Molecular mechanisms of inherited thoracic aortic disease – from gene variant to surgical aneurysm

Elizabeth Robertson · Candice Dilworth · Yaxin Lu ·
Brett Hambly · Richmond Jeremy

Received: 21 September 2014 / Accepted: 10 November 2014 / Published online: 6 December 2014
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Abstract Aortic dissection is a catastrophic event that has a high mortality rate. Thoracic aortic aneurysms are the clinically silent precursor that confers an increased risk of acute aortic dissection. There are several gene mutations that have been identified in key structural and regulatory proteins within the aortic wall that predispose to thoracic aneurysm formation. The most common and well characterised of these is the *FBN1* gene mutation that is known to cause Marfan syndrome. Others less well-known mutations include TGF- β 1 and TGF- β 2 receptor mutations that cause Loeys–Dietz syndrome, Col3A1 mutations causing Ehlers–Danlos Type 4 syndrome and Smad3 and-4, ACTA2 and MYHII mutations that cause familial thoracic aortic aneurysm and dissection. Despite the variation in the proteins affected by these genetic mutations, there is a unifying pathological end point of medial degeneration within the wall of the aorta characterised by vascular smooth muscle cell loss, fragmentation and loss of elastic fibers, and accumulation of proteoglycans and glycosaminoglycans within vascular smooth muscle cell-depleted areas of the aortic media. Our understanding of these

mutations and their post-translational effects has led to a greater understanding of the pathophysiology that underlies thoracic aortic aneurysm formation. Despite this, there are still many unanswered questions regarding the molecular mechanisms. Further elucidation of the signalling pathways will help us identify targets that may be suitable modifiers to enhance treatment of this often fatal condition.

Keywords Marfan · Fibrillin · Transforming growth factor · Vascular smooth muscle · Aortopathy

Abbreviations

ARB	Angiotensin II receptor blockers
BAV	Bicuspid aortic valve
ECM	Extra-cellular matrix
EDS	Ehlers–Danlos syndrome
fTAAD	Familial thoracic aortic aneurysm and dissection
LAP	Latency-associated peptide
LDS	Loeys–Dietz syndrome
LLC	Larger latent complex
LTBP	Latent TGF- β binding protein
MAGP	Microfibril-associated peptide
MFS	Marfan syndrome
MiRNA	MicroRNA
MMP	Matrix metalloproteinases
NO	Nitric oxide
SLC	Small latent complex
TAA	Thoracic aortic aneurysm
TAAD	Thoracic aortic aneurysm and dissection
TGF- β	Transforming growth factor β
TGF- β R	Transforming growth factor β receptor
TIMP	Tissue inhibitors of metalloproteinases
VSMC	Vascular smooth muscle cell

Elizabeth Robertson and Candice Dilworth have contributed equally to this work

“Special Issue: Biophysics of Human Heart Failure”

E. Robertson · R. Jeremy
Cardiology Department, Royal Prince Alfred Hospital, Sydney,
Australia

C. Dilworth · Y. Lu · B. Hambly
Pathology Discipline and Bosch Institute, Sydney Medical School,
University of Sydney, Sydney, Australia

C. Dilworth (✉)
The University of Sydney, Level 4 West, Charles Perkins Centre
D17, Sydney, NSW 2008, Australia
e-mail: cdil6356@uni.sydney.edu.au

Introduction

For many years, the aorta was considered a relatively passive conduit structure, simply sharing the depredations of age and atherosclerotic disease with other major arteries. In recent years, this view has changed radically, as we have begun to understand more clearly not only the key functional role played by the aorta in mediating the interaction of the heart and the vasculature but, more importantly, the complex molecular mechanisms required for homeostasis of structure and function of the aortic wall. The cause for these new insights has been the discovery of gene variants associated with inherited aortic disease. These heritable aortic diseases carry with them significant mortality, associated with sudden and catastrophic dissection or rupture. Currently, the only effective way to avoid this outcome is highly invasive prophylactic aortic replacement. A major thrust of current research focuses on the molecular and cellular pathogenesis of the disease, in an effort to identify medical therapy that may ultimately avoid the need for high-risk invasive surgery. The emergence of angiotensin receptor blockade as a therapeutic option in Marfan syndrome has provided a stimulus to identify further potential targets for manipulation of pathological signalling that underlies the development of congenital thoracic aortopathy. This review explores aspects of the molecular and cellular processes that underlie aneurysm formation in an effort to identify new therapeutic targets.

Normal structure and function of the aorta

The aorta is the most proximal segment of the vasculature to the heart and has important haemodynamic roles, including both blood conduit and pressure buffering functions (Safar et al. 1990). These functions are dependent upon the closely regulated and well-maintained microstructure of the aortic wall (El-Hamamsy and Yacoub 2009, Boudoulas et al. 2012). As with most mammalian arteries, the aorta has a basic triple-layer anatomy, with the intima, media and adventitia (inner to external layer) each characterised by a specific microstructure and function (Rubin et al. 2008).

The monocellular intimal layer of endothelial cells connects to a collagenous (type IV) basal membrane that adjoins the blood vessel lumen (Rubin et al. 2008; Kumar et al. 2010). The media is composed of vascular smooth muscle cells (VSMC) intercalated between multiple layers of fenestrated elastic fibres that associate with collagen (type I and type III) and contain fibrillin-1 microfibrils and proteoglycans, which together form the extra-cellular matrix (ECM) that surrounds the VSMC (El-Hamamsy and Yacoub 2009, Mohamed 2011). The adventitia is composed of loose connective tissue, primarily longitudinally arranged collagen fibres (type I and type III), which serve to provide overall rigidity to the aorta (Rubin

et al. 2008; El-Hamamsy and Yacoub 2009). Two thick elastic laminae, the internal and external, separate these three layers from each other and function to deliver additional structural support and elasticity to the aorta (Rubin et al. 2008; Grotenhuis and de Roos 2011).

The lamellar unit

The basic microstructural unit of the aortic wall has been described as the lamellar unit. This unit consists of a single VSMC together with adjacent collagen and elastin fibres, and associated microfibrils, embedded in the glycosaminoglycan-rich ECM (Baldock et al. 2002). The key functional attributes of the lamellar unit include both tensile strength and elastic distensibility, allowing the aorta to withstand the pulsatile force of left ventricular ejection during systole. Elastin and collagen fibres are comprised of microfibril subunits in both the media and adventitia. Specifically, it is the elastin fibres, together with associated proteoglycans such as versican, biglycan and perlecan, that are responsible for the malleability of the arterial wall (Montes 1996), while collagen fibres provide static strength and load resistance properties (Fratzl 2008; Schriebl et al. 2012). The structural subdivision of these fibres into fibrils contributes to aortic wall flexibility and minimises the risk of aortic rupture, as the blood pressure is primarily opposed by this flexibility and fissures cannot easily propagate across multiple concentric collagen fibrils (Fratzl et al. 1998; Ottani et al. 2001).

Thus, the biomechanical properties of the aorta reflect the complex structure and interactions that occur within and between lamellar units, and between additional components of the ECM and microfibrillar network (Wolinsky and Glagov 1967).

Fibrillin-1 microfibrils

Fibrillin-1 is a calcium-binding glycoprotein that is secreted by fibroblasts and VSMC (Rossi et al. 2010). It polymerises to form insoluble microfibrils, which are a major component of connective tissue matrices and are widely distributed throughout the body (Zhang et al. 1995; Faivre et al. 2007). The microfibrils are structured in an anti-parallel head-to-tail arrangement (Jensen et al. 2009, 2012; Ramirez and Sakai 2010) and are located in the extracellular space in juxtaposition to both the elastin laminae and the endothelium (Jeremy et al. 2013).

The structural and cell-signalling properties of fibrillin-1 appear to have essential roles in sequestration of TGF- β , modulating elastin deposition and mediating the environmental cues necessary for subsequent normal tissue development and homeostasis in the mammalian aorta (Fedak et al. 2002). An important architectural feature of fibrillin-1 is the presence of many Ca^{2+} binding EGF domains. These domains control

the tertiary protein structure and stiffness (Faivre et al. 2007). Additionally, fibrillin-1 binds via an RGD sequence to specific integrins ($\alpha 5\beta 3$, $\alpha 5\beta 6$ and $\alpha 5\beta 1$) on VSMC. This binding appears to be essential in facilitating mechanotransduction signalling between the endothelium and matrix proteins to the VSMC (Jovanovic et al. 2008; Jeremy et al. 2013).

Molecular mechanisms of wall homeostasis

Haemodynamic stress (shear stress on the endothelium as a result of turbulent pulsatile flow) and strain are believed to be the major stimuli for growth and remodelling (Kassab et al. 2002). The mechanotransduction responsible for this regulation has been a subject of particular interest and is important to developing an understanding of the pathophysiological mechanisms proposed in heritable aortopathy. Specifically, on a molecular level, there are several signalling pathways through which this regulatory homeostasis is achieved, and of these the most vital appears to be the TGF- β signalling pathway and its associated downstream intracellular signalling events (Jeremy et al. 2013). Additionally, VSMC synthetic activity plays an essential role in aortic wall maintenance in response to haemodynamic stress and strain.

The TGF- β signalling pathway

TGF- β isoforms (TGF- $\beta 1$, TGF- $\beta 2$ and TGF- $\beta 3$) are constitutively expressed, pleiotropic cytokines involved in the regulation of many cell functions including proliferation, differentiation and apoptosis (Isogai et al. 2003; Kaartinen and Warburton 2003). TGF- β plays a fundamental role in maintaining ECM homeostasis by functioning concomitantly as both a tissue growth promoter and suppressor, and by governing VSMC phenotypic transformation (Forte et al. 2010, 2013; Jeremy et al. 2013).

Many cell types, including VSMC and macrophages, secrete TGF- β in a latent form, resulting in significant inactive quantities being sequestered in normal tissue. Latent TGF- β consists of the mature growth factor bound by non-covalent interactions to the TGF- β propeptide (latency-associated peptide; LAP) (Isogai et al. 2003). This dimeric complex is referred to as the small latent complex (SLC) (Massam-Wu et al. 2010). Once synthesised, the SLC is secreted into the ECM (Saharinen et al. 1999) where TGF- β is rendered biologically inactive, anchored to latent TGF- β binding protein 1 (LTBP-1) via LAP, as part of a larger latent complex (LLC) (Fig. 1a) (Isogai et al. 2003; Massam-Wu et al. 2010). These multimeric protein complexes bind directly to microfibrils through sequence complementary between the LTBP-1 C-terminus and fibrillin-1 monomers (Gelb 2006; Choudhury

et al. 2009; Jondeau et al. 2011), acting as a reservoir for the release of TGF- β .

In healthy individuals, the bioavailability of active TGF- β is highly controlled through sequestration of the TGF- β /LTBP complex to fibrillin-1 microfibrils and associated glycoproteins (microfibril-associated glycoprotein; MAGP-1 and MAGP-2), latent TGF- β binding proteins (LTBP) and fibulins (Gelb 2006; Choudhury et al. 2009; Jondeau et al. 2011), to ensure appropriate remodelling in response to normal shear stress occurs. Recent evidence indicates that the associated glycoproteins, particularly fibulin and MAGP, play a role in stabilising binding of the TGF- β /LTBP complex to fibrillin-1 and in suppressing the release of TGF- β until it is required (Jeremy et al. 2013).

The fibulin protein family contains 5 members, with fibulin-4 appearing to play a significant role in microfibril binding interactions. Fibulin-4 is localised to the microfibrils (Yanagisawa et al. 2002) and is highly expressed in the outer medial layers adjacent to the adventitia in large blood vessels, including the aorta (Huang et al. 2010; Moltzer et al. 2011). Importantly, the fibulin-4 C-terminus can bind the TGF- β /LTBP complex via the LTBP-1 N-terminus (Massam-Wu et al. 2010; Jeremy et al. 2013). When fibulin-4 is associated with fibrillin-1 (Choudhury et al. 2009; Ono et al. 2009), it forms a secure ternary complex between LTBP-1, fibrillin-1 and itself (Fig. 1a). This interaction is believed to stabilise the sequestration of TGF- β .

In contrast, MAGP-1 competitively inhibits the C-terminus of LTBP-1 from interacting with fibrillin-1 (Fig. 1b). This allows proteases, such as plasmin, to aid the release of the TGF- β SLC by cleavage of the LTBP–LAP bond, after receiving certain physiological stimuli, such as mechanical stimuli, pH changes and signals from other cytokines (Jeremy et al. 2013). Once released, free active TGF- β binds to the TGF- β receptor 2 (TGF- β R2), recruiting and activating the TGF- β receptor 1 (TGF- β R1), inducing a signalling cascade of intracellular protein phosphorylation, including receptor associated Smads (Smads 2 and 3) and the MAP kinase system, amongst others (Fig. 2) (El-Hamamsy and Yacoub 2009; Forte et al. 2013).

Smad2 and Smad3 phosphorylation recruits Smad4 to form a Smad2/3–Smad4 complex, which translocates to the nucleus, allowing for downstream activation of gene transcription (specific activators or repressors via the canonical pathway) (Neptune et al. 2003; El-Hamamsy and Yacoub 2009). This transcription regulation results in increased ECM protein secretion and suppressed matrix degradation, through upregulated collagen deposition (Ignatz and Massague 1986; Kahari et al. 1992) and matrix metalloproteinase (MMP) inhibition by tissue inhibitors of metalloproteinase (TIMP) (Kwak et al. 2006). At the same time, the Smad2/3–Smad4 complex can act as a transcription factor for signalling through the non-canonical p38 mitogen activated kinase pathway (MAPK/

Fig. 1 Proposed model of TGF- β sequestration and release. **a** The proposed theory behind TGF- β sequestration. The small latent complex binds to LTBP-1 forming the LLC. LLC binds through the C-terminal of LTBP-1 to fibrillin-1 microfibrils. This bond is stabilised through the formation of a ternary complex via fibulin-4 interactions with the N-terminus of LTBP-1. **b** The proposed theory behind TGF- β release. MAGP-1 inhibits binding of LTBP-1 C-terminus to fibrillin-1, thereby enhancing the release of active TGF- β . (Adapted from Massam-Wu et al. 2010)

ERK), triggering apoptosis and causing an upregulation in MMP secretion, which results in ECM destruction through proteolysis (Fig. 2) (Sinpitaksakul et al. 2008). Whilst the mechanism through which apoptosis occurs is largely unknown, the relative balance between downstream signal activation and resulting gene transcription will dictate the overall consequences arising from TGF- β pathway stimulation.

Fig. 2 Illustration of the multiple signalling pathways for TGF- β in the VSMC. In the normal state, TGF- β is sequestered by microfibrils, bound to fibrillin-1 through LTBP-1 and fibulin-4 interactions, until its required release. Mechanical stimuli encourage MAGP-1 to competitively bind fibrillin-1, inhibiting binding of the TGF- β /LTBP-1 complex (LLC). Once released, free active TGF- β can interact with its cell surface

Vascular smooth muscle cells

The VSMC also contribute to wall maintenance. VSMC can respond to haemodynamic stress, although knowledge of the factors underpinning the mechanism of response is incomplete. The VSMC can reversibly switch between an inactive, contractile phenotype, and an active, synthetic phenotype, the latter characterised by proliferation and ECM synthesis (Beamish et al. 2010). Switching between these two phenotypes is, in part, regulated by the endothelium. The endothelium serves as a sensor of haemodynamic shear stress, resulting in the release of nitric oxide (NO) or the activation of TGF- β signalling in response to blood flow and stretch (Kuhlencordt et al. 2001). The endothelial release of NO favours a contractile phenotype. Normally, TGF- β signalling supports a contractile phenotype; however, endothelial secretion of perlecan in response to cyclic stretch of endothelial cells inhibits TGF- β signalling to contractile VSMC, resulting in a shift towards the synthetic VSMC phenotype (Jeremy et al. 2013).

In the normal aorta, the distension of the elastic laminae is balanced against the physiological contractile response of VSMC (Jeremy et al. 2013), resulting in low level remodelling of the ECM. This balance is achieved through mechanotransduction via the binding of actin-linked cell surface integrins to key ECM proteins. It is hypothesised that, in response to excessive stretching of the aortic wall, actin-myosin cycling places strain upon the intermediary proteins, namely talin and vinculin (Goldman and Wurzel 1992; Jeremy et al. 2013), that link the actin-myosin complex to the integrins. This strain results in deformation and displacement of the linkers from the complex, allowing for conformational change, opening new sites for protein phosphorylation and

receptors, binding to the TGF- β R heterodimer and activating the canonical pathway (Smad) or non-canonical pathway (MAPK/ ERK) in the correct ratio, resulting in balanced activation of transcription factors. Pathological levels of free active TGF- β disrupts the homeostatic balance between the two pathways to favour signalling through the non-canonical pathway. (Adapted from (Ramirez and Sakai 2010; Jeremy et al. 2013)

growth factor activation in response to haemodynamic stress (Jeremy et al. 2013). Intrinsic tone in the VSMC, dictated by actin–myosin interactions, determines an operating set-point for ideal balance between haemodynamic stimulus to aortic remodelling.

Additionally, the synthetic phenotype of the VSMC contributes to homeostatic wall maintenance through the upregulation of actin and myosin expression, and through increased production of plasminogen activators and matrix remodelling enzymes such as MMP and associated glycosaminoglycans (GAG) and proteoglycans (Forte et al. 2010; Jeremy et al. 2013). Major GAG within the ECM include the large molecule versican, as well as the smaller decorin and biglycan. Whilst versican appears to mediate VSMC growth in response to growth factors, both decorin and biglycan are associated closely with the microfibrillar network and can bind TGF- β and, thus, may regulate growth factor availability. The proteoglycan perlecan is a key factor for maintaining vascular homeostasis via the inhibition of VSMC proliferation, and is thus assumed to assist in maintaining vascular homeostasis (Iozzo 1998; Garl et al. 2004).

Inherited aortic aneurysm disease

Recent progress in discovering the gene variants associated with familial aortic aneurysm and dissection has given further insights into the mechanisms of aortic growth, remodelling and homeostasis. To date, mutations in more than 40 genes have been associated with heritable aortic aneurysm formation. These mutations appear to cluster in several protein systems: (1) the ECM microfibrils (fibrillin-1, elastin, collagen III, fibulin-4); (2) the VSMC contractile apparatus (actin, myosin heavy chain, myosin light chain kinase); and (3) the TGF- β signalling pathway (TGF- β 2, TGF- β R1 and 2, Smad3, Smad 4), and the endothelium (NOTCH1, Jagged1). The investigation of several clinical syndromes associated with mutations in specific genes allows us to examine in more detail the roles of key proteins in aortic pathology.

Marfan syndrome

Marfan syndrome (MFS) (OMIM 154700) is a disorder of connective tissue that is due to mutations in the FBN1 gene (OMIM 134797), resulting in the production of abnormal fibrillin-1 (Hayward and Brock 1997; Judge and Dietz 2008; Ogawa et al. 2011). The predominant cardiovascular manifestation is thoracic aortic aneurysm (TAA), most frequently localised to the Sinuses of Valsalva and proximal aorta, although dilatation may extend more distally (Milewicz et al. 2005). Histopathologically, MFS is characterised by medial degeneration (Romaniello et al. 2014). Especially prominent

are a high number of connective tissue interruptions, particularly thickened elastic lamellae and the accumulation of abundant extracellular glycosaminoglycan-rich material, with VSMC showing a prevalent synthetic phenotype (Fig. 3) (Romaniello et al. 2014). The contribution of FBN1 mutations to this histological appearance is reinforced by histological similarities in the aorta of FBN1 mutant mice (Kim et al. 2014).

Loeys–dietz syndrome

Loeys–Dietz syndrome (LD) (OMIM 609192) is also a disorder of connective tissue that bears phenotypic similarity to MFS, but is caused by mutations in the genes encoding the 2 subunits of the TGF- β R heterodimer: subunit 1 (*TGFBR1*) (OMIM 190181) or subunit 2 (*TGFBR2*) (OMIM 190182) (Karttinen and Warburton 2003; Loeys et al. 2005, 2006;

Fig. 3 Pathophysiological features of inherited aortic disease. **a** Normal histological appearance of the aorta, showing normally aligned VSMC anchored by layers of elastic lamellae (composed of elastin and collagen). Additionally, structural support is provided by fibrillin-1 microfibrils. **b** Abnormal histological architecture that is seen in inherited aortic diseases, showing degradation of the elastic lamellae by MMP and apoptosis of the VSMC. (Adapted from Fedak et al. 2002; Verma and Siu 2014)

Lemaire et al. 2007; Attias et al. 2009; Boileau et al. 2012). Phenotypically, the TGF- β R mutations result in a predisposition to aneurysm formation that is more severe compared with MFS. Histologically, aortic tissue samples from these patients closely resemble that of MFS with medial degeneration and elastin fragmentation (Loeys et al. 2005). Additionally, a significant excess of aortic wall collagen, increased connective tissue growth factor and Smad2 have been observed.

Ehlers–Danlos syndrome

Ehlers–Danlos syndrome (EDS) Type IV (OMIM 130050) is a connective tissue disorder caused by a mutation in Col3A1 (OMIM 120180) that results in the production of abnormal pro- α 1-chain of collagen type III, which is abundant in the aortic wall. The mutation results in decreased thermal stability, reduced secretion, and abnormal proteolytic processing of the pro- α 1 chain, that subsequently causes the histological appearance of reduced medial thickness (Boutouyrie et al. 2004). The abnormally low medial thickness results in a higher wall stress compared to normal and, in an elastic artery, is thought to increase the risk of arterial dissection and rupture (Boutouyrie et al. 2004). Unlike MFS and LDS, in EDS the thoracic aorta is not the most common site of vascular involvement, with the entire vascular tree being significantly affected by frequent spontaneous rupture and dissection (Pyeritz 2014).

Familial non-syndromal thoracic aortic aneurysm and dissection

Thoracic aortic aneurysm and dissection (TAAD) has also been shown to occur outside of the previously identified syndromes, but clustered within families (Milewicz et al. 1998). To date, there have been several causative genes identified using large family pedigree studies. These mutations include mutations in proteins that are known modulators of the TGF- β signalling pathway, such as Smad3 (OMIM 603109) and Smad4 (OMIM: 600993) (Regalado et al. 2011; van de Laar et al. 2011). In addition, there have been several gene mutations identified in proteins of the contractile apparatus of VSMC cells such as ACTA2 (OMIM 102620), which encodes the VSMC-specific isoform of α -actin (ACTA2) (Guo et al. 2009), and MYH11 (OMIM 160745), which encodes the VSMC major specific contractile isoform of myosin heavy chain (Kaartinen and Warburton 2003; Zhu et al. 2006; Morisaki et al. 2009; Boileau et al. 2012; Teekakirikul et al. 2013). Histologically, these patients are similar to MFS and LD in that they have medial degeneration with reduced elastin content and elastic fibre fragmentation (Lindsay and Dietz 2011; van de Laar et al. 2011).

Bicuspid aortic valve

Bicuspid aortic valve (BAV) is a common congenital abnormality affecting approximately 1 per 100 people, and is characterised by the presence of two, rather than three aortic valve semi lunar cusps, either as a result of fusion of the right and left cusps or fusion of the right and non coronary cusps. In rare cases, the leaflets are symmetrical and there is no raphe. Dilatation of any or all segments of the proximal aorta from the aortic root to the aortic arch, called bicuspid aortopathy, is also present in approximately 50 % of patients with BAV (Fedak et al. 2002). Histologically, medial degeneration is again seen, with loss of smooth muscle and fibrosis that causes collapse of the elastic lamina, with an increase in mucopolysaccharides and disarray of the elastic fibres within the media (Fedak et al. 2002). The resulting matrix disruption and elastin and lamellar fragmentation lead to increased apoptosis of VSMC and disruption of the media layer, adversely affecting the structural integrity and flexibility of the aorta (Verma and Siu 2014).

Genetic studies have established familial clustering amongst patients, suggesting a Mendelian pattern of inheritance that is thought to be autosomal dominant (Teekakirikul et al. 2013) with variable expressivity and reduced penetrance. However, a definitive causative gene has not yet been identified (Huntington et al. 1997; Fedak et al. 2002; Siu and Silversides 2010; Braverman 2011; Padang et al. 2012). There is evidence of association of BAV with mutations in *NOTCH1* (OMIM 190198), which encodes a transmembrane receptor that has a role in determining cell outcomes in organogenesis (Garg 2006; Mordi and Tzemos 2012). Several other genetic loci have been postulated, although the responsible gene/s at each loci have not yet been identified (Siu and Silversides 2010; Mordi and Tzemos 2012).

Pathogenesis of aneurysm formation

Fibrillin-1 and the lessons learned from MFS

Two mechanisms have been proposed to explain how the mutations in the fibrillin-1 protein cause the pathology in MFS. The first mechanism is associated with a negative dominance, where defective fibrillin-1 molecules do not allow for the necessary formation of fibrillin-1 polymers, rendering the ECM microfibrils structurally abnormal (Dietz et al. 1993; Eldadah et al. 1995). This structural irregularity may cause detachment of the VSMC from the elastic laminae, with subsequent accelerated VSMC apoptosis and release of pathological MMP (Padang et al. 2012). This would result in weakened and disordered elastic fibre formation in aortic tissue, together with disruption of the microfibril network

connecting the elastic lamellae to the adjacent VSMC which compromises the structural integrity of the aortic wall (Jones and Ikonomidis 2010). The second mechanism proposes haploinsufficiency, in which reduction of total fibrillin-1 production below a specific critical threshold results in connective tissue weakness, because the affected allele, including the mutated *FBN1* gene, is not functionally transcribed (Judge et al. 2004; Canadas et al. 2010; Jondeau et al. 2011) or produces a truncated protein that is not incorporated into ECM structures or is selectively and prematurely degraded.

A MFS mouse model has shown the aortic elastic fiber length decreased in 3- and 9-month-old MFS mice compared to controls in all three aortic sections (root, arch and descending), with significantly lower elastin orientation indices in MFS ascending aorta (Cui et al. 2014). The orientation of elastic fibers determines their resistance to strain (Lillie et al. 1998), implying that the MFS aorta has an impaired load bearing capacity. Furthermore, Chung et al. have demonstrated endothelial dysfunction in MFS, where endothelium-dependent relaxation in the thoracic aorta is impaired, likely owing to the downregulation of Akt/eNOS-induced production of NO (Chung et al. 2007). Hence, shear stress induced NO production may be an important contributor to the pathogenesis of thoracic aneurysm manifestations in MFS.

Abnormal TGF- β signalling in MFS and LD

Fibrillin-1 may determine the bioavailability of active TGF- β . In MFS, it is hypothesised that reduced or defective forms of fibrillin-1 lead to failed matrix sequestration of the LLC, with subsequent excessive activation and signalling of TGF- β (Fig. 2) (El-Hamamsy and Yacoub 2009). This excessive TGF- β signalling can activate the alternate (non-canonical) p38 mitogen activated kinase (MAPK/ERK) pathway in VSMC, leading to increased production of plasminogen activators and upregulation of MMP transcription, resulting in ECM degradation (Kaartinen and Warburton 2003; Kim et al. 2004; Carta et al. 2009). Transgenic mice models of MFS offer support for a cause-and-effect relationship between excessive TGF- β signalling and the observed clinical manifestations (Neptune et al. 2003; Ng et al. 2004; Habashi et al. 2006; Jondeau et al. 2011). Furthermore, the treatment of MFS mouse models with anti-TGF- β antibodies rescues the animal and prevents the development of aortic root aneurysm (Habashi et al. 2006). Histopathology demonstrated that treatment with anti-TGF- β antibodies revealed reduced fragmentation of the elastic fibres and a decreased rate of aortic root dilatation (Neptune et al. 2003; Ng et al. 2004; Habashi et al. 2006; Jondeau et al. 2011).

A defective mutation in either of the TGF- β receptor genes is predicted to result in impaired receptor function and, hence, diminished TGF- β signalling (Loeys et al. 2006; Boileau et al. 2012). However, aneurysmal aortic tissue of LDS patients

with either *TGFBR1* or *TGFBR2* mutations has an unexpected increase in TGF- β signalling (Loeys et al. 2005; El-Hamamsy and Yacoub 2009). As both *TGFBR1* and *TGFBR2* knock-out mouse models are lethal and the haploinsufficient mouse model did not exhibit cardiovascular abnormalities, it has been concluded that the underlying molecular mechanism in LDS is likely to be unregulated TGF- β signalling (Larsson et al. 2001; Carvalho et al. 2007; Choudhary et al. 2009). Analysis of TGF- β signalling in the aortic wall in vivo of transgenic mice (overexpressing mutant *TGFBR2*) revealed upregulation of Smad2 phosphorylation and TGF- β target gene output. This finding matched aneurysm pathology of increasing severity and corresponded with upregulation of TGF- β 1 ligand expression. Suppression of Smad2 phosphorylation and TGF- β 1 expression paralleled the therapeutic efficiency of the angiotensin II (AngII) type 1 receptor antagonist losartan (Gallo et al. 2014).

Further support for the TGF- β pathway as a disease modifier has been shown in several studies that have demonstrated that total serum TGF- β 1 levels are significantly increased in MFS as well as BAV-aortopathy compared to control, with an increasing trend in LDS and fTAAD (Matt et al. 2009; Franken et al. 2013; Kim et al. 2013; Ogawa et al. 2013; Hillebrand et al. 2014). Additional analysis demonstrated that the increased serum levels were associated with mutations in proteins associated with the TGF- β signalling pathway, (i.e. *FBN1* and *NOTCH1* mutations compared to control), and was not as proportionally upregulated in mutations affecting the contractile elements within the VSMC (i.e. *ACTA2*/*MYH11* mutations). Evidence has also been presented that, in MFS patients, plasma TGF- β 1 positively correlates with aortic diameter as well as the growth rate of the aortic root (Franken et al. 2013).

TGF- β pathway and lessons from angiotensin II receptor blockers

AngII binds to two receptors within mammals, type 1 (AT1) and type 2 (AT2). AT1 receptors mediate most AngII actions in adulthood, such as vasoconstriction, renal salt and water retention and aldosterone release, and occur in both peripheral tissues and the brain (Stroth and Unger 1999). In adult mammals, AT2 have been implicated in anti-proliferation and apoptosis pathways (Stroth and Unger 1999). The binding of AngII to AT1 receptors within the aortic wall causes proliferation of VSMC, favours fibrosis, increases the expression of MMP-2 and MMP-9 and reduces apoptosis (Keane and Pyritz 2008). These effects are mediated in part by an increase in TGF- β release, with subsequent increase in downstream signalling effects via MAPK/ ERK activation (Keane and Pyritz 2008; Matt et al. 2009; Bunni et al. 2011). In contrast to AT1 receptors, when AngII binds to AT2 receptors,

it has an anti-proliferative effect within the aortic wall (Keane and Pyritz 2008).

Increased apoptosis is thought to contribute to the pathogenesis of aortic root aneurysms in MFS. TGF- β is known to induce apoptosis via the Smad and MAPK/ ERK pathways, however, the mechanism underlying this induction is largely unknown (Merk et al. 2012). Mice studies have shown that AngII infusion promotes development of ascending aortic aneurysms (AA) in wild type mice via elastin fragmentation and transmural medial breaks of the ascending aorta. CD45⁺ haematolymphoid cells accumulated in the adventitia but were minimally present in the media. These findings were similar to the pathology that was observed in tissues obtained from patients with ascending TAA. Therefore, it has been postulated that AngII promotes ascending TAA through region-specific changes, independent of co-morbidities such as hypercholesterolemia or raised systolic blood pressure (Rateri et al. 2014).

Losartan, a commonly used anti-hypertensive drug with AngII blocking effects, also has the ability to inhibit TGF- β signalling in the aorta due to crossover between the intracellular signalling cascades that are activated by the AT1R and the TGF- β R complex (Brooke et al. 2008). Losartan has been shown to prevent the fragmentation of elastic fibres and ameliorate aortic root dilatation in MFS mice by restoring muscle architecture and permitting regeneration (Habashi et al. 2006; Cohn et al. 2007). Studies in human MFS patients have demonstrated significantly reduced plasma TGF- β levels in those patients treated with losartan compared to untreated age matched controls (Brooke et al. 2008). In addition, increased apoptosis in samples of the aortic wall of MFS patients was reduced when AT2 receptor, but not AT1 receptor, blockade occurred (Nagashima et al. 2001). Several clinical studies have shown that, following treatment with losartan, the mean rate of change in aortic root dilatation decreased significantly compared to control (Brooke et al. 2008; Groenink et al. 2013).

New insights – the role of microRNA

MicroRNA (miRNA) are small non-coding RNA molecules (22 nucleotides) that primarily function as post-transcriptional regulators of gene expression via messenger RNA silencing (Ambros 2004; Bartel 2004). Recent research has demonstrated that miRNA dysregulation is associated with the pathogenesis of many cardiovascular disorders (Urbich et al. 2008). In a mouse model of MFS, upregulation of miR-29b has been shown to be associated with aortic wall remodelling and increased aneurysm formation, through increased apoptosis and altered ECM synthesis/deposition in the ascending aorta (Merk et al. 2012). Nuclear factor κ B activation represses miRNA-29b activity and was found to be decreased in the aortic wall of MFS mice. In addition, TGF- β blockade with

losartan decreased miR-29b levels, which supports the current notion of MFS being a dysregulation of TGF- β signalling and the use of angiotensin receptor blockers (ARB) as a treatment modality.

Other miRNAs have been shown to be crucial in regulating VSMC functions, such as miR-143/145, miR-1, miR-21, miR-221, miR-24, and members of the miR-29 family (including miR-29a, -b and -c) (Boettger et al. 2009; Cordes et al. 2009; Davis et al. 2009; Elia et al. 2009; Xin et al. 2009; Boon et al. 2011). These miRNA have been implicated in VSMC growth, differentiation and function. Whether they are altered in aneurysm formation and the mechanisms through which they may act have yet to be elucidated.

Conclusion

All inherited forms of TAA disease exhibit surprising similarities in both phenotype and histological appearance within the aorta, despite the varied abnormalities that occur within the molecular pathogenesis. The common association between these diseases appears to an abnormality within the TGF- β signalling pathway. This observation has focused current therapeutic efforts on the inhibition of this abnormal signalling, with the strongest evidence supporting the use of ARB. A deeper understanding of the signalling abnormalities in the various forms of congenital thoracic aortopathy may identify other targets that will lead to more effective therapy for this similar yet heterogeneous group of diseases.

Acknowledgements We do not have any acknowledgements to make. This article does not contain any studies with animals performed by any of the authors. This article does not contain any studies with human participants performed by any of the authors.

All authors declare no conflict of interest Elizabeth Robertson declares no conflict of interest. Candice Dilworth declares no conflict of interest. Yaxin Lu declares no conflict of interest. Brett Hambly declares no conflict of interest. Richmond Jeremy declares no conflict of interest.

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