Engl J Med 355(8):788–798
60. Biddinger A et al (1997) Familial thoracic aortic dilatations and dissections: a case control study. J Vasc Surg 25(3):506–511
61. Hagan PG et al (2000) The International Registry of Acute Aortic Dissection (IRAD): new insights into an old disease. JAMA 283(7):897–903
62. Guo DC et al (2009) Mutations in smooth muscle alpha-actin (ACTA2) cause coronary artery disease, stroke, and Moyamoya disease, along with thoracic aortic disease. Am J Hum Genet 84(5):617–627
63. Milewicz DM et al (2010) De novo ACTA2 mutation causes a novel syndrome of multisystemic smooth muscle dysfunction. Am J Med Genet A 152A(10):2437–2443
64. Loscalzo ML et al (2007) Familial thoracic aortic dilation and bicommissural aortic valve: a prospective analysis of natural history and inheritance. Am J Med Genet A 143A(17):1960–1967
65. Zhu L et al (2006) Mutations in myosin heavy chain 11 cause a syndrome associating thoracic aortic aneurysm/aortic dissection and patent ductus arteriosus. Nat Genet 38(3):343–349
66. Isselbacher EM (2005) Thoracic and abdominal aortic aneurysms. Circulation 111(6):816–828
67. Denton CP et al (2003) Fibroblast-specific expression of a kinase-deficient type-II transforming growth factor beta (TGFbeta) receptor leads to paradoxical activation of TGFbeta signaling pathways with fibrosis in transgenic mice. J Biol Chem 278(27):25109–25119
68. Wong SH et al (2000) Endoglin expression on human microvascular endothelial cells association with betaglycan and formation of higher order complexes with TGF-beta signalling receptors. Eur J Biochem/FEBS 267(17):5550–5560

69. Di Guglielmo GM et al (2003) Distinct endocytic pathways regulate TGF-beta receptor signalling and turnover. Nat Cell Biol 5(5):410–421
70. Nakao A et al (1997) Identification of Smad7, a TGFbeta-inducible antagonist of TGF-beta signalling. Nature 389(6651):631–635
71. van de Laar IM et al (2011) Mutations in SMAD3 cause a syndromic form of aortic aneurysms and dissections with early-onset osteoarthritis. Nat Genet 43(2):121–126
72. Loeys B, De Paepe A (2008) New insights in the pathogenesis of aortic aneurysms. Verh K Acad Geneeskd Belg 70(2):69–84
73. Coucke PJ et al (2006) Mutations in the facilitative glucose transporter GLUT10 alter angiogenesis and cause arterial tortuosity syndrome. Nat Genet 38(4):452–457
74. Shprintzen RJ, Goldberg RB (1982) A recurrent pattern syndrome of craniosynostosis associated with arachnodactyly and abdominal hernias. J Craniofac Genet Dev Biol 2(1):65–74
75. Greally MT et al (1998) Shprintzen-Goldberg syndrome: a clinical analysis. Am J Med Genet 76(3):202–212
76. Akutsu K et al (2007) Phenotypic heterogeneity of Marfan-like connective tissue disorders associated with mutations in the transforming growth factor-beta receptor genes. Circ J 71(8):1305–1309
77. Greally MT (1993) Shprintzen-Goldberg syndrome. In: Pagon RA et al (eds) Gene reviews. University of Washington, Seattle
78. Robinson PN et al (2005) Shprintzen-Goldberg syndrome: fourteen new patients and a clinical analysis. Am J Med Genet A 135(3):251–262
79. Ades LC et al (1995) Distinct skeletal abnormalities in four girls with Shprintzen-Goldberg syndrome. Am J Med Genet 57(4):565–572
80. Ades LC et al (2006) FBN1, TGFBR1, and the Marfan-craniosynostosis/mental retardation disorders revisited. Am J Med Genet A 140(10): 1047–1058
81. Ng CM (2004) TGF- -dependent pathogenesis of mitral valve prolapse in a mouse model of Marfan syndrome. J Clin Invest 114(11):1586–1592
82. Girdauskas E et al (2011) Transforming growth factor-beta receptor type-II mutation in a patient with bicuspid aortic valve disease and intraoperative aortic dissection. Ann Thorac Surg 91(5):e70–e71
83. Rendu H (1896) Epistaxis repetees chez un sujet porteur de petits angiomes cutanes et muquez. Gazette des Hopitaux Civils et Militaires (Paris) 135:1322
84. Osler W (1901) On a family form of recurring epistaxis, associated with multiple telangectases of the skin and mucous membranes. Bull Johns Hopkins Hosp 12:333
85. Weber F (1907) Multiple hereditary developmental angiomata (telangiectases) of the skin and mucous membranes associated with recurring hemorrhages. Lancet 2:160–162
86. Kjeldsen AD, Vase P, Green A (1999) Hereditary haemorrhagic telangiectasia: a population-based study of prevalence and mortality in Danish patients. J Intern Med 245(1):31–39
87. Bideau A et al (1989) Epidemiological investigation of Rendu-Osler disease in France: its geographical distribution and prevalence. Popul Engl Sel 44(1):3–22
88. Shovlin CL et al (2008) Primary determinants of ischaemic stroke/brain abscess risks are independent of severity of pulmonary arteriovenous malformations in hereditary haemorrhagic telangiectasia. Thorax 63(3):259–266
89. Bourdeau A, Dumont DJ, Letarte M (1999) A murine model of hereditary hemorrhagic telangiectasia. J Clin Invest 104(10):1343–1351
90. Wallace GM, Shovlin CL (2000) A hereditary haemorrhagic telangiectasia family with pulmonary involvement is unlinked to the known HHT genes, endoglin and ALK-1. Thorax 55(8):685–690
91. Cole SG et al (2005) A new locus for hereditary haemorrhagic telangiectasia (HHT3) maps to chromosome 5. J Med Genet 42(7):577–582
92. Plauchu H et al (1989) Age-related clinical profile of hereditary hemorrhagic telangiectasia in an epidemiologically recruited population. Am J Med Genet 32(3):291–297
93. Pasculli G et al (2005) Capillaroscopy of the dorsal skin of the hands in hereditary hemorrhagic telangiectasia. QJM 98(10):757–763
94. Fulbright RK et al (1998) MR of hereditary hemorrhagic telangiectasia: prevalence and spectrum of cerebrovascular malformations. AJNR Am J Neuroradiol 19(3):477–484
95. Cottin V et al (2004) Pulmonary arteriovenous malformations in patients with hereditary hemorrhagic telangiectasia. Am J Respir Crit Care Med 169(9):994–1000
96. Piantanida M et al (1996) Hereditary haemorrhagic telangiectasia with extensive liver involvement is not caused by either HHT1 or HHT2. J Med Genet 33(6):441–443
97. Govani FS, Shovlin CL (2009) Hereditary haemorrhagic telangiectasia: a clinical and scientific review. Eur J Hum Genet 17(7):860–871
98. Shovlin CL et al (2007) Elevated factor VIII in hereditary haemorrhagic telangiectasia (HHT): association with venous thromboembolism. Thromb Haemost 98(5):1031–1039
99. Cirulli A et al (2006) Patients with Hereditary Hemorrhagic Telangectasia (HHT) exhibit a deficit of polymorphonuclear cell and monocyte oxidative burst and phagocytosis: a possible correlation with altered adaptive immune responsiveness in HHT. Curr Pharm Des 12(10):1209–1215
100. Shovlin CL et al (2000) Diagnostic criteria for hereditary hemorrhagic telangiectasia (Rendu-Osler-Weber syndrome). Am J Med Genet 91(1):66–67
101. Faughnan ME et al (2011) International guidelines for the diagnosis and management of hereditary haemorrhagic telangiectasia. J Med Genet 48(2):73–87

102. McAllister KA et al (1994) Endoglin, a TGF-beta binding protein of endothelial cells, is the gene for hereditary haemorrhagic telangiectasia type 1. Nat Genet 8(4):345–351
103. Berg JN et al (1997) The activin receptor-like kinase 1 gene: genomic structure and mutations in hereditary hemorrhagic telangiectasia type 2. Am J Hum Genet 61(1):60–67
104. Johnson DW et al (1996) Mutations in the activin receptor-like kinase 1 gene in hereditary haemorrhagic telangiectasia type 2. Nat Genet 13(2):189–195
105. Andrabi S et al (2011) SMAD4 mutation segregating in a family with juvenile polyposis, aortopathy, and mitral valve dysfunction. Am J Med Genet A 155A(5):1165–1169
106. Letarte M et al (2005) Reduced endothelial secretion and plasma levels of transforming growth factor-beta1 in patients with hereditary hemorrhagic telangiectasia type 1. Cardiovasc Res 68(1):155–164
107. Roman BL et al (2002) Disruption of acvrl1 increases endothelial cell number in zebrafish cranial vessels. Development 129(12):3009–3019
108. Oh SP et al (2000) Activin receptor-like kinase 1 modulates transforming growth factor-beta 1 signaling in the regulation of angiogenesis. Proc Natl Acad Sci U S A 97(6):2626–2631
109. Seki T, Yun J, Oh SP (2003) Arterial endothelium-specific activin receptor-like kinase 1 expression suggests its role in arterialization and vascular remodeling. Circ Res 93(7):682–689
110. Stefansson S et al (2001) Inhibition of angiogenesis in vivo by plasminogen activator inhibitor-1. J Biol Chem 276(11):8135–8141
111. Fernandez LA et al (2005) Blood outgrowth endothelial cells from Hereditary Haemorrhagic Telangiectasia patients reveal abnormalities compatible with vascular lesions. Cardiovasc Res 68(2):235–248
112. Goumans MJ et al (2003) Activin receptor-like kinase (ALK)1 is an antagonistic mediator of lateral TGFbeta/ALK-5 signaling. Mol Cell 12(4):817–828
113. Davies RJ et al (2012) BMP type-II receptor deficiency confers resistance to growth inhibition by TGF-beta in pulmonary artery smooth muscle cells: role of proinflammatory cytokines. Am J Physiol Lung Cell Mol Physiol 302(6):L604–L615
114. West J et al (2004) Pulmonary hypertension in transgenic mice expressing a dominant-negative BMPRII gene in smooth muscle. Circ Res 94(8):1109–1114
115. Kimura N et al (2000) BMP2-induced apoptosis is mediated by activation of the TAK1-p38 kinase pathway that is negatively regulated by Smad6. J Biol Chem 275(23):17647–17652
116. Trembath RC et al (2001) Clinical and molecular genetic features of pulmonary hypertension in patients with hereditary hemorrhagic telangiectasia. New Engl J Med 345(5):325–334
117. You HJ et al (2007) The type-III TGF-beta receptor signals through both Smad3 and the p38 MAP kinase pathways to contribute to inhibition of cell proliferation. Carcinogenesis 28(12):2491–2500
118. Hawinkels LJ, Ten Dijke P (2011) Exploring anti-TGF-beta therapies in cancer and fibrosis. Growth Factors 29(4):140–152
119. Rico MC et al (2008) Thrombospondin-1 and transforming growth factor beta are pro-inflammatory molecules in rheumatoid arthritis. Transl Res 152(2):95–98
120. Rico MC et al (2010) The axis of thrombospondin-1, transforming growth factor beta and connective tissue growth factor: an emerging therapeutic target in rheumatoid arthritis. Curr Vasc Pharmacol 8(3):338–343
121. Wahl SM, Chen W (2005) Transforming growth factor-beta-induced regulatory T cells referee inflammatory and autoimmune diseases. Arthritis Res Ther 7(2):62–68
122. Ohtsuka K et al (1998) Decreased production of TGF-beta by lymphocytes from patients with systemic lupus erythematosus. J Immunol 160(5):2539–2545
123. Mageed RA, Prud'homme GJ (2003) Immunopathology and the gene therapy of lupus. Gene Ther 10(10):861–874
124. Hiratzka LF et al (2010) ACCF/AHA/AATS/ACR/ASA/SCA/SCAI/SIR/STS/SVM guidelines for the diagnosis and management of patients with Thoracic Aortic Disease: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines, American Association for Thoracic Surgery, American College of Radiology, American Stroke Association, Society of Cardiovascular Anesthesiologists, Society for Cardiovascular Angiography and Interventions, Society of Interventional Radiology, Society of Thoracic Surgeons, and Society for Vascular Medicine. Circulation 121(13):e266–e369
125. Coady MA, Rizzo JA, Elefteriades JA (1999) Developing surgical intervention criteria for thoracic aortic aneurysms. Cardiol Clin 17(4):827–839
126. Coady MA et al (1999) Surgical intervention criteria for thoracic aortic aneurysms: a study of growth rates and complications. Ann Thorac Surg 67(6):1922–1926, discussion 1953–1958
127. Elefteriades JA (2002) Natural history of thoracic aortic aneurysms: indications for surgery, and surgical versus nonsurgical risks. Ann Thorac Surg 74(5):S1877–S1880, discussion S1892–S1898
128. Lobato AC, Puech-Leao P (1998) Predictive factors for rupture of thoracoabdominal aortic aneurysm. J Vasc Surg 27(3):446–453
129. Svensson LG et al (1989) Appraisal of adjuncts to prevent acute renal failure after surgery on the thoracic or thoracoabdominal aorta. J Vasc Surg 10(3):230–239
130. Svensson LG et al (1993) Experience with 1509 patients undergoing thoracoabdominal aortic operations. J Vasc Surg 17(2):357–368, discussion 368–370
131. Bobik A (2006) Transforming growth factor-betas and vascular disorders. Arterioscler Thromb Vasc Biol 26(8):1712–1720
132. Ikonomidis JS et al (2006) Expression of matrix metalloproteinases and endogenous inhibitors within

ascending aortic aneurysms of patients with Marfan syndrome. Circulation 114(1 Suppl):I365–I370
133. Kapoun AM et al (2006) Transforming growth factor-beta receptor type 1 (TGFbetaRI) kinase activity but not p38 activation is required for TGFbetaRI-induced myofibroblast differentiation and profibrotic gene expression. Mol Pharmacol 70(2):518–531
134. Hu Y et al (2006) Role of extracellular signal-regulated kinase, p38 kinase, and activator protein-1 in transforming growth factor-beta1-induced alpha smooth muscle actin expression in human fetal lung fibroblasts in vitro. Lung 184(1):33–42
135. Greenberg RK et al (2008) Contemporary analysis of descending thoracic and thoracoabdominal aneurysm repair: a comparison of endovascular and open techniques. Circulation 118(8):808–817
136. Makaroun MS et al (2008) Five-year results of endovascular treatment with the Gore TAG device compared with open repair of thoracic aortic aneurysms. J Vasc Surg 47(5):912–918
137. Yetman AT, Bornemeier RA, McCrindle BW (2005) Usefulness of enalapril versus propranolol or atenolol for prevention of aortic dilation in patients with the Marfan syndrome. Am J Cardiol 95(9):1125–1127
138. Kubo A et al (2000) Angiotensin II regulates the cell cycle of vascular smooth muscle cells from SHR. Am J Hypertens 13(10):1117–1124
139. Habashi JP et al (2006) Losartan, an AT1 antagonist, prevents aortic aneurysm in a mouse model of Marfan syndrome. Science 312(5770):117–121
140. Habashi JP et al (2011) Angiotensin II type 2 receptor signaling attenuates aortic aneurysm in mice through ERK antagonism. Science 332(6027):361–365
141. Judge DP et al (2011) Mitral valve disease in Marfan syndrome and related disorders. J Cardiovasc Transl Res 4(6):741–747
142. Holm TM et al (2011) Noncanonical TGFbeta signaling contributes to aortic aneurysm progression in Marfan syndrome mice. Science 332(6027):358–361
143. Milewicz DM, Carlson AA, Regalado ES (2010) Genetic testing in aortic aneurysm disease: PRO. Cardiol Clin 28(2):191–197
144. Sood S et al (1996) Mutation in fibrillin-1 and the Marfanoid-craniosynostosis (Shprintzen-Goldberg) syndrome. Nat Genet 12(2):209–211
145. Kosaki K et al (2006) Molecular pathology of Shprintzen-Goldberg syndrome. Am J Med Genet A 140(1):104–108, author reply 109–110
146. van Steensel MA et al (2008) Shprintzen-Goldberg syndrome associated with a novel missense mutation in TGFBR2. Exp Dermatol 17(4):362–365
147. Stheneur C et al (2008) Identification of 23 TGFBR2 and 6 TGFBR1 gene mutations and genotype-phenotype-Investigations in 457 patients with Marfan syndrome type-I and II, Loeys-Dietz syndrome and related disorders. Hum Mutat 29(11):E284–E295
148. Kuhnel TS et al (2005) Clinical strategy in hereditary hemorrhagic telangiectasia. Am J Rhinol 19(5):508–513
149. Buscarini E et al (2006) Liver involvement in hereditary hemorrhagic telangiectasia: consensus recommendations. Liver Int 26(9):1040–1046
150. Cohen JH et al (2005) Cost comparison of genetic and clinical screening in families with hereditary hemorrhagic telangiectasia. Am J Med Genet A 137(2):153–160
151. Abdalla SA, Letarte M (2006) Hereditary haemorrhagic telangiectasia: current views on genetics and mechanisms of disease. J Med Genet 43(2):97–110
152. Rifkin DB, Todorovic V (2010) Bone matrix to growth factors: location, location, location. J Cell Biol 190(6):949–951
153. Atsawasuwan P et al (2008) Lysyl oxidase binds transforming growth factor-beta and regulates its signaling via amine oxidase activity. J Biol Chem 283(49):34229–34240
154. Fernandez LA et al (2006) Hereditary hemorrhagic telangiectasia, a vascular dysplasia affecting the TGF-beta signaling pathway. Clin Med Res 4(1):66–78
155. Gleason TG (2005) Heritable disorders predisposing to aortic dissection. Semin Thorac Cardiovasc Surg 17(3):274–281
156. Pannu H et al (2005) Mutations in transforming growth factor-beta receptor type-II cause familial thoracic aortic aneurysms and dissections. Circulation 112(4):513–520
157. Callewaert BL et al (2008) Arterial tortuosity syndrome: clinical and molecular findings in 12 newly identified families. Hum Mutat 29(1):150–158
158. van de Laar IM et al (2012) Phenotypic spectrum of the SMAD3-related aneurysms-osteoarthritis syndrome. J Med Genet 49(1):47–57