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

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Abstract

Marfan Syndrome (MFS) and Loeys-Dietz Syndrome (LDS) represent heritable connective tissue disorders that segregate 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. With overwhelming evidence of the involvement of aberrant Transforming Growth Factor-beta (TGF-β) signaling in MFS and LDS, this signaling pathway may represent the common link in the relationship between connective tissue disorders and their associated cardiovascular complications. To further explore this hypothetical link, this chapter will review the TGF-β signaling pathway, the heritable connective tissue syndromes related to aberrant TGF-β signaling, and will 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) · Ehlers-Danlos syndrome (EDS) · 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) · Transforming growth factor-β (TGF-β) · Endoglin · Mitral valve · Arteriovenous

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malformation · SMAD · TGF-β receptor · BMP receptor · Activin receptor-like kinase (ALK) · Mitogen-activated protein kinase (MAPK) · Extracellular signal related kinase (ERK) · Fibrillin · Curacao diagnostic criteria · Genetic testing · Beta blockers · Losartan · Doxycycline · Integrins

Abbreviations

AAT	Aortic Aneurysm Thoracic
ACE	Angiotensin Converting Enzyme
AKT	Protein Kinase B
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
ATIIR	Angiotensin II Receptor Type II
ATIR	Angiotensin II Receptor Type I
ATS	Arterial Tortuosity Syndrome
AVM	Arteriovenous Malformation
BMP	Bone Morphogenetic Protein
BMPR1A	Bone Morphogenetic Protein Receptor 1A
BMPR2	Bone Morphogenetic Protein Receptor 2
Co-SMAD	Common SMAD; SMAD-4
CTGF	Connective Tissue Growth Factor
ECM	Extracellular Matrix
ERK1/2	Extracellular Signal-Regulated Kinase 1/2
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	TGF-β Large Latent Complex
LTBP	Latent Transforming Growth Factor-β 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
PI3K	Phosphoinositide-3-Kinase
RA	Rheumatoid Arthritis
R-SMAD	Receptor SMAD
SARA	SMAD Anchor for Receptor Activation
SGS	Shprintzen-Goldberg Syndrome
SLC	TGF-β Small Latent Complex
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
TGFBR1	Transforming Growth Factor-Beta Receptor, Type-I gene
TGFBR2	Transforming Growth Factor-Beta Receptor, Type-I gene
TGF-β	Transforming Growth Factor-Beta
TGF-βRI	Transforming Growth Factor-Beta Receptor, Type-I protein
TGF-βRII	Transforming Growth Factor-Beta Receptor, Type-II protein
TIMPs	Tissue Inhibitors of Matrix Metalloproteinases
TRAF6	Tumor Necrosis Factor Receptor Associated Factor 6

7.1 Introduction

The connective tissue comprises the most abundant and widely distributed primary tissues within the body. Its key functions include protecting, supporting, and insulating our major organs, as well as serving as a fuel reserve by sequestering important growth factors and metabolites. Due to its key role in multi-organ support, it is not surprising that there are over 200 disorders,

both non-genetic and hereditary (including some autoimmune disorders), that impact the structure, function, and integrity of the connective tissue. The Transforming Growth Factor-Beta (TGF-β) superfamily of signaling molecules, is a large structurally related, and functionally overlapping family of signaling proteins including the TGF-βs, the bone morphogenetic proteins, the growth and differentiation factors, and the activins and inhibins. Overall, this family includes approximately 30 different ligands, 7 different type-I receptors, 5 different type-II receptors, and 8 different signaling intermediates (SMAD proteins) (Jones et al. 2009). Among other functions, these factors play key roles in regulating the cellular responses that maintain the balance between extracellular matrix deposition and degradation. Perturbations in homeostatic signaling have been associated with numerous human diseases involving multiple organ systems, of which disorders of the cardiovascular system often carry grave life-threatening consequences.

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 aortic valve regurgitation (Judge and Dietz 2005). In the early 90's, Dietz and Pyeritz (Dietz and Pyeritz 1995) identified key mutations in the fibrillin-1 (FBN-1) gene that were associated with MFS and its related disorders. Fibrillin-1 is a 350 kDa fibular glycoprotein comprised of a series of epidermal growth factor-like motifs, many of which contain calcium-binding sequences, termed calcium-binding EGF (cbEGF) repeats (Yuan et al. 1997). These cbEGF modules are arranged in tandem, separated by cysteine-rich motifs (8-cys/TB) that have high homology to the family of latent transforming growth factor-beta (TGF-β) binding proteins (LTBPs) (Yuan et al. 1997; Ramirez and Pereira 1999). Fibrillin monomers self-assemble into macroaggregates forming the basic structures on which mature elastin fibers are synthesized from tropoelastin subunits. Accordingly, it was postulated that the FBN-1 mutations within the aorta result in a weakened and disordered microfibril network connecting the elastic lamellae to the adjacent interstitial cells, and that this weakening pre-disposed patients to the primary cardiovascular manifestation of MFS, ascending aortic aneurysm, the primary cause of mortality in patients with MFS (Jones and Ikonomidis 2010; Pereira et al. 1999). Dietz and Pyeritz (Dietz and Pyeritz 1995) however, went further to suggest that MFS may be caused by more than just a disordered microfibril matrix, suggesting that the inability of fibrillin to sequester latent TGF-β may play a prominent role in its many pathological manifestations. This was followed by a series of seminal studies in which a TGF-β neutralizing antibody was used in animal models to demonstrate that antagonizing the activation of the TGF-β signaling could reverse the lung, skeletal muscle, mitral valve, and aortic dysfunction associated with MFS (Habashi et al. 2006; Neptune et al. 2003; Ishibashi et al. 2004).

Subsequent to this revelation, in 2005, Loeys and Dietz described a cohort of patients with a connective tissue disorder that significantly overlapped with the phenotype of MFS but included a greater cardiovascular risk (Loeys et al. 2005). This disorder was designated Loeys-Dietz syndrome (LDS, OMIM #609192), and was characterized by the enhanced development of aortic aneurysms and dissections that occur at a younger age and smaller aortic diameter. Both disorders exhibit a marfanoid habitus (*pectus deformity*), arachnodactyly (*elongated fingers*), scoliosis, and dolichostenomelia (*elongated limbs*), heart valve prolapse with significant regurgitation, and aortic aneurysm with dissection. Interestingly, this group of patients presented with mutations in the type-I (TGF-βRI) or type-II (TGF-βRII) TGF-β receptors (Loeys et al. 2005). Moreover, despite mutated receptors incapable of propagating signals, patients with Loeys-Dietz syndrome (LDS) paradoxically exhibited indications of increased TGF-β signaling: enhanced phosphorylation of SMAD-2, a key intracellular mediator of TGF-β signaling, as well as elevated expression of collagen and connective tissue growth factor (CTGF), common indicators of TGF-β pathway activation (Loeys et al. 2005).

As examples, MFS and LDS represent connective tissue disorders that are significantly affected by altered TGF-β signaling and display significant 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, with respect to heritable connective tissue syndromes that present with significant cardiovascular complications and will discuss the pathogenic contribution of TGF-β to these syndromes.

7.2 TGF-β, Signaling Pathways, and Physiological Effects

Transforming growth factor-β is a soluble cytokine secreted by many cell-types within the body. The different three TGF-β isoforms (TGF-β 1, 2, 3) are produced as 55 kDa precursor proteins that homodimerize and bind to an inhibitory peptide fragment called the Latency Associated Peptide, or LAP, to form the Small Latent Complex (SLC). The SLC then associates with a Latent Transforming Growth Factor-Beta Binding Protein (LTBP), to form the Large Latent Complex (LLC), which is then secreted from the cell and targeted for sequestration in the extracellular matrix (Gentry et al. 1988; Lawrence et al. 1984; Zeyer and Reinhardt 2015). Fibrillin-1, comprised of multiple cbEGF motifs arranged in tandem with 8-cys/TB repeats, has high homology to the family of LTBPs, and provides a landing point to sequester the LLC within the matrix (Yuan et al. 1997; Pereira et al. 1999; Isogai et al. 2003). Thus, the ECM serves to sequester and concentrate TGF-β in locations where it may be rapidly activated when needed (Ramirez and Rifkin 2003). Indeed, the ECM is no longer thought to be a passive structural support but rather a dynamic regulator of growth factor bioavailability and signaling (Brekken and Sage 2001; Hynes 2009).

7.2.1 "Canonical" TGF-β Signaling

Mature TGF-β is activated through several different known mechanisms including: proteolysis of the LTBP or LAP by the action of several matrix metalloproteinases (MMP-2, MMP-9, MMP-13, MMP-14); interaction of the LAP with thrombospondin-1; exposure of the LAP to extracellular reactive oxygen species; a decrease in extracellular matrix pH effectively causing degradation of the LAP; or through interaction with a family of transmembrane protein receptors called the integrins, including $\alpha_v\beta_1$, $\alpha_v\beta_3$, $\alpha_v\beta_6$, and $\alpha_v\beta_8$ (Annes et al. 2003; Costanza et al. 2017; Patsenker et al. 2009; Ramirez and Rifkin 2009; Reed et al. 2015; Roth et al. 2013). Once released from the LAP, the TGF-β homodimer interacts with a family of TGF-β receptors initiating a signaling cascade. The TGF-β receptors have been subdivided into three families. The type-I and type-II receptors belong to a superfamily of tissue- and cell- specific transmembrane serine/threonine kinase receptors. The type-III receptors, also known as accessory receptors, including endoglin and betaglycan, contain transmembrane monomers that lack the kinase domain, but function to bind and present the TGF-β homodimer to the type-I and type-II receptor complex (Wrana et al. 1992).

In the "Canonical TGF-β Signaling Pathway" TGF-β binds to a homodimer of the type-II receptor, which auto-activates its serine/threonine kinase domain, resulting in the autophosphorylation of the type-II receptor. The phosphorylated type-II receptor, recruits a homodimer of the type-I receptor, and activates the type-I receptor through a transphosphorylation event. The activated type-I receptor then recruits and phosphorylates one of a class of cytoplasmic mediators, the receptor regulated SMADs (R-SMADs), these intracellular signaling intermediates were named for their homologues in *Caenorhabditis elegans* (SMA genes) and *Drosophila* (MAD

genes; mothers against decapentaplegic). The activated R-SMAD then recruits and binds to a common-mediator SMAD (co-SMAD; SMAD-4) forming a complex revealing a nuclear localization signal, and is then shuttled into the nucleus where, upon interaction with transcriptional modifiers (activators or repressors), it forms a competent transcription complex capable of inducing or repressing numerous TGF-β-dependent genes (Fig. 7.1) (Jones et al. 2009; Shi and Massague 2003).

7.2.2 Alternate "Noncanonical" TGF-β Signaling

In addition to the canonical signaling pathway, a growing body of evidence now supports the hypothesis that TGF-β signaling can proceed by alternative mechanisms that bypass key mediators in the classical pathway (Moustakas and Heldin 2005). For example, these noncanonical signaling responses include: 1) signals propagated directly by type-II receptors without type-I receptor

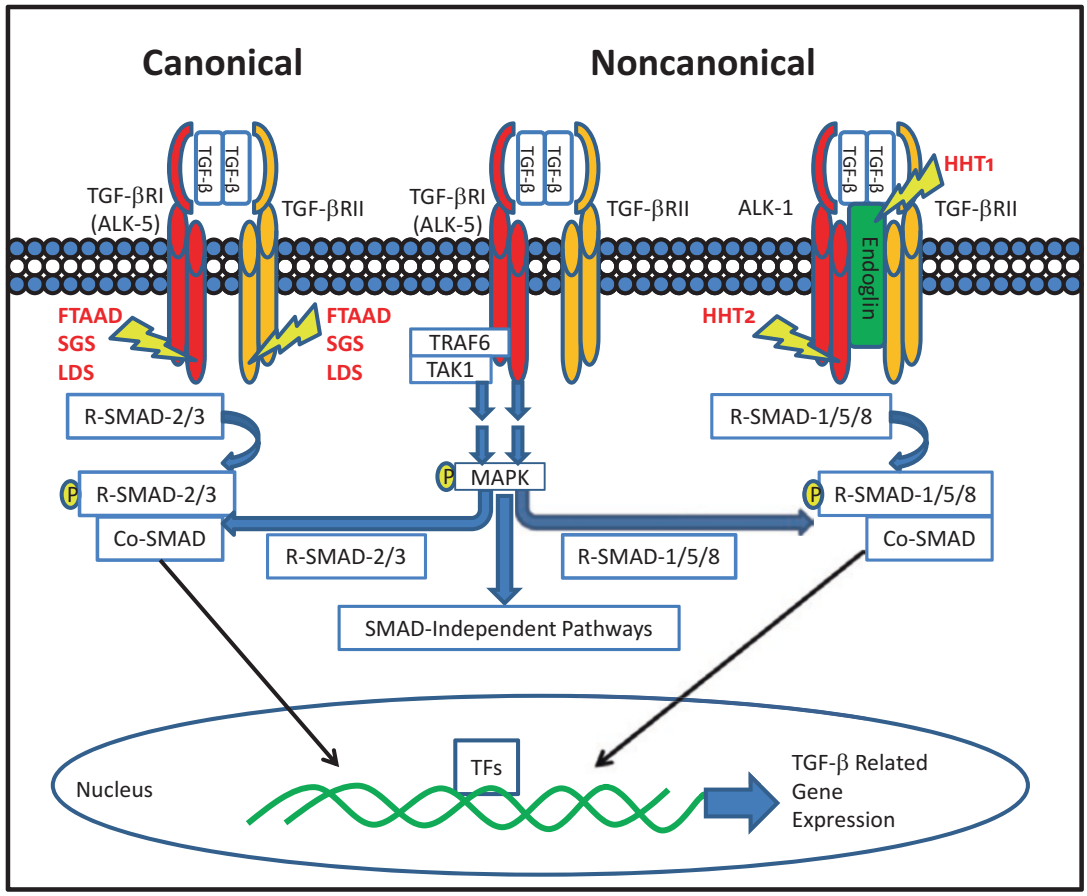

Fig. 7.1 Canonical, Noncanonical, and Endoglin/ALK-1 signaling pathways. Both Canonical and Endoglin/ALK-1 TGF-β signaling is mediated by the phosphorylation of distinct R-SMAD proteins. Nuclear translocation requires Co-SMAD binding in both pathways. The R-SMAD and Co-SMAD complex, once translocated to the nucleus, associates with other transcriptional regulatory factors to either repress or activate TGF-β-dependent gene expression. The noncanonical pathway is mediated by TGF-βRI, TRAF6, and TAK1, resulting in the phosphorylation of other intracellular signaling intermediates such as ERK1/2, JNK, and p38 MAPK. These MAPK-dependent signals can re-enter the SMAD-dependent pathway through direct activation of the SMADs, or they can mediate downstream signaling through SMAD-independent pathways. The lightning bolts represent mutations to the indicated proteins causative of the syndromes listed in red

involvement; 2) direct type-I receptor signals in the absence of R-SMAD activation; 3) R-SMAD signaling to parallel pathways in the absence of co-SMAD involvement; and 4) activation of R-SMADs by other signaling mediators in response to TGF-β, but not as a result of direct interaction with TGF-β receptors (Jones et al. 2009).

The downstream intracellular mediators of these alternative pathways are not as well understood as the role of the SMAD proteins in the classical pathway. Studies of noncanonical signaling in FBN1 deficient mice have proven helpful in this regard. Carta et al. demonstrated *in vivo* that p38 mitogen-activated protein kinase (p38 MAPK) mediated the phosphorylation of SMAD-2/3, which was attenuated by treatment with p38 MAPK inhibitors. This suggested that R-SMAD activation/phosphorylation could occur in a manner independent of TGF-βRI activity (Carta et al. 2009). Subsequent studies examining the role of Tumor Necrosis Factor-Receptor Associated Factor 6 (TRAF6), an E3 ubiquitin ligase, demonstrated association with TGF-βRI in a TGF-β-dependent manner (Yamashita et al. 2008; Sorrentino et al. 2008), however, this complex was found to recruit and activate a secondary intracellular kinase called TGF-β Associated Kinase 1 (TAK1), which was shown to subsequently activate p38 MAPK (Fig. 7.1) (Yamashita et al. 2008; Sorrentino et al. 2008). In addition to p38 MAPK, TGF-β can activate many other signal pathways independent of R-SMAD mediation including: Extracellular-signal Regulated Kinase 1 and 2 (ERK1/2) (Lee et al. 2007), c-Jun N-terminal Kinase (JNK) (Yamashita et al. 2008; Sorrentino et al. 2008), and the Phosphoinositide-3-Kinase (PI3K), in the protein kinase B (AKT)-mediated cellular survival pathway (Wilkes et al. 2005). Importantly, the canonical and noncanonical pathways appear to exert differential effects on the connective tissues within the ECM. Thus, through combinatorial receptor interactions, multiple regulatory mechanisms, and alternative signaling pathways, the complexity of signaling through this pathway is dramatically amplified, contributing to the myriad complex signaling outcomes reported for TGF-β.

7.2.3 Cellular Responses to TGF-β Signaling

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 a well-established modulator of ECM structure and composition (Bertolino et al. 2005; Massague 2000). Stimulation of the canonical TGF-β pathway has been well accepted as a primary driver of increased extracellular matrix protein production and deposition (*collagen and elastin*) (Ignotz and Massague 1986). In addition, while driving matrix production the pathway simultaneously functions to attenuate the expression of matrix proteolytic enzymes (*matrix metalloproteinases (MMPs)*) (Yan and Boyd 2007), and enhances the expression of the tissue inhibitors of MMPs (TIMPs) (Kwak et al. 2006). Alternatively, stimulation of the noncanonical pathways has been associated with enhanced matrix degradation through increased MMP-dependent proteolysis (Kim et al. 2004) and enhanced MMP activation via plasminogen activators (Laiho et al. 1986). This is supported by work from Kim et al. demonstrating that the activation of p38 MAPK-mediated signaling has been associated with increased MMP-2 and -9 production and release in breast cancer cells (Kim et al. 2004). In similar fashion, *in vitro* expression of MMP-13 in rat osteoblasts was found to be dependent on p38 MAPK, involving SMAD-2 activation and ERK1/2 signaling (Selvamurugan et al. 2004).

The complexity of this signaling pathway highlights the importance of TGF-β as a key regulatory factor in maintaining the homeostatic balance within the ECM, and further underscores how dysregulation of this pathway could play a dominant role in connective tissue disorders and disease. Accordingly, regulation of this pathway is highly critical at multiple levels. An example of this regulation is the inhibitory feedback pathway involving I-SMADs (*inhibitory SMAD6 and SMAD7*) (Park 2005). SMAD6 exerts its effects by binding directly to type-I receptors and blunt-

ing R-SMAD phosphorylation (Imamura et al. 1997). SMAD6 also inhibits signaling by competing with SMAD-4 for R-SMAD binding sites, reducing nuclear translocation (Hata et al. 1998). In addition, SMAD7 inhibits TGF-β signaling by targeting both type-I and type-II receptors for ubiquitination and subsequent degradation, through the recruitment of SMAD Ubiquitination Regulatory Factors 1 and 2 (Wicks et al. 2006; Ebisawa et al. 2001; Kavsak et al. 2000). Lastly, there are many other regulatory proteins that influence the bioavailability of TGF-β, such as proteoglycans decorin and biglycan, which bind and scavenge its availability for signaling (Droguett et al. 2006; Lopez-Casillas et al. 1994; Stander et al. 1999).

This group of TGF-β receptors and intermediates is a small part of a larger "superfamily" of growth factors and receptors (Feng and Derynck 2005). 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 (ALK-2), a type-I BMP receptor, are associated with fibrodysplasia ossificans progressive (FOP), a skeletal dysplasia characterized by progressive heterotopic bone formation (Pignolo et al. 2011). Dysregulation of the activin and inhibin signaling has yet to be 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 (Werner and Alzheimer 2006).

7.3 TGF-β Signaling and Connective Tissue Disorders

In addition to MFS and LDS, several heritable connective tissue disorders have been associated with mutations in the TGF-β signaling pathway including Ehlers-Danlos Syndrome (EDS), Familial Thoracic Aortic Aneurysms and Dissections (FTAAD), Shprintzen-Goldberg Syndrome (SGS), and Hereditary Hemorrhagic Telangiectasia (HHT), among others (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. These disorders will be explored in more detail below.

7.3.1 Ehlers-Danlos Syndrome

The Ehlers-Danlos syndromes (EDS) are a clinically diverse and genetically heterogeneous group of inherited connective tissue disorders primarily characterized by causal mutations in genes encoding collagen, its modifying enzymes, or other proteins involved in extracellular matrix formation (Cortini et al. 2019). These mutations lead to a loss of structural integrity within different organ systems, and are commonly characterized by joint hypermobility, skin hyperextensibility, and tissue fragility. To date, there have been 13 different subtypes of EDS defined based on diverse clinical presentation and genetic heterogeneity (Malfait et al. 2017). Of these 13 subtypes, two have been associated with altered TGF-β signaling, hypermobile EDS (Type-III) and Vascular EDS (Type-IV). Ehlers-Danlos syndrome type III (EDS, OMIM #130020), also known as Hypermobile Ehlers-Danlos Syndrome (hEDS), is the most prevalent subtype of EDS, and is primarily characterized by marked joint hypermobility in the absence of skeletal deformity, with a milder involvement of the skin as typically observed in the classical and vascular types of EDS. Commonly, patients with hEDS present with softer "velvety" skin, that displays reduced thickness, and increased fragility. In addition, an impaired wound healing response often results in atrophic scarring. While the primary underlying genetic basis of hEDS has yet to be identified, several candidate genes have been identified, most without definitive evidence proving causality (Gensemer et al. 2020). Of these candidate genes, TNXB (Tenascin X) may further highlight the association between aberrant TGF-β signaling and cardiovascular complications in hEDS.

Table 7.1 TGF-β Related Heritable Connective Tissue Disorders

Connective Tissue Syndrome	Associated Mutations	Connective Tissue Manifestations	Cardiovascular Manifestations	References
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 and Dietz (2005)) Dietz (1993))
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); Loeys et al. (2006))
Ehlers-Danlos Syndrome (EDS), Type-III and Type-IV	Tenascin X TGF-βRII COL3A1 OMIM# 130020 OMIM# 130050	Hypermobile joints, reduced skin thickness, increased skin fragility, atrophic scarring, aberrant TGF-β signaling	Enhanced levels of circulating TGF-β, aortic and small vessel aneurysm, left ventricular dysfunction, heart failure, mitral valve insufficiency	Gensemer et al. (2020)) Morissette et al. (2014)) Sylvan et al. (2013)) Superti-Furga et al. (1988) Boutouyrie et al. (2004)
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) Van Steensel et al. (2008)
Familial thoracic aortic aneurysm and dissection syndrome (FTAAD)	AAT1–11 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 (2005) Pannu et al. (2005)
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)

(continued)

Table 7.1 (continued)

Connective Tissue Syndrome	Associated Mutations	Connective Tissue Manifestations	Cardiovascular Manifestations	References
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; telangiectasias; pulmonary artery stenoses and aneurysms	Callewaert et al. (2008) Coucke et al. (2006)
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 and Shovlin (2009) Fernandez et al. (2006)

Tenascin X is an extracellular matrix protein that plays an essential role in collagen deposition by regulating the spacing between collagen fibrils. Interestingly, Tenascin X-deficiency has been associated with increased expression and abundance of TGF-β ligands, elevated TGF-β signaling, activation of signaling intermediates (SMAD-1/5/8), and increased MMP-13 production (Morissette et al. 2014). While enhanced levels of circulating TGF-β resulting in signaling through the classical TGF-β pathway, are hard to reconcile with the aberrant wound healing and atrophic scarring observed in hEDS, these results may suggest that the signaling proceeds through a non-canonical TGF-β pathway, dominated by the activation of SMAD-1/5/8. Reports by Bertolino and Jones, have suggested that TGF-β ligands (TGF-β 1, 2, 3) can signal through a second Type-I TGF-β receptor, Activin receptor-Like Kinase 1 (ALK-1), when recruited to TGF-βRII, resulting in the activation of a different set of intracellular R-SMADs (SMAD-1/5/8) (Jones et al. 2009; Bertolino et al. 2005; Jones et al. 2008). Interestingly, activation of this alternative pathway may be associated with ECM degradation by leading to enhanced MMP-13 production, resulting in the diminished collagen network.

In another report by Sylvan and coworkers, a patient with a complex medical history including a previous diagnosis of type-III EDS, mitral valve replacement surgery at age 18, left ventricular dysfunction, congestive heart failure, and pulmonary issues was identified (Sylvan et al. 2013). The patient presented with multiple small vessel aneurysms, and upon further genetic testing, a mutation in the TGF-βRII was identified, while confirming the absence of any COL3A1 mutations. While this report represents a single case study, the presence of a TGF-β receptor mutation in the absence of COL3A1 mutations (*commonly observed in Type-IV EDS; vascular EDS*) and in the absence of an LDS phenotype, may also suggest TGF-β receptor mutations can contribute to hEDS.

Ehlers-Danlos Syndrome type IV (EDS, OMIM #130050), also known as vascular type EDS, primarily affects the skin and large arteries, and can lead to medial degenerative disease of the aorta resulting in acute dissection. The original cause was linked to a 3.3-kb DNA deletion in one allele of the type III procollagen gene (COL3A1). This mutation, which results in a truncated procollagen monomer is characterized by decreased thermal stability, resistance to proteolytic processing, and reduced secretion

(Superti-Furga et al. 1988). Ultimately, in the aorta, it results in a diminished collagen network, with a low intimal-medial thickness, increased wall stress, and an increased propensity for acute dissection and rupture (Boutouyrie et al. 2004). As with many connective tissue disorders on this spectrum, diagnosis of EDS type IV can be difficult since there is significant phenotypic overlap with patients presenting with LDS. Loeys et al. (Loeys et al. 2006), while characterizing 52 LDS-affected families for mutations in TGFBR1 and TGFBR2 genes also assessed a cohort of EDS type IV patients that lacked the COL3A1 gene mutations and the craniofacial features of the typical LDS patient. Twelve EDS type IV probands were identified containing TGF-βRI or TGF-βRII mutations, suggesting a possible reclassification of this group as LDS type 2. One could speculate that EDS type IV patients with COL3A1 mutations display enhanced TGF-β signaling to compensate for the loss of type III collagen within the aorta. Nonetheless, these results further bolster the association between cardiovascular complications in connective tissue disorders involving aberrant TGF-β signaling.

7.3.2 Familial Thoracic Aortic Aneurysm and Dissection Syndrome (FTAAD)

Classically, FTAAD was defined as a collection of inherited genetic disorders, leading to thoracic aortic aneurysms or aortic dissections, characterized by medial necrosis of the proximal ascending aorta (Nicod et al. 1989). Diagnosed patients typically had a first degree relative who also had aortopathy, however diagnosis was often clouded by dominate inheritance with variable penetrance (Milewicz et al. 1998). Medial necrosis is described as degeneration and fragmentation of elastic fibers, loss of smooth muscle cells, with the presence of interstitial pools of basophilic ground substance. This typically occurs in the absence of a known genetic syndrome, like MFS. Aortic disease in these families is characterized by aneurysms involving the ascending aorta often leading to type I and II aortic dissections in the absence of hypertension (Guo et al. 2001). Since its original description, FTAAD has been localized to multiple genetic loci, now with at least 11 independently associated genes identified in the Online Mendelian Inheritance in Man (OMIM) database: Aortic Aneurysm Thoracic (AAT) 1–11. Interestingly, four of these mutations, AAT3 (OMIM#608967; TGF-βRII), AAT5 (OMIM#610380; TGF-βRI), AAT9 (OMIM#601103; MFAP5) and AAT10 (OMIM#617168; LOX), were found in genes capable of affecting the connective tissue directly. AAT3 and AAT5 were found to be associated with mutations in TGF-β-receptors (receptors -II and -I respectively) and have subsequently been grouped under the heading of Loeys-Dietz Syndrome as LDS2 and LDS1, to simplify classification overall. AAT9, was associated with a mutation in the Microfibrillar Associated Protein 5 (MFAP5) gene, which encodes Microfibril-Associated Glycoprotein 2, a small proteoglycan involved in the assembly and/or maintenance of elastic fibers (Barbier et al. 2014), while AAT10, was found to encode Lysyl Oxidase (LOX), a protein that plays a critical role in initiating the crosslinking of collagens and elastin. In each case, the initial precipitating event was often incidentally discovered as aortic dilatation, dissection, or sudden death (Attias et al. 2009; Bruno et al. 1984; Von Kodolitsch et al. 2004). 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 exacerbated by contributing risk factors such as 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 (Pannu et al. 2005). Diagnosing this group of patients remains complicated because of the variable penetrance within a family group.

With new and refined genetic technologies, additional genes are being added to this list on a

regular basis, underscoring the importance of the connective tissue compartment, and further defining the link between dysregulation of extracellular matrix and cardiovascular complications.

7.3.3 Shprintzen-Goldberg Syndrome (SGS)

In 1982, Shprintzen and Goldberg first described their eponymous heritable connective tissue syndrome in two patients (Shprintzen and Goldberg 1982). Shprintzen-Goldberg syndrome is characterized by anomalies of the head/face, skeleton, brain, and cardiovascular system (Greally et al. 1998). Shprintzen-Goldberg syndrome has since been recognized as part of a group of phenotypically overlapping syndromes associated with TGF-β receptor mutations (i.e. LDS and FTAAD) affecting connective tissues and the cardiovascular system (Akutsu et al. 2007). However, SGS has been linked to mutations in TGF-β RI and RII, 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 TGF-β receptor, or a connective tissue component like fibrillin-1. 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 is 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 (Greally et al. 1998). These impairments are known to occur simultaneously with brain abnormalities: communicating hydrocephalus, dilated lateral ventricles, and Arnold-Chiari formation type-I (Robinson et al. 2005). 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 (Loeys et al. 2006), however, hypertelorism (seen in LDS), myopia, and exophthalmos are commonly observed (Greally et al. 1998). In addition, several skeletal anomalies have been identified, appearing in early childhood (Ades et al. 1995). The major skeletal characteristic is scaphocephaly (*boat shaped skull*) with craniosynostosis (*premature fusion of skull*) (Ades et al. 1995). In fact, SGS has been referred to as marfanoid habitus with craniosynostosis (Ades et al. 2006). Many of the other skeletal findings associated with MFS and LDS are likewise observed in SGS: dolichostenomelia, arachnodactyly, scoliosis, pectus excavatum or carinatum (*hollowed or pigeon chest*), joint hypermobility, and contracture of the proximal joints of the hand (Loeys et al. 2006). 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 (Greally 1993). Additional characteristic findings include: minimal subcutaneous fat, hypotonia, obstructive apnea, defects in the abdominal wall musculature with hernias, hyperelastic skin, and cryptorchidism (Greally 1993).

The cardiovascular defects in SGS are mostly limited to the heart valves. Mitral and/or aortic valve regurgitation is commonly observed (Greally et al. 1998). Mitral Valve Prolapse (MVP), often seen in MFS, occurs commonly, as well (Greally 1993). Given that FBN1 mutations have been associated with an increase in TGF-β release and signaling, Ng et al. examined the association of aberrant TGF-β pathway signaling and the pathogenesis of MVP using a mouse model of MFS (Ng et al. 2004). Changes in mitral architecture were observed to be temporally and spatially linked with increased TGF-β activation resulting in enhanced proliferation/growth of valve cells and decreased apoptosis. 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 well. While common in MFS, LDS, and FTAAD, aortic root dilatation and aneurysm has been previously described in SGS but is not present in most affected individuals (Loeys et al. 2005). The presence of aortic dilatation may therefore represent overlap with one of these phenotypically

similar syndromes. Aortic valve pathology has also been linked to TGF-βRII mutations. An SGS patient with a bicuspid aortic valve and an ascending aortic aneurysm, which later dissected, was found to have a mutation in TGF-βRII (Girdauskas et al. 2011). Thus, both the mitral and aortic valvular manifestations of SGS may be due to mutations in TGF-β receptors, and alterations in the signaling pathway.

7.3.4 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 (Macri et al. 2020; Sys and Van Den Hoogen 2005). HHT is most commonly caused by mutations within TGF-β receptors, disrupting TGF-β signaling, and inducing 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 (Bideau et al. 1989; Kjeldsen et al. 1999; Shovlin et al. 2008). 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 (Bourdeau et al. 1999; Cole et al. 2005; Wallace and Shovlin 2000).

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 (Pasculli et al. 2005; Plauchu et al. 1989). 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 arteriovenous malformations (AVMs), vascular abnormalities resulting from malformed connections between arteries and veins in the visceral organs (Kjeldsen et al. 1999).

While 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%) (Cottin et al. 2004; Fulbright et al. 1998; Piantanida et al. 1996). A further pulmonary manifestation of HHT is severe pulmonary artery hypertension (PAH) arising mainly from 2 sources: (1) high output heart failure secondary to hepatic AVM shunting and (2) primary PAH without signs of heart failure (Govani and Shovlin 2009).

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 (Shovlin et al. 2007). Reports of defects in adaptive immunity and a mononuclear cell infiltrate around telangiectasias 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 (Cirulli et al. 2006).

The Curacao diagnostic criteria are based on international consensus and used to diagnose HHT with a score that gauges the likelihood of its

presence (Faughnan et al. 2011; Shovlin et al. 2000). 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. The presence of more than two criteria is evidence of "definitive" 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.

Mutations in at least five genes have been directly associated with the development of HHT. These are subdivided based on the gene loci involved (Govani and Shovlin 2009). HHT1 and 2 are the major subtypes linked to mutations within endoglin (HHT1, OMIM#187300) (Mcallister et al. 1994) and ALK-1 (HHT2, OMIM#600367) (Berg et al. 1997; Johnson et al. 1996). Additionally, mutations in SMAD4 result in 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 SMAD-4 (Govani and Shovlin 2009). However, a family with a history of juvenile polyposis, aortopathy, and mitral valve dysfunction, was described by Andrabi and coworkers, suggesting co-segregation of these phenotypes with a mutation in SMAD-4 (Andrabi et al. 2011). Aortopathy and mitral valve defects are more typically related to MFS and LDS, not HHT, though case reports of large vessel aneurysms in HHT do exist (Andrabi et al. 2011) The presence of these features associated with SMAD4 mutations further supports the role of dysfunctional TGF-β signaling in the common pathogenesis of disorders. Furthermore, it provides a spectral link between the vascular features of MFS and LDS (aortopathy, aneurysm, and mitral valve defects) 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 (Letarte et al. 2005). Interestingly, homozygous ALK-1 mutations in zebrafish and mice produce embryonic lethality and exhibit severely dilated vessels (*including the aorta*) and abnormal vessel fusion (Oh et al. 2000; Roman et al. 2002). 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 highly expressed in the developing endothelium of arteries (Seki et al. 2003). Taken together, these observations support the role of TGF-β signaling in early vascular development and dilatation.

As mentioned above, TGF-β ligands are capable of signaling through two distinct type-I receptors, TGF-βRI and ALK-1, when complexed with TGF-βRII, resulting in different groups of SMAD proteins being activated intracellularly; TGF-βRI activates SMAD-2/3, whereas ALK-1 activates SMAD-1/5/8 (Fig. 7.1) (Bertolino et al. 2005). Endoglin plays an interactive role with ALK-1 and is required for TGF-β-dependent ALK-1 signaling, through mediating/facilitating the binding of TGF-β to the ALK-1 receptor (Bertolino et al. 2005). TGF-β stimulation of the endoglin/ALK-1 pathway activates SMAD-1/5/8 and has been associated with endothelial proliferation and migration, both essential to angiogenesis. Alternatively, signaling through ALK-5 pathway activates SMAD-2/3 and produces an opposite response, inhibiting proliferation and migration (*quiescence*) (Stefansson et al. 2001). 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 can regulate the expression of ALK-5, such that decreased ALK-1 signaling leads a reduction in ALK-5 signaling, as evidenced by an 80% decrease in ALK-5 mRNA transcripts as measured in peripheral blood endothelial cells from

HHT1 and HHT2 patients (Fernandez et al. 2005). 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 (Goumans et al. 2003). Alternatively, similar to LDS in which increased TGF-β signaling was described despite TGF-β -RI and -RII 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*) was observed in <2% of HHT patients, and was identical to inherited primary PAH due to a loss of function mutation in Bone Morphogenetic Protein Receptor 2 (BMPR2); another type-II receptor in the TGF-β superfamily (Govani and Shovlin 2009). 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 (Davies et al. 2012; Kimura et al. 2000; West et al. 2004). When examining families with both HHT and PAH, a suggestive linkage between BMPR2 and ALK-1 was identified (Trembath et al. 2001). 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. Mutations in endoglin, facilitating signaling through ALK-1, may also result in PAH. Type-III TGF-β receptors have been implicated in the TGF-β-mediated growth inhibition in myoblasts, with dependence on SMAD-3 activation and p38 MAPK signaling (Roman et al. 2002; You et al. 2007). 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.

7.3.5 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, or scleroderma, rheumatoid arthritis (RA), and systemic lupus erythematosus (SLE). The severity of scleroderma, a disease of excessive fibrosis of vessels, organs, and particularly the skin, has been associated with increased levels of TGF-β signaling (Hawinkels and Ten Dijke 2011). Patients with rheumatoid arthritis (RA), often present with elevated plasma levels of both thrombospondin-1 and TGF-β; both of which are associated with early onset atherosclerosis commonly observed in RA (Rico et al. 2008; Rico et al. 2010). Other studies have provided evidence that TGF-β can play a role in suppressing immune function directly and stimulating T-cell conversion to a suppression phenotype (Wahl and Chen 2005). Interestingly, TGF-β production is decreased in SLE (Ohtsuka et al. 1998). Thus, a lack of TGF-β may contribute to SLE through diminished ability to suppress the immune system (Mageed and Prud'homme 2003). Due to its relationship with fibrosis and immune modulation, TGF-β may plausibly be involved in many autoimmune connective tissue disorders though its exact roles remain to be clarified.

Further 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 display symptoms overlapping with the other TGF-β receptor mutation syndromes. A mutation in SMAD-3 was recently linked to a heritable syndrome of vascular aneurysms, arterial tortuosity (*twisted, corkscrew like arteries*), skeletal/craniofacial abnormalities with osteoarthritis, and is referred to as Aneurysm-Osteoarthritis Syndrome (AOS) (Van De Laar et al. 2011). Similarly, arterial tortuosity syndrome (ATS), characterized by tortuosity of medium to large vessels and aneu-

rysms, has associated with loss-of-function mutations in SLC2A10 (Solute Carrier Family 2, Facilitated Glucose Transporter Member 10) (Coucke et al. 2006; Loeys and De Paepe 2008). Increased TGF-β signaling is also believed to be associated with this syndrome, as vascular smooth muscle cells from ATS patients exhibit decreased production of decorin, a small leucine rich proteoglycan known to bind and sequester TGF-β in the ECM (Coucke et al. 2006). The decorin promoter contains a glucose response element which is less active, resulting in fewer functional glucose transporters, creating a decorin deficiency that can result in increased TGF-β ligand abundance and signaling (Coucke et al. 2006).

7.4 TGF-β-Directed Therapy as a Prime Target for Connective Tissue Disorders

The evidence presented above highlights the indominable role of TGF-β in connective tissue disorders with cardiovascular complications. Understanding the mechanistic underpinnings of these disorders may allow for the development of specific therapies designed to interrupt aberrant TGF-β signaling and perhaps diminish the primary complication leading to mortality in many of these disorders. The following examples are provided as potential evidence to support therapeutic options targeted at attenuating the TGF-β signaling.

In a very short period of time, significant progress has been made in understanding the cause, clinical pathogenesis, and clinical management of MFS (Pyeritz 2016). The dilation of the aortic root and its dissection or rupture, are the primary manifestations of MFS that drive mortality in these patients. Thus, much effort has been focused on how to treat these sequelae in hope of attenuating aneurysm disease. As a first line therapy for all aneurysm patients (with MFS or not) β-blockers (e.g. atenolol) are used to regulate systemic blood pressure as well as dP/dt (rate–rise time) of the cardiac pulse wave as it moves through the aorta. This therapy was chosen based on the rationale that regulating systemic blood pressure is likely advantageous to prevent rupture and dissection of an aneurysm. In an effort to build on this principle, Habashi et al. attempted treating MFS mice (C1039G/+), which spontaneously develop ascending/aortic root aneurysms, with another class of antihypertensives, the angiotensin-II receptor blockers (ARBs), specifically losartan (Habashi et al. 2006). Losartan became a drug of interest for several reasons. Previous studies by Daugherty and coworkers, discovered that treating mice that had been induced to develop abdominal aortic aneurysms (AAAs) with Angiotensin-II (AngII) infusion, using an AngII **type-I** specific receptor (AT1-receptor) blocker (losartan), could attenuate AAA formation, while treatment with an Ang-II **type-II** specific receptor (AT2-receptor) inhibitor (PD123319), enhanced aortic pathology and accelerated aneurysm development (Daugherty et al. 2001). Taken together, this suggested that signaling through the AT1-receptor had deleterious consequences, accelerating aortic pathology, while signaling through the AT2-receptor may have been protective. More importantly, many studies had suggested that stimulation of the AT1-receptor could enhance the expression and activation of TGF-β ligands and receptors (Everett et al. 1994; Fukuda et al. 2000; Naito et al. 2004; Wolf et al. 1999), therefore, inhibition of AT1-receptors may be advantageous toward reducing overall TGF-β signaling. Not surprisingly, when Habashi and colleagues treated MFS (C1039G/+) mice with losartan, aortic dilation and the phosphorylation of SMAD-2 were both attenuated (Habashi et al. 2006). Furthermore, losartan was also shown to rescue the TGF-β-dependent skeletal muscle defects and the alveolar septation defects observed in the MFS mice (Judge et al. 2011). Subsequent studies by Carta et al. (Carta et al. 2009) and Rodriguez-Vita et al. (Rodriguez-Vita et al. 2005) went further to demonstrate that AngII could stimulate the phosphorylation of SMAD-2, independent of signaling through TGF-βRI, resulting in enhanced production of CTGF. Importantly, losartan treatment was able

to attenuate both the phosphorylation of SMAD-2 and the accumulation of CTGF, implicating an additional role for an unknown downstream mediator in the AT1-receptor pathway. These data not only linked TGF-β to aortic pathology, but also clearly demonstrate that the inhibition of the AT1-receptor is capable of attenuating TGF-β signaling.

Xiong and colleagues discovered that doxycycline, an FDA approved tetracycline antibiotic with non-specific MMP-inhibitory activity, could delay rupture of the ascending aorta in fibrillin-1 under-expressing mice (mgR/mgR mouse model) (Xiong et al. 2008). This was directly associated with doxycycline's ability to inhibit matrix metalloproteinases (MMP) -2 and − 9. Further, the study showed that doxycycline enhanced the preservation of elastic fiber integrity, normalized aortic wall stiffness, and prevented vessel wall weakening when compared to β-blocker therapy alone (Chung et al. 2008). When compared head-to-head with losartan, doxycycline was shown to inhibit both MMP-2 activation and the phosphorylation of ERK1/2 (Xiong et al. 2012). Interestingly, the action of doxycycline wasn't attributed to its effects on MMP activity, but rather to its secondary effect of inhibiting MMP-2-dependent TGF-β release. Accordingly, further studies of doxycycline alone and in combination with β-blockers or ARBs, should be considered in the treatment of other TGF-β-dependent connective tissue disorders.

Finally, progress in understanding the process of TGF-β release and activation as mediated by membrane integrins should be further explored as a potential therapy in the treatment of TGF-β-dependent connective tissue disorders. Recent reports have demonstrated roles for integrin αvβ1 and αvβ3 in the activation and release of TGF-β, in processes leading to the development of fibrosis. Newly developed integrin-specific inhibitors such as C8 (αvβ1 specific) (Reed et al. 2015) or Cilengitide (αvβ3/αvβ5 specific) (Patsenker et al. 2009; Roth et al. 2013; Li et al. 2013) have been used to attenuate TGF-β activation and its downstream signaling response.

Together, as the role of TGF-β signaling in connective tissue disorders continues to be elucidated, it is important to note that therapeutic advances targeted at attenuating aberrant TGF-β signaling events may provide significant benefit by enhancing quality and quantity of life, given that the cardiovascular-associated disorders are often the most life-threatening manifestations of these disorders.

7.5 Genetic Testing

As reported herein, identifying and diagnosing heritable connective tissue disorders is not without significant challenges. As noted, many of the disorders have significantly overlapping symptomology. This is further complicated by their often-dominant inheritance with variable penetrance, leaving the patient and their physician to examine family history and the overt phenotypic symptoms in an effort to make an accurate diagnosis. Accordingly, the use of genetic testing through new technologies such as Genome Wide Association Studies (GWAS) and whole exome Next Generation Sequencing (NGS), provides some hope in identifying mutations that may be associated with a given disorder. The interpretation of the genetic sequencing data alone, however, continues to provide significant challenges due to a large number of sequence variants and private mutations, further complicated by the fact that many of the missense mutations responsible for disease often resemble benign genetic variation. Thus, clinical symptoms and common phenotypes are often used in combination with NGS results to narrow down the possibilities, identifying the specific underlying genetic variants that cause disease. As reported by Pope and coworkers, the presence of cardiovascular disease with a documented family history, is a suitable indication for NGS, based on the presence of the large number of causative sequence variants that are well associated with heritable connective tissue disorders (Pope et al. 2019). Together, the phenotype and genotype can be used diagnostically with a much greater chance of identifying the underlying cause of disease. Once identified, then familial inheritance can be back confirmed, and specific treatment plans can be made. Thus, this section

will discuss the role of genetic testing in some of the heritable connective tissue disorders identified above.

7.5.1 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 potential 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 (Milewicz et al. 2010). 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 (Milewicz et al. 2010). 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 (Milewicz et al. 2010). This recommendation is particularly relevant because only ~30% percent of FTAAD patients will have one of the 11 known associated mutations (Biddinger et al. 1997; Coady et al. 1999; Pomianowski and Elefteriades 2013).

7.5.2 Genetic Testing for SGS

SGS is clinically suspected when an individual present 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 (Greally et al. 1998). 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 with other heritable connective tissue disorders, and the proclivity for 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 (Kosaki et al. 2006; Sood et al. 1996). A later genetic study of multiple SGS patients found no FBN1 mutations (Ades et al. 2006). 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 significant phenotypic overlap between SGS and LDS (Van Steensel et al. 2008). In a study describing TGF-β receptor mutation phenotypes, a TGFBR1 mutation was identified in a patient with clinically diagnosed SGS (Stheneur et al. 2008). Current criteria do not require the identification of a specific mutation to diagnose SGS, though identification of an FBN1 or TGFBR mutation may help to confirm the diagnosis.

7.5.3 Genetic Testing for HHT

In those cases where HHT is symptomatic, patient management is based on the standard of care for each of the individual symptoms/conditions as if they occurred alone in a normal patient. Arteriovenous malformations are treated dependent on their location and based upon an expert clinical consultation pertaining to the organ involved, the primary diagnosis of HHT, or both. Embolotherapy is the preferred and most definitive therapy for AVMs (Shovlin et al.

2008), though surgical resection or arterial ligation are alternative options (Faughnan et al. 2011).

Patients with HHT are typically identified in childhood with a progression of subtle clinical signs increasing with age. Therefore, determining whether a newborn has inherited HHT is not possible clinically and if suspected, requires genetic testing for confirmation (Cohen et al. 2005). Genetic testing for HHT involves the examination of the endoglin, ALK-1, and SMAD-4 genes; these mutations account for more than 80% of all HHT cases (Govani and Shovlin 2009). However, this means the diagnosis for a substantial portion, approximately 20% of individuals with HHT, cannot be confirmed or excluded by molecular genetic testing. In most instances, a positive genetic test does not alter the recommended course of treatment or screening. However, it may alert the patient to be more vigilant. For example, if a SMAD-4 mutation (*associated with juvenile polyposis*) was detected, it would be critical to know whether there was a family history of GI polyps and/or malignancy. This would instruct the patient to be more rigorous about GI screening (Abdalla and Letarte 2006). For this reason, genetic testing and counseling are of great potential benefit to HHT suspected and affected families.

7.6 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 pathway intermediates. Many of these syndromes including MFS, LDS, and EDS exhibit concomitant cardiovascular manifestations that provide a phenotypic readout of the presence of disease and are described in more detail in dedicated chapters in this volume. While significant progress has been made in understanding their underlying mechanisms, the refinement of treatment strategies remains an area of critical need. In particular, the study of TGF-β receptor mutation syndromes holds great promise in this regard for the treatment of both connective tissue and cardiovascular disorders. Mutations in FBN1 or TGF-β receptors 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, EDS, HHT, SGS, AOS and ATS) exhibit a spectrum of cardiovascular defects including aortic or arterial aneurysm, dilatation or dissection, mitral valve disease, arterial tortuosity, and primary PAH (Fig. 7.2). Their pathogeneses emphasize a common theme, that normal TGF-β signaling is integral to the normal development and homeostasis of connective tissues and the cardiovascular system. This signaling superfamily contains potent regulators of many cell-types within the mesoderm-derived tissues. Thus, perturbations within the signaling pathway are uniquely situated to produce defects in these tissue types. 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 disorders with cardiovascular complications involving fibrillinopathies and mutated TGF-β receptor syndromes, supports this notion of a common signaling pathway (Loeys et al. 2005). Indeed, these mutated receptor phenotypes are even recapitulated by mutations in downstream TGF-β pathway components (e.g. SMAD-4 in HHT, SMAD-3 in LDS and FTAAD) (Van De Laar et al. 2011; Abdalla and Letarte 2006).

Our new understanding of causal signaling disturbances in these disorders significantly improves the treatment prospects for highly morbid cardiovascular manifestations and debilitating connective tissue defects. Through amassing evidence derived from a combination of understanding the symptomology, the familial inheritance, and sequencing results, it may be the indominable role of TGF-β that holds the key to discovering novel therapeutic strategies for these patients.

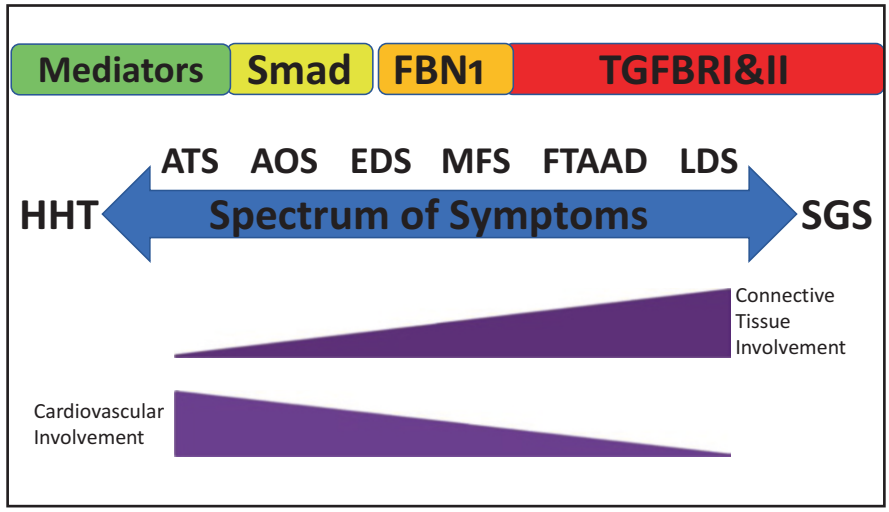

Fig. 7.2 Known heritable connective tissue disorders with cardiovascular involvement that associate with gene mutations related to aberrant TGF-β signaling. These disorders are arranged based on their increasing level of either connective tissue involvement or cardiovascular involvement, and notably share a spectrum of common symptoms, which support their related pathophysiologies

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