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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 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 (TGFB1) mutations, and discuss the pathogenic contribution of TGF- β to these syndromes with a primary focus on the cardiovascular system.

Keywords

Aorta; aneurysm; extracellular matrix; collagen; metalloproteinase; Shprintzen-Goldberg syndrome; thoracic aortic aneurysm and dissection syndrome; hereditary hemorrhagic telangiectasia (HHT); Marfan syndrome (MFS); Loeys-Dietz syndrome (LDS); Aortic Aneurysm Thoracic (AAT); Aneurysm-Osteoarthritis syndrome (AOS); arterial tortuosity syndrome (ATS); primary pulmonary hypertension; fibrodysplasia ossificans progressive (FOP); familial thoracic aortic aneurysm and dissection syndrome (FTAAD); Moyamoya disease; transforming growth factor- β (TGF- β); endoglin; signaling Pathway; mitral valve; arteriovenous malformation; Smad; TGF- β receptor; BMP receptor; activin receptor-like kinase (ALK); mitogen-activated protein kinase; fibrillin; Curacao diagnostic criteria; genetic testing; vascular imaging for aortic aneurysm; endovascular aortic repair (EVAR); beta blockers; angiotensin converting enzyme (ACE) inhibitors; losartan; genetic testing; embolotherapy

7.1 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 Chapter 5 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 Chapter 6). 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 TGFBRI mutations, and discuss the pathogenic contribution of TGF- β to these syndromes with a primary focus on the cardiovascular system.

7.2 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].

7.2.1 “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 predominantly forms homodimers that can now interact with and 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 TGFBR1) 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 (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 TGFBR1, results in the phosphorylation of Smad 2 or 3; while activation of the type-I receptor ALK-1 results in the phosphorylation of Smad1, 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] (Figure 7.1).

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 TGFBR1 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].

7.2.2 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 TGFBR1 [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 TGFBR1 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] (Figure 7.1). 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.

7.3 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 7.1). 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.

7.3.1 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 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 TGFBR1 and TGFBR2, 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 ($>1.5\times$ 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 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].

7.3.1.1 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 TGFBR1'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.

7.3.2 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 TGFBR1 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 associated with TGF- β signaling-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 TGFBR2 mutations. An SGS patient with a BAV and an ascending aortic aneurysm that later dissected was found to have a mutation in TGFBR2 [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.

7.3.3 Hereditary Hemorrhagic Telangiectasia (HHT)

Originally described in the 19th 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 5000 to 1 in 8000, 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 percent 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 2 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 was 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 TGFBR1) in endothelial cells [43]. Signaling through TGFBR1 activates Smads2/3, while ALK-1 is unique among type-I receptors in that it activates Smads 1, 5 or 8 (Figure 7.1). 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 TGFBR1 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.

7.3.4 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.

7.4 CURRENT STANDARD OF CARE FOR TAAs AND GENETIC TESTING

7.4.1 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 $> \text{or} = 1 \text{ cm/year}$) 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 $>4.4 \text{ cm}$ 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 the losartan 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.

7.4.2 Genetic Testing

7.4.2.1 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 7 known associated mutations [54].

Prenatal testing is possible, but it is not possible to determine the severity of disease a child with the mutation may inherit. 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.

7.4.2.2 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 3 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 must be identified to diagnose SGS, though identification of an FBN1 or TGFBR mutation may suggest it.

7.4.2.3 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 normal patient. 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.

7.5 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 (Figure 7.2). 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 mesodermally 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 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.

List of 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
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

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FIGURE 5.1.

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 to either repress or activate TGF-Beta related gene expression. The noncanonical pathway is mediated by TGFBR1, 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.

FIGURE 5.2.

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.

TABLE 7.1

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)	TGFBR1 & 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: TGFBR1 OMIM#610380 AAT3: TGFBR2 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; protrusion 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 telangiectasias; 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]