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Review Article

The genetic basis of thoracic aortic disease: The future of aneurysm classification?

M Yousuf Salmasi [1,*], Shehani Alwis [2], Shabnam Cyclewala [2], Omar A. Jarral [1], Heba Mohamed [2], David Mozalbat [2], Christoph A. Nienaber [2], Thanos Athanasiou [1], Deborah Morris-Rosendahl [3], on behalf of the Members of the London Aortic Mechanobiology Working Group[*]

[1] Department of Surgery and Cancer, Imperial College London, UK
[2] Royal Brompton and Harefield Foundation Trust, UK
[3] Clinical Genetics and Genomics Laboratory, Royal Brompton Hospital, London, UK

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ABSTRACT

The expansion in the repertoire of genes linked to thoracic aortic aneurysms (TAA) has revolutionised our understanding of the disease process. The clinical benefits of such progress are numerous, particularly helping our understanding of non-syndromic hereditary causes of TAA (HTAAD) and further refinement in the subclassification of disease. Furthermore, the understanding of aortic biomechanics and mechanical homeostasis has been significantly informed by the discovery of deleterious mutations and their effect on aortic phenotype. The drawbacks in genetic testing in TAA lie with the inability to translate genotype to accurate prognostication in the risk of thoracic aortic dissection (TAD), which is a life-threatening condition. Under current guidelines, there are no metrics by which those at risk for dissection with normal aortic diameters may undergo preventive surgery. Future research lies with more advanced genetic diagnosis of HTAAD and investigation of the diverse pathways involved in its pathophysiology, which will i) serve to improve our understanding of the underlying mechanisms, ii) improve guidelines for treatment and iii) prevent complications for HTAAD and sporadic aortopathies.
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1. Introduction

Since the discovery of the causative gene (FBN1) for the mutation in Marfan Syndrome 29 years ago, mutations in numerous other genes have been linked, with varying degrees of strength, to both the hereditary and sporadic causes of thoracic aortic aneurysms (TAA) and dissection (TAAD). The linkage of recognised features of connective tissue disease, including those outlined in the Ghent nosology for Marfan Syndrome (Table 1), to specific genetic malformations has been a vital step in understanding the pathophysiology of TAA and TAAD. In addition, common single nucleotide polymorphisms (SNPs) and genomic copy number variants (CNVs) in some of these same genes have demonstrated an increased risk of TAAD in the general population. By linking mutations to their corresponding coded proteins, the roles of extra-cellular matrix (ECM) proteins such as elastin, integrins, fibrillins and other glycoproteins have been revealed. Furthermore, the interactions of the vascular smooth muscle cell (VSMC; or SMC) with ECM proteins and downstream intracellular signalling, including transforming growth factor-β (TGFβ) pathways, have been found to be critical for the normal biomechanical function of the aorta and explain patterns of disease in the case of genetic abnormalities.

The wide availability of whole exome sequencing has prompted the incorporation of genetic testing into risk assessment for TAA and TAAD. Over 50 genes have been made available on diagnostic panels for aortopathy assessment in numerous institutions. Clinically, the use of panel testing is either triggered by the incidental finding of a dilated thoracic aorta or relevant mutations identified in an affected family member. This has the potential to guide risk assessment for the timing of intervention.

2. Genetic basis of disease: aortic mechanical homeostasis

The contractile units in the SMCs attach to the ECM through focal adhesions associated with integrins on the cell surface (also

* Corresponding Author. 10th Floor QEQM Building, St Mary's Campus, South Wharf Road, London, W2 1NY, UK.
E-mail address: y.salmasi@imperial.ac.uk (M.Y. Salmasi).
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[*] Members of the London Aortic Mechanobiology Working Group are listed at the end of the article.

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Table 1
Ghent diagnostic nosology, adapted from Dean et al., 2018.[66]

System	Major Criterion	Involvement
Skeletal	At least 4 of the following features: Pectus carinatum Pectus excavatum requiring surgery Upper lower segment ratio of <0.86 or arm span:height>1.05 Wrist and thumb signs Scoliosis >20° or spondylolisthesis Reduced elbow extension (<170°) Pes planus Protrusio acetabulae	2 of the major features, or 1 major and 2 of the following: Pectus excavatum Joint hypermobility High palate with dental crowding Characteristic face
Ocular	Lens dislocations (ectopia lentis)	Flat cornea Increased axial length of globe (causing myopia) Hypoplastic iris or ciliary muscle (causing decreased miosis)
Cardiovascular	Dilatation of the aortic root Dissection of the ascending aorta	Mitral valve prolapse Dilatation of the pulmonary artery, below age 40 Calcified mitral annulus, below age 40 Other dilatation or dissection of the aorta
Pulmonary	None	Spontaneous pneumothorax Apical blebs
Skin/Integument	None	Striae atrophicae Recurrent or incisional hernia
Dura	Lumbosacral dural ectasia	None
Genetic findings	Parent, child or sibling meets these criteria independently FBN1 mutation known to cause Marfan syndrome Inheritance of DNA marker haplotype linked to Marfan syndrome in the family	None

called dense plaques). The integrin receptors bind to microfibrils that surround the elastin fibres. This continuous connection between intracellular actomyosin components and elastin fibres has been termed the “elastin–contractile unit”.[1]

Integrins can self-assemble into moderately tight bundles and interact with other integrins on the SMC cell surface and other matrix molecules within the microenvironment, including TGFβ.[2] Therefore, their contribution to aortic wall integrity is both due to their mechanical strength and through the regulation of cell signalling pathways.[3] Abnormalities in the essential structure of fibrillin disrupt the integrity of the aortic wall that can be appreciated through histological analysis of samples from the ascending comparing the organised lamellar structure of a healthy aorta to medial degeneration seen in aortopathy (Fig. 1).

Within the VSMC, recognised intracellular signalling pathways link mechanical stimuli from the cell surface to gene regulatory activity at the nucleus. These include mitogen-activated protein kinase (MAPK) and SMAD, which follow downstream from angiotensin-II (Ang-II), TGFβ and other growth factor receptors. Ang-II and TGFβ are important in ageing[4] and hypertension[5] since their ability to potentiate VSMC phenotype switching toward a synthetic function[6] can adversely increase the production of matrix, leading to increased thickness and stiffness of the wall. A global increase in structural stiffness can further increase central pulse pressures and pulse wave velocities[5,7] that pathologically increase proximal aortic loading.

The appreciation of proteins in the aortic medial ECM has increased in recent years through discoveries in mutations in genes coding for these proteins and the resulting aortopathy. Proteins concerned with the structural integrity of the aortic wall can be subdivided according to the following families (Table 2):

1. ECM proteins
2. Cytoskeletal proteins
3. TGF signalling-related proteins
4. Membrane proteins

3. Syndromic TAAs

Syndromic TAAs are defined as aneurysms that occur in conjunction with a range of associated anomalies affecting various body systems. TAAs are a main feature of some syndromes, namely Marfan, Loeys–Dietz and Ehlers–Danlos syndromes. In other cases, such as BAV disease and Turner syndrome, TAAs are a possible manifestation.

3.1. Marfan Syndrome and mutations in Fibrillin-1

MFS is an autosomal dominant heritable disorder of connective tissues with prominent involvement of skeletal, ocular, cardiovascular and pulmonary organ systems. The estimated prevalence ranges from 1/5000 to 1/10,000, and approximately 75% cases of MFS have an affected parent and the remaining 25% of probands are sporadic.

3.1.1. Fibrillin function in the aortic wall

In 1991, mutations in the fibrillin-1 (FBN1) gene were identified as the cause of Marfan Syndrome.[8] This involved initial cloning of the fibrillin-coding DNA and mapping via in-situ hybridisation onto chromosome 15. This concept was strengthened by identifying de novo fibrillin gene mutations in patients with sporadic disease. To date, almost 1000 different genetic mutations have been identified.

The structure of fibrillin is cysteine rich, which may be critical for microfibril assembly and may be the means of interaction with TGFβ. They also possess multiple epidermal growth factor (EGF)-like domains that bind calcium.[9] Interestingly, fibrillins possess a very similar structure to another family of proteins in the matrix-termed latent TGFβ-binding proteins (LTBPs), which are also cysteine rich and function as signalling molecules to direct cell behaviour. Specifically, LTBPs directly interact with TGFβ, sequestering it within the tissue microenvironment and thus regulate its bioavailability (Fig. 2).

3.1.2. Mechanism of disease

Fibrillins contain the known integrin-interacting sequence RGD to support cell adhesion and can be viewed as a physical tether between local cells and their matrix. In the aorta, this contributes to tissue stability. The loss of these vital cell-matrix connections displayed a number of key features: i) elastin

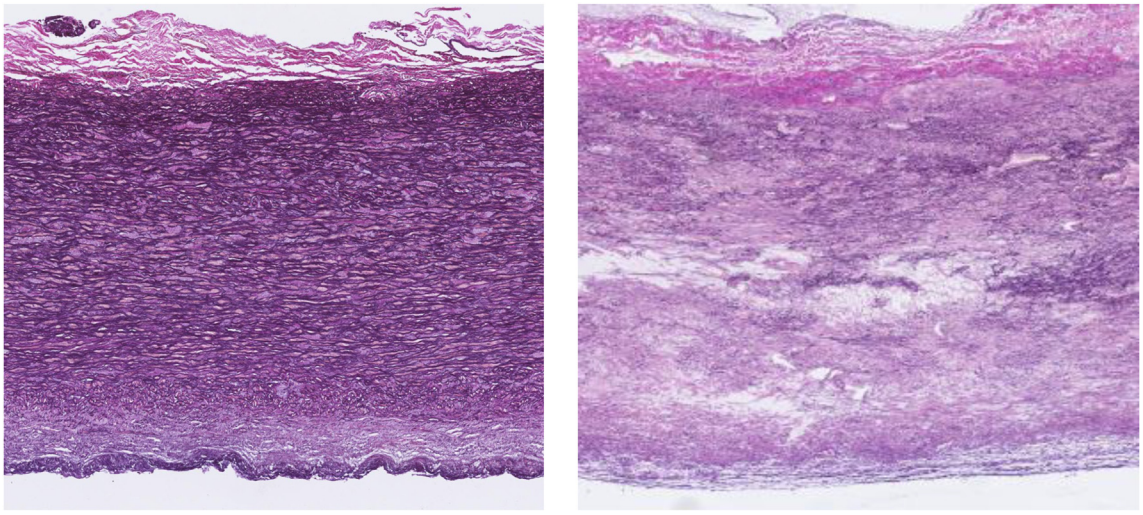

Figure 1. Histological images of the ascending aortic wall demonstrates healthy aortic media (A) and severe degeneration of the aortic media from a patient with aortopathy (B).

Table 2
Summary of genes associated with TAA phenotypes.

Gene	Associated Protein	Locus	OMIM	Syndrome
Extracellular matrix proteins				
FBN1	Fibrillin-1	15q21.1	134797	MFS, MASS phenotype (8)
EFEMP2	Fibulin-4	11q13.1	604633	AR cutis laxa[67]
ELN	Elastin	7q11.23	130160	AD cutis laxa[68]
COL3A1	Collagen 3 α-1	2q32.2	120180	EDS vascular type[69]
COL5A1	Collagen 5 α-1	9q34.3	120215	EDS classic type
COL1A1	Collagen 1 α-1	17q21.33	120150	Osteogenesis Imperfecta[70]
COL1A2	Collagen 1 α-2	7q21.3	120160	Osteogenesis Imperfecta, EDS, valvular type[70]
PLOD1	lysyl hydroxylase 1	1p36.22	153454	EDS, kyphoscoliotic[71]
TGFB signalling pathway				
TGFBR1	TGFβ receptor-1	9q22.33	190181	LDS[20]
TBFBR2	TGFβ receptor-2	3p24–25	190182	LDS, FTAAD (*TAAD2*)[19,72]
TGFB2	TGFβ –2	1q41	190220	LDS[72]
TGFB3	TGFβ –3	14q24.3	190230	LDS-like syndrome
SMAD3	SMAD family member 3	15q22.33	603109	LDS with osteoarthritis[22]
SKI	v-SKI sarcoma oncogene homolog	1p36.33-p3632	182212	Shprintzen–Goldberg Syndrome[73]
SLC2A10	glucose transporter 10	20q13.12	606145	Arterial tortuosity Syndrome[33]
Cytoskeletal/SM contraction apparatus proteins				
ACTA2	α-smooth muscle actin	10q23.31	102620	Familial aortic aneurysm with levido reticularis and iris floccule[49]
MYH11	smooth muscle myosin	16p13.11	160745	Familial aortic aneurysm with patent ductus arteriosus[51,52]
MYLK	myosin light chain kinase	3q21.1	600922	Familial aortic aneurysm and dissection[53]
PRKG1	protein kinase, cGMP-dependent, type I	10q11.23	176894	Familial aortic aneurysm and dissection[54]
PTPN11	SH2 domain-containing protein tyrosine phosphatase-2	12q24.13	176876	Noonan's syndrome[34] and LEOPARD syndrome[74]
Other proteins				
NOTCH1	Notch 1	9q34-q35	190198	BAV/TAAD
KCNJ2		17q23.1–q24.2	600681	BAV/Coarctation
LOX	Lysyl oxidase	5q23.1	153455	[75]

degradation, ii) increase MMP activity leading to further matrix degeneration and iii) SMC loss through apoptotic mechanisms. These features stem from alteration of SMC phenotype in an apparent attempted repair mechanism to sustain loss of matrix, though the processes unfortunately mediate further degeneration of the matrix, contributing to weakening of the vessel. This emphasises the need for VSMCs to be physically linked to their matrix in order to sense the ongoing needs of the ECM for homeostasis: a constant dynamic dialogue and exchange of information.

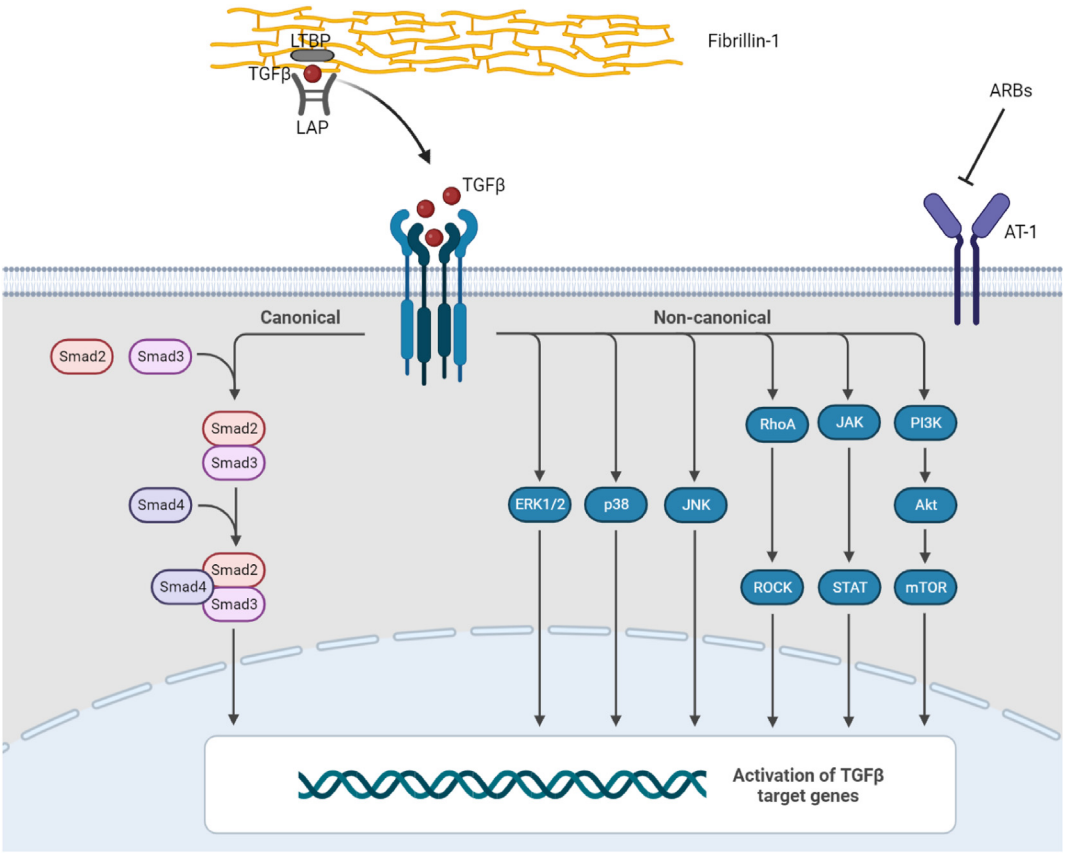

Figure 2. Canonical and non-canonical TGFβ signalling. In this figure, latent TGFβ activation and intracellular pathways are depicted. TGFβ is sequestered in the extracellular matrix by latent TGFβ-binding protein (LTBP) and fibrillin. In the intracellular space, SMAD-mediated canonical signalling (red) and non-canonical (green) p38, extracellular signal-regulated kinase (ERK1/2) and Jun N-terminal associated kinase (JNK1)-mediated pathways are shown.

3.1.3. *Types of FBN1 mutations*

The biomechanical function of FBN1 is dependent upon its proper expression, assembly and incorporation.[10] The majority of FBN1 mutations in Marfans are point mutations, though frame-shift, nonsense and splice-site mutations also exist, in addition to whole-exon or even whole-gene deletions.[11,12] This strongly suggests the loss of function of the FBN1 protein as the relevant mechanism. Substitutions of cysteine residues that alter the disulphide bonds within the EGF or cysteine-binding modules are the most frequent mutations. These structural alterations impair the organization of the fibrillin into microfibril bundles. In a mouse model of MFS, the FBN1 knockout, the appearance of the aorta displays elastin degradation and disorganization along with considerable loss of FBN1[13]. FBN1 knockout mice die within one year of birth due to aortic dissection and rupture: aneurysms initiates within 2 weeks of birth and continue to enlarge with time.[14]

In addition, *FBN1* mutations substantially impact fibrillin's ability to bind and sequester TGFβ, leading to increased free TGFβ and excessive signalling of this pathway.[15] A number of trials have therefore attempted to use inhibitors of TGFβ signalling, namely Losartan, to halt TAA progression in MFS, particularly in children,[16,17] though its efficacy in comparison to beta-blockade was not established.[18]

3.2. *Loeys-Dietz Syndrome and Mutations in TGFβ signalling-related genes*

Loeys-Dietz syndrome (LDS) is an autosomal dominant disorder linked to mutations in the genes coding for the receptors for TGFβ (*TGFBR1* and *TGFBR2*).[19,20]

3.2.1. *Phenotype of patients with LDS*

Patients with LDS show many phenotypic features that overlap with those of MFS such as scoliosis, arachnodactyly, pectus deformity and importantly aortic aneurysm centered at the sinuses of Valsalva. In addition to this, the syndrome is associated with an array of other extra-aortic manifestations, including cleft palate, craniosynostosis, translucent skin and a bifid uvula. Among the original 52 families described, 75% of affected individuals had LDS type I with craniofacial manifestations (widely spaced eyes, bifid uvula/cleft palate, craniosynostosis); 25% of individuals had LDS type II with systemic manifestations of LDS type I but minimal or absent craniofacial features. The natural history of LDS is characterized by aggressive arterial aneurysms (mean age at death 26.1 years) and a high incidence of pregnancy-related complications, including uterine rupture and death.

Aortic dissection has been observed as early as early childhood and at aortic dimensions that do not confer the risk of dissection or rupture in other aortopathies. Compared to MFS, the arterial involvement is more diffuse with arterial tortuosity present in most individuals, affecting the head and neck vessels.

3.2.2. *Role of TGFβ pathways in LDS*

TGFβ signalling is enhanced in LDS, which is thus detected by elevated connective tissue growth factor (CTGF) expression and nuclear *SMAD2* localisation.[19,20] The up-regulation of CTGF could in-turn propagate excessive TGFβ signalling via attenuation of the inhibitor *SMAD7*, a key component of the TGFβ feedback loop (Fig. 2).

Evidence suggests that the signalling cascade distal to the TGFβ receptor is key to its pathogenesis. LDS-causing mutations in the

TGFBR1 and *TGFBR2* genes, mostly missense changes, cluster around the receptor kinase domains, which are regions of the receptor protein that potentiate canonical TGFβ signalling. Interestingly, the loss of function mutations in *TGFBR1* cause Ferguson-Smith Disease[21] and not LDS, which have entirely different clinical manifestations (Ferguson-Smith Disease presents with multiple spontaneous epitheliomas and not the features of LDS, including TAA and TAAD).

TGFBR1 and *TGFBR2* are activated in tandem leading to phosphorylation of receptor proteins such as SMAD2 and SMAD3, which translocate to the nucleus as transcriptional modulators. Patients with the loss of function mutations in *SMAD3* were found to show the complete phenotypic picture of LDS.[22] Despite this, evidence suggests that the overactivation of TGFβ signalling is the pathogenic process leading to aneurysm formation and LDS. Tissue obtained from patients with *SMAD3* or *TGFB2* mutations obtained at the time of aortic surgery show the characteristic signature of increased rather than decreased TGFβ signalling (increased phosphorylation of SMAD proteins and increased expression of TGFβ ligands).[22,23]

3.2.3. Similarities with MFS

[24] Histologically, LDS and MFS also have similar appearances, namely medial degeneration with little evidence of inflammation, including findings of elastin fragmentation and increased collagen deposition.[25] The similarity in MFS can be described by the commonality in effects on the TGFβ pathway: both conditions lead to the overactivation of TGFβ signalling (mutations in the FBN1 protein in MFS lead to the increased chemical availability of TGFβ). Also, the activation of the TGFβ pathway potentiates inhibitors of tissue proteases such as plasminogen activator inhibitor-1 (PAI-1) and tissue inhibitors of the MMPs (TIMPs). MMPs are essential for normal remodelling and organised matrix deposition, as seen in other tissues such as the skeletal system. Therefore, the result of enhanced activation of cascades involving TGFβ and CTGF is a disorganised tissue remodelling process leading to compromised mechanical integrity. As well as LDS, studies have also shown higher circulating TGFβ in MFS patients compared to healthy controls.[26] Following treatment with Losartan, circulating TGF levels were also found to decline.[27]

3.3. Ehlers-Danlos Syndromes

Collagen is a vital structural protein of the aortic wall, particularly type III collagen, which predominates in the aortic media and is synthesised by VSMCs. Collagen fibre strength is dependent on its cross-links formed between adjacent helical domains.

A heterogeneous group of connective tissue disorders, many types of Ehlers-Danlos Syndromes (EDS) are known to cause serious vascular complications and aneurysmal disease, all of which are linked to the molecular structure and assembly of fibrillar collagen. According to the Villefranche nosology,[28] the classic, hypermobile, vascular and kyphosclerotic types (formerly types I/II, III, IV and VI) are associated with aortic enlargement.

EDS vascular type is characterized by thin, translucent skin, easy bruising, characteristic facial appearance, arterial, intestinal and uterine fragility. Vascular rupture or dissection and gastrointestinal perforation are extremely common (up to 70% of effected members). Arterial rupture may occur with or without a preceding aneurysm. Given the extreme friability and fragility of aortic tissue, patients with type IV Ehlers–Danlos syndrome are at a greater risk of adverse outcomes following vascular surgery than patients with other connective tissue disorders. Median survival of patients is 48 years.[29] Inheritance is autosomal dominant, and diagnosis is confirmed by abnormal type III collagen biosynthesis and/or the identification of a mutation in COL3A1: this is the only gene known to be associated with EDS vascular type. More than 70 mutations have been identified.[30,31]

EDS kyphoscoliotic type is characterized by kyphoscoliosis, joint laxity and muscle hypotonia. Affected individuals are at risk of rupture of medium- and large-sized arteries, including the aorta, which can be preceded by dilatation. These individuals have a deficient activity of the enzyme procollagen-lysine, 2-oxoglutarate 5-dioxygenase 1 (*PLOD1* or lysyl hydroxylase 1). Mutations in *PLOD1*, the gene encoding the enzyme lysyl hydroxylase 1, are causative, and inheritance is autosomal recessive.

3.4. Other syndromes associated with TAA

TAA is a common phenotype in other genetic conditions as well, but very few are due to monogenic mutations. TAAs occur in 40% of patients with Turner Syndrome.[32] The syndrome is characterized by a 45, XO aneuploid chromosomal constitution. Its phenotypic characteristics include short stature and gonadal disgenesis. In addition to aortic dilatation, bicuspid aortic valve and aortic coarctation are associated. The loss of function mutations in *SLC2A10* can cause arterial tortuosity syndrome, with similarly high TGFβ signalling as seen in tissues from patients with MFS and LDS.[33] Although infrequently observed, TAA has also been reported in Noonan syndrome[34] and neurofibromatosis type 1 (NF1).[35]

3.4.1. Bicuspid aortic valve

Bicuspid aortic valve (BAV) disease is the most common cardiovascular malformation in humans (1–2%), with a gender bias of 2:1, males versus females.[36] TAAs affect about 40% of patients with BAVs and have a considerable association with increased risk of dissection and rupture.[37] BAV occurs as a result of defective endocardial endothelial-to-mesenchymal transition during embryonic development – a process that relies strongly on *NOTCH1* signalling. The association between BAV-related aortopathy and Notch 1 signalling has been noted,[38] though only a very small portion of patients with both BAV and TAA have any mutations in *NOTCH1* or any of the known aortopathy genes.[39] Despite this, it is widely observed that the BAV-associated TAA syndrome follows an autosomal-dominant pattern of inheritance, albeit with incomplete penetrance and variable expressivity.[40,41]

Whilst, to date, there is no single gene mutation known to cause BAV, there are two possible theories in the debate surrounding the causative mechanisms of TAA and TAAD in patients with BAV disease. Firstly, aneurysmal disease may stem from the effects of altered hemodynamics through the BAV, including eccentric flow patterns and altered stress acting on the aortic wall.[10] Secondly, this may be due to an inherent defect in the wall of the ascending aorta, which contributes to the loss of biomechanical strength and leads to dilatation and aneurysm formation. BAV patients overall have been found to have larger diameter aortic roots and ascending aortas compared to age- and sex-matched controls.[42] Current guidelines stipulate intervention on BAV-associated aneurysms earlier than non-BAV aneurysms, at a size cut-off of 50 mm (compared to the wider 55 mm recommendation).[43]

Excised aneurysmal tissue of BAV patients display similar histopathology to that of connective tissue disorders (discussed above). These included medial degeneration, loss of VSMCs, accumulation of proteoglycans and increased MMP activity. As with other TAAs, those occurring in BAV patients involve ECM degradation, which results in a weakened biomechanical response.

4. Familial thoracic aortic aneurysms (non-syndromic TAA)

Up to 20% of patients referred for repair of thoracic aneurysm or dissection have familial clustering of the disease.[44] The existence of hereditary TAA with no other syndromic manifestations was recognised over thirty years ago,[45] though their increased recognition in recent years has stemmed from the ongoing discovery of novel causative genes. Termed Familial Thoracic Aortic Aneurysms and Dissection (or FTAAD), these conditions follow an autosomal pattern of inheritance with decreased penetrance and variable expression.[46] Of associated genes, *TGFBR1* and *TGFBR2* have been found as a cause of FTAAD, as cases with TAA and no other manifestations of LDS. When *TGFBR2* is the affected gene in FTAAD, the condition is called TAA2.

A common collection of causative genes of FTAAD (*ACTA2, MYH11, MYLK, PRKG1*) all code for SMC contractile proteins. In normal subjects, SMCs express the smooth muscle-specific isoform of α-actin (encoded by *ACTA2*) that oligomerises to form thin filaments.[47] The thick filaments of the cytoskeleton are composed of a SM-specific isoform of myosin heavy chain dimer, β-myosin-heavy-chain (βMHC) (encoded by *MYH11*) and 4 light chains. The thick and thin filaments are assembled into contractile units anchored to focal adhesions on the cell membranes and dense bodies in the cytoplasm[48](Fig. 3). Actomyosin contractility and VSMC linkage with the ECM are vital for appropriate aortic mechanical homeostasis, which relies on the cross-linkage between actin and myosin filaments.

4.1. ACTA2 mutations

ACTA2 mutations were first identified in a large family affected by non-syndromic TAAs that have an autosomal-dominant inheritance mode and low penetrance (0.48%).[49] *ACTA2* mutations are the most commonly identified cause of FTAAD. Patients are predisposed to TAA with few outward physical manifestations, though they may present with livedo reticularis (mottled discolouration of skin) or mydriasis with iris floccule (benign cyst like lesions at the pupillary border of the iris) and other stigmata of systemic smooth muscle dysfunction. Some patients have also been associated with a premature onset of coronary artery disease and ischemic stroke,[50] suggesting a widespread disruption of mechanotransduction pathways in vascular tissue. *ACTA2* mutations likely affect local adaptation of cellular and extracellular responses to mechanical stresses. Analysis of aneurysmal aortic tissue shows a decreased number of VSMCs, fragmentation and loss of elastic fibres, as well as an accumulation of proteoglycans in the media. In non-FTAAD forms of *ACTA2* mutations, patients can still present with livedo reticularis, iris floccule, a patent ductus arteriosus and non-thoracic aneurysms.

ACTA2 is a highly conserved gene and relatively invariant in the general population. To date, more than 40 mutations have been identified that predispose to FTAAD,[48] the majority being missense mutations. *ACTA2* mutations disrupt amino acids located in all 4 subdomains of actin and are likely to produce structurally altered actin monomers. This means that for FTAAD to manifest, *ACTA2* mutations need to alter the vital structure of the smooth muscle isoform of α-actin: there is no evidence that *ACTA2* variants leading to haploinsufficiency predispose to thoracic aortic disease.

4.2. MYH11 mutations

MYH11 mutations have been linked to familial TAA and patent ductus arteriosus formation.[51] These mutations are usually in-frame deletions in *MYH1*1 that appear to inhibit the inclusion of a coiled-coil structure by βMHC. This leads to a failure of myosin thick filaments to be assembled. Subsequent analysis of three unrelated families with TAA and patent ductus arteriosus identified missense point mutations in the *MYH11* gene in two families (the remaining family had a TGFβR2 mutation).[52] As well as focal areas of VSMC loss and the loss of elastic fibres and collagen, histological studies also show VSMC disarray and focal areas of cell hyperplasia.[52] Also of note, as observed with *ACTA2* mutations, there was increased vascularity and marked dysplasia in the adventitial vasa vasorum, which seemed to invade the media.[52]

4.3. Mutations in regulator kinases of SMC actin-myosin

4.3.1. MYLK mutations

Mutations in *MYLK* (the gene encoding myosin-light-chain kinase) were identified by candidate gene analysis as rare causes of FTAAD,[53] usually as heterozygous loss-of-function mutations. These cause either haploinsufficiency or alter the calmodulin-binding domain, thus impairing the activation of kinase function. This leads to a disruption in the regulation of actin-myosin interaction and impaired actomyosin cross-bridge cycling for SMC contraction.

4.3.2. PRKG1 mutations

Another important kinase protein, cGMP-dependent protein kinase 1, is an important regulator protein for actin-myosin function. A missense mutation in its encoding gene (*PRKG1*) has been associated with FTAAD.[54] PKG-1α is activated in SMCs when nitric oxide stimulates soluble guanylyl cyclase to increase cellular cGMP levels. The activated PKG-1α regulates many cellular systems that lead to the relaxation of SMCs, including phosphorylation of the regulatory myosin-binding subunit. *MYLK* and *PRKG1* mutations are predicted to decrease aortic SMC contraction as a result of decreased phosphorylation and increased dephosphorylation of the RLC, respectively.[48,54] PKG1 also regulates vascular tone in vivo, and murine models of *PRKG1* knockout or PKg1α mutations demonstrate severe hypertension.[55,56]

5. Sporadic thoracic aortic disease

Patients with TAA or TAAD and without a family history of the disease or syndromic features represent 80% of cases and are termed sporadic TAA and dissection (STAAD). The controversy remains whether STAAD patients should undergo genetic testing and what the value of positive results may be. Our increasing knowledge in the effects of germline mutations on target proteins crucial to aortic wall integrity has informed aortopathy specific genetic databases and acts as an important reference when identifying sporadic mutations, including SNPs, associated with the disease. Importantly, genetic testing in many patients with STAAD give rise to variants of genes not previously shown to be pathogenic. Large databases exist to compare the relevance of the variance to the general population, with most variants being of unknown significance (VUS).

5.1. Gene wide association studies

Genetic testing in large patient groups with known phenotypes have provided further information on SNPs that may be linked with TAA and TAAD. Genome-wide association studies (GWAS) were most notably conducted in 800 STAAD patients (with strict exclusion of patients with syndromic features). Interestingly, the only GWAS peak for common variants associated with STAAD was found in SNPs of the FBN1 gene, located on chromosome 15,[57] conferring a ~2 fold increase in risk of developing the disease. Thus, both rare

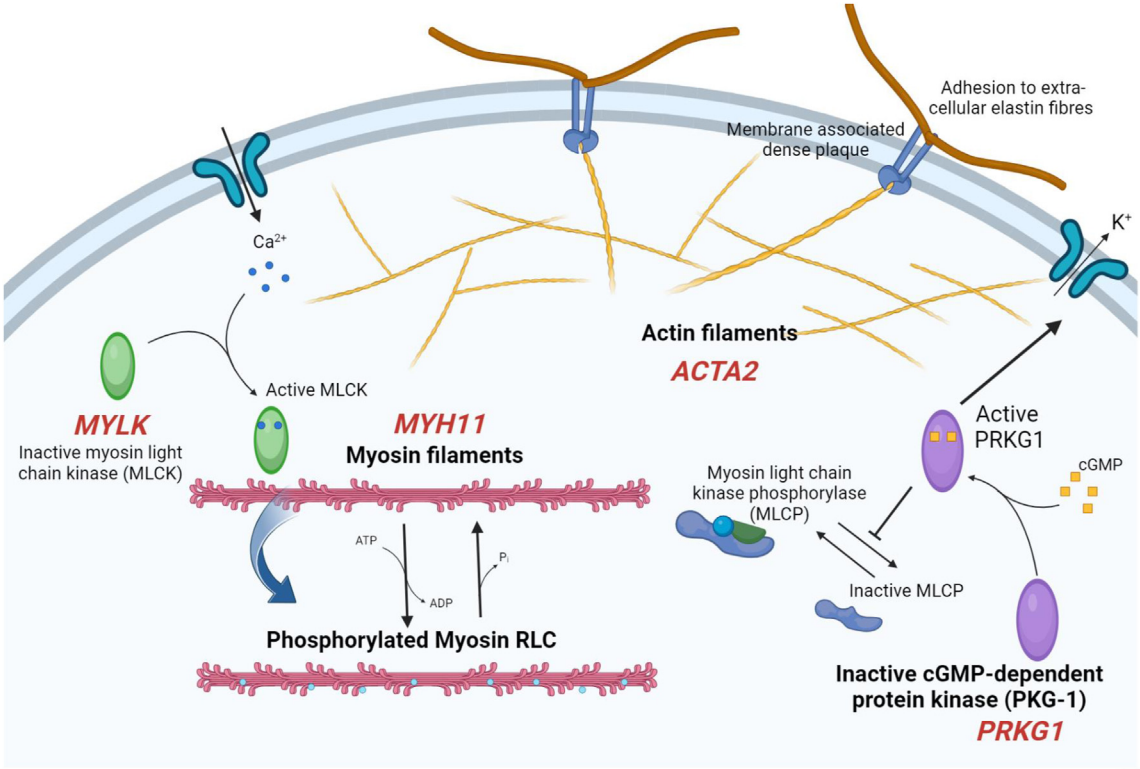

Figure 3. Signalling pathways involved in VSMC contraction/relaxation. Mutated genes causing heritable thoracic aortic disease (red font) disrupt critical proteins involved in SMC contraction. The pathway converges at the common step of regulatory light chain (RLC) phosphorylation.

pathogenic variants and common variants in *FBN1* predispose to TAA and TAAD.

As well as this, SNPs associated with the *MYH11* gene have found to be disease-causing, whereby CNV analyses of the same STAAD-patient GWAS data on (800 patients) identified a >12-fold increased risk specifically for aortic dissections that was associated with a recurrent duplication of 16p13.1, MYH11.[58] These initial studies indicate that genetic variants in known TAA/TAAD-related genes, FBN1 and MYH11, contribute to the pathogenesis of sporadic TAAs and dissections.

In another GWAS type study, exome sequencing was performed using samples from 355 STAAD cases with acute TAAD and <56 years of age (also with exclusion of patients with syndromic features). The frequency of individuals with pathogenic rare variants in any of the known disease-causing genes in this dissection cohort was 9.3%, 4% of which were *FBN1* mutations.[59]

6. Clinical applicability of genetic testing for TAA and TAAD

Genetic testing for rare disease-causing variants in hereditary TAA and TAAD genes is available worldwide. Selecting patients suitable for testing should be considered when an adequate suspicion of hereditary aortopathy exists. Referrals for testing will be triaged by the Genomic Laboratory; testing should be targeted at those where a genetic or genomic diagnosis will guide management for the proband or family. The criteria used to select patients, as outlined in the National Genomic Medicine Service Directory, is based on the incidence of TAA/TAAD at a young age, the presence of a family history and clinical features of connective tissue disease (Table 3).

The rationale for genetic testing is crucial because individuals can be identified as at-risk for TAAD and associated mortality/morbidity is preventable, including for potentially at-risk family members. Powerful information for aortic and other vascular disease management can also be collected.

6.1. Variants of "unknown" significance

For the ease of understanding, the genetic basis of TAA and TAAD has been typically classified as syndromic (part of a set of clinical findings) or non-syndromic (occurring as an isolated finding), as it has been classified in much of the contemporary literature on this subject. However, the distinction between the two entities is becoming increasingly blurred: current data indicates that variants in hereditary TAA genes can lead to a phenotypic spectrum that ranges from syndromic to non-syndromic risk for TAA/TAAD. In addition, some variants confer a high penetrant risk for disease, whereas other variants in the same gene confer a low penetrant risk for disease.[60]

Another important recent finding is that the frequency of VUSs identified in disease-associated genes in patients with TAAD was found to be significantly higher than in controls,[59] with 30% of the cases having 1 to 3 VUSs. The burden of VUSs was the greatest for *ACTA2, TGFBR2, TGFBR1* and *MYLK*.[59]

Functional assays and bioinformatics analyses support the hypothesis that some of these VUSs are disease-predisposing variants that trigger disease in a random manner or when other risk factors are present (namely hypertension) but are not in isolation highly penetrant pathogenic variants. The future therefore lies in the improvement of classification for VUSs in known hereditary TAA/TAAD genes so that accurate clinical assessment for the risk of TAAD in the general population can be conducted.[60]

Causal mutations are frequently identified in MFS and LDS, compared to FTAAD, where the causal mutation is identified in less than 10% of cases. The process of ascribing pathogenicity to a VUS is a complex process and should follow the ACMG guidelines[61] Over

Table 3
Eligibility criteria for genetic testing in aortopathy. Adapted from the NHS Genomic Medicine Service for Aortopathies.

Patients must satisfy one of the following to be considered for targeted genetic sequencing	
1	Thoracic aortic aneurysm (z score >2 for body surface area) or dissection with onset before age 50
2	Thoracic aortic aneurysm (z score >2 for body surface area) or dissection with onset before age 60 AND a first degree relative with thoracic aortic aneurysm or dissection
3	Thoracic aortic aneurysm (z score >2 for body surface area) or dissection before age 60 AND absence of classical cardiovascular risk factors
4	Thoracic aortic aneurysm (z score >2 for body surface area) or dissection before age 60 AND features suggestive of aortopathy, e.g., iris flocculi, arterial tortuosity,
5	Clinical features suggestive of Loeys-Dietz syndrome
6	Features of Marfan syndrome giving a systemic Ghent score of ≥7, following assessment by a clinical geneticist or specialist with expertise in aortopathy
7	High clinical suspicion of a condition predisposing to aortic/arterial disease AND diagnostic testing for other conditions such as Ehlers–Danlos syndrome (where indicated) has not identified a causative mutation

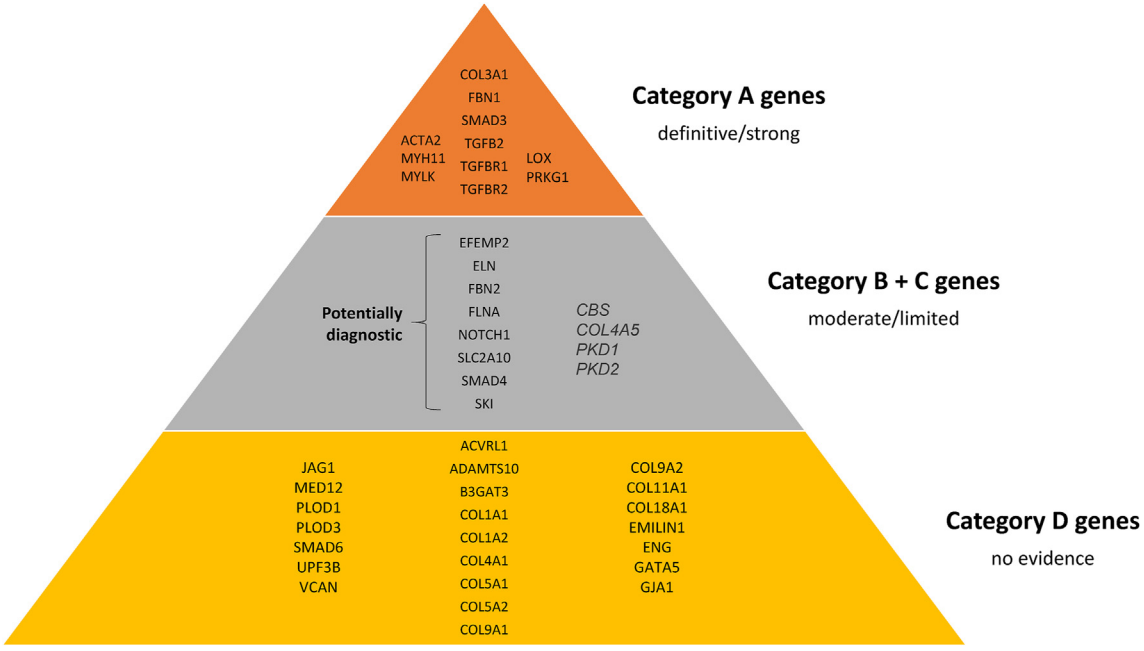

Figure 4. Classification of genes associated with TAA and TAAD. The above classification was curated using the semi-quantitative ClinGen framework to assess presumed gene-disease relationships between 53 candidate genes and hereditary forms of the disease. Adapted from Renard et al., 2018[62].

50 genes have been made available on diagnostic panels for aortopathy. The impact of many of these variants on the pathogenesis of TAA remains poorly understood, resulting in identification of many VUSs. The average aortopathy panel offered by commercial companies and clinical laboratories consists of 25 genes, many of which have limited to no clinical evidence. In this light, a balance is required between i) the act of curating genes on diagnostic panels to prevent ambiguous reports (to minimise diagnostic confusion) and ii) broadening the efforts to acquire more genetic data relating to potential disease causing (including polygenic) mechanisms. The appropriate assimilation of genetic information and diagnostic panels may help limit the emergence of VUS results and consequent burden of uncertainty, whilst still identifying at-risk patients and family members to guide disease management.[62]

6.2. ClinGen consortium

The human genome comprises approximately 20,000 protein-coding genes (see OMIM website in Web Resources), of which about 3,000 have been reported in association with at least one Mendelian disease.[63]

Efforts to help classify genetic diseases and facilitate more knowledgeable utilisation of genomic variants in clinical and research settings culminate in thorough appraisal of available evidence through expert panels. The NIH-funded Clinical Genome Resource (ClinGen) has developed a framework providing structured tools to define and evaluate the clinical validity of gene-disease pairs across a variety of Mendelian disorders, thus informing clinical utility and allowing for meaningful patient counselling and clinical decision making.[64] The framework provides a semi-quantitative measurement for the strength of evidence of a gene-disease relationship that correlates to a qualitative classification: “Definitive,” “Strong,” “Moderate,” “Limited,” “No Reported Evidence” or “Conflicting Evidence.”

In a recent classification analysis by Renard et al.[62] (achieving a highly cited JACC publication), 53 HTAAD gene-disease pairs were curated by an expert team. The genes are classified into pre-specified tiers based on the clinical, genetic and experimental evidence, along with discussion and consensus of the experts (Fig. 4). These clinically validated genes can be used to prioritize genes for research, inform which genes should be included in aortopathy disease panels and results returned from exome/genome sequencing and provide guidance for which genes to exclude from routine clinical reporting. For these purposes, HTAAD was defined as either thoracic aortic enlargement with evidence of progression to the life-threatening complication of acute aortic dissection or thoracic aortic dissection (TAD) in the absence of aneurysm formation.[62]

Only 11 genes (*ACTA2, COL3A1, FBN1, MYH11, SMAD3, TGFB2, TGFBR1, TGFBR2, MYLK, LOX, PRKG1*) were deemed to have "Definitive" or "Strong" gene-disease association (categorised as A1 and A2, respectively). The remainder had limited or no clinical evidence, or unknown significance. Genes with limited clinical evidence include *CBS, COL4A5, PKD1 and PKD2*, which are well-known causes for congenital conditions with a very low incidence of TAAD, and may be diagnosed based on pathology in other systems that have a greater clinical impact than the rare aortic pathology, such as renal abnormalities. Whilst not exclusive, the result of this gene curation effort may provide useful information to guide clinical laboratories in the development and interpretation of genetic testing for patients with aortic disease.[62] The process of curating a genetic variant into a bone fide pathogenic identity is complex and involves an assessment of the resulting genetic effect of the mutation (e.g., missense vs nonsense vs loss of function) and thorough correlation with other pathogeneic variants of the affected gene reported in the literature. An example of this process has been recently reported by the present authors.[65]

Although genetic panels are increasing in size, most tests may be customised and tailored to include only genes with solid clinical evidence. Although the same panel may be run, narrowing the panel to genes of significance to the individual patient may allow for personalised medicine.

7. Conclusion

Under current guidelines, there are no metrics by which those at risk for aortic dissection with normal aortic diameters may undergo preventive surgery: even a patient with a known pathogenic mutation is only offered intervention once the aorta reaches a size threshold on imaging surveillance. In patients with sporadic TAA or TAAD, the underlying pathology may be due to polygenic or sporadic mutations with similar pathophysiology as monogenic conditions such as MFS, LDS or smooth muscle contraction defects. However, current databases and diagnostic panels are far from offering relevant explanations for several VUSs, or link these to the disease pathogenesis, or indeed to provide a measure of risk for dissection. Future research lies with more advanced genetic diagnoses of HTAAD and investigation of the diverse pathways involved in its pathophysiology, which will serve to improve our understanding of the underlying mechanisms, improve guidelines for treatment and prevent complications for HTAAD and sporadic aortopathies.

Conflicts of interest

None to declare.

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