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Review

Genetic basis of hereditary thoracic aortic aneurysms and dissections

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ABSTRACT

Recent advances in DNA sequencing technology have identified several causative genes for hereditary thoracic aortic aneurysms and dissections (TAADs), including Marfan syndrome (MFS), Loeys–Dietz syndrome, vascular Ehlers–Danlos syndrome, and familial non-syndromic TAADs. Syndromic TAADs are typically caused by pathogenic variants in the transforming growth factor-β signal and extracellular matrix-related genes (e.g. *FBN1*, *TGFBR1*, *TGFBR2*, *SMAD3*, *TGFB2*, and *COL3A1*). On the other hand, approximately 20% of the non-syndromic hereditary TAADs result from altered components of the contractile apparatus of vascular smooth muscle cells, which are encoded by *ACTA2*, *MYH11*, *MYLK*, and *PRKG1* genes; however, the remaining 80% cannot be explained by previously reported candidate genes. Moreover, the relationship between the genotype and phenotype of TAADs has extensively been reported to investigate better methods for risk stratification and further personalized treatment strategies. With regard to MFS-causing *FBN1*, recent reports have shown significantly increased risk of aortic events in patients carrying a truncating variant or a variant exhibiting a haploinsufficient-type effect, typically comprising nonsense or small insertions/deletions resulting in out-of-frame effects, compared to those carrying a variant with dominant negative-type effect, typically comprising missense variants. Therefore, cardiologists are required to have sufficient knowledge regarding the genetics of hereditary TAADs for providing the best clinical management, with an appropriate genetic counseling. In the current review, we present current advances in the genetics of hereditary TAADs and discuss the benefits and limitations with respect to the use of this genetic understanding in clinical settings.

Contents

Introduction

Improved technology and decreased costs for DNA sequencing have identified the predisposition genes in hereditary thoracic aortic aneurysm and dissection (HTAAD), including those associated with Marfan syndrome (MFS), Loeys–Dietz syndrome (LDS), vascular Ehlers–Danlos syndrome (vEDS), and familial non-syndromic TAAD, and such genetic tests to guide precision medicine have been covered by health insurance in Japan since 2016. The ClinGen Aortopathy Expert Panel have classified 11 causative genes (*FBN1*, *TGFBR1*, *TGFBR2*, *SMAD3*, *TGFB2*, *COL3A1*, *ACTA2*, *MYH11*, *MYLK*, *LOX*, and *PRKG1*) for HTAAD as category A

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Table 1
Genes associated with familial thoracic aortic aneurysm [1].

Category	Gene symbol	Disease	OMIM No.
Category A1 and recently identified (uncertain) genes, associated with syndromic TAAD			
A1	*FBN1*	Marfan syndrome (MFS)	154700
A1	*TGFBR1*, *TGFBR2*, *SMAD3*, *TGFB2*	Loeys–Dietz syndrome (LDS), type 1–4	609192, 610168, 613795, 614816
Uncertain	*TGFB3*, *SMAD2*	Loeys–Dietz syndrome (LDS), type 5 and 6	615582, unassigned
Uncertain	*BGN*	LDS-like and MFS-like	301870
A1	*COL3A1*	Ehlers–Danlos syndrome (EDS), vascular type	130050
Category A2 and recently identified (uncertain) genes, associated with non-syndromic TAAD			
A2	*ATCA2*	Aortic aneurysm, familial thoracic 6	611788
A2	*MYH11*	Aortic aneurysm, familial thoracic 4	132900
A2	*MYLK*	Aortic aneurysm, familial thoracic 7	613780
A2	*PRKG1*	Aortic aneurysm, familial thoracic 8	615436
A2	*LOX*	Aortic aneurysm, familial thoracic 10	617168
Uncertain	*FOXE3*	Aortic aneurysm, familial thoracic 11	617349
Uncertain	*HCN4*	Sick sinus syndrome 2	163800
Uncertain	*MAT2A*	Aortic dilatation, bicuspid aortic valve	Unassigned
Uncertain	*MFAP5*	Aortic aneurysm, familial thoracic 9	616166
Category B: genes without significant risks of progression or aortic dissection			
B	*SKI*	Shprintzen–Goldberg syndrome	182212
B	*FBN2*	Congenital contractural arachnodactyly	121050
B	*FLNA*	Periventricular nodular heterotopia	300049
B	*ELN*	Cutis laxa, autosomal dominant	123700
B	*EFEMP2 (FBLN4)*	Cutis laxa, autosomal recessive, type IB	604633
B	*SLC2A10*	Arterial tortuosity syndrome	208050
B	*NOTCH1*	Bicuspid aortic valve with aneurysm	109730
B	*SMAD4*	Juvenile polyposis/hereditary hemorrhagic telangiectasia syndrome	175050
Category C: low risk genes for TAAD			
C	*COL4A5*	Alport syndrome, X-linked	301050
C	*PKD1*, *PKD2*	Polycystic kidney disease 1 and 2	173900, 613095
C	*CBS*	Homocystinuria	236200
Category D: some experimental data may suggest a link with TAAD, but no clinical evidence is available.			
D	**Gene lists:** *ACVRL1*, *ADAMTS10*, *B3GAT3*, *COL1A1*, *COL1A2*, *COL4A1*, *COL5A1*, *COL5A2*, *COL9A1*, *COL9A2*, *COL11A1*, *COL18A1*, *EMILIN1*, *ENG*, *GATA5*, *GJA1*, *JAG1*, *MED12*, *PLOD1*, *PLOD3*, *SMAD6*, *UPF3B*, *VCAN*		

TAAD, thoracic aneurysm and dissection.

genes in 2018 (Table 1) [1]; this classification should trigger aortic imaging, medical therapy, and screening of family members for the variant with the goal of preventing acute aortic dissections, and thus cardiologists are required to have sufficient knowledge regarding the genetics of HTAAD for providing the best clinical management. In the current review, we present and discuss current advances in the genetics of HTAAD for its diagnosis and long-term management, with a focus on the category A genes.

Marfan syndrome

MFS is an autosomal dominant heritable disorder of the 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 [2]. Recent advances in surgical therapies for TAADs have improved life expectancy from approximately 30 years to >70 years; however, the comprehensive management strategies for preventing multiple organ system disorders remain inadequate to relieve lifetime anxiety and enhance the quality of life (QOL) of patients, despite the administration of β-blockers and angiotensin II receptor blockers, such as losartan [2].

The diagnosis can be made according to the 2010 revised Ghent criteria (Table 2) [3], which place particular focus on the cardiovascular manifestations (aortic root aneurysm and/or dissection), ectopia lentis, and molecular genetic testing of *FBN1*. *FBN1* spans about 230 kb of genomic DNA containing 66 exons (NM_000138.4), and >3000 different pathogenic variants have been identified and are distributed throughout the entire region of the gene [2]. In addition, we have identified 243 pathogenic and likely pathogenic variants (mutations) from 288 Japanese MFS families at our institute until February 2019. We have observed that missense mutations affecting/creating cysteine residues are the major variant type (85/243; 35.0%), followed by other missense (44/243; 18.1%), nonsense (44/243; 18.1%), in-frame and out-of-frame deletion/insertions (44/243; 18.1%), and splice (26/243; 10.7%) mutations, which is consistent with data previously reported [4,5].

The encoded fibrillin-1 is a major component of elastic fibers that are widely expressed in structural elements of the affected organs and tissues, and MFS is traditionally considered to result from the structural weakness of the connective tissue. However, recent reports on molecular mechanisms of MFS have indicated that fibrillin-1 interacts with latent transforming growth factor-β (TGF-β) binding protein-1 (LTBP) to regulate TGF-β activity (Fig. 1A), and therefore defective fibrillin-1 results in the structural weakness and excessive activity of TGF-β signaling—both of which contribute to the complicated pathogenesis [2]. Fibrillin-1 contains 7 TGF-β binding protein-like (TB) domains and 47 epidermal growth factor (EGF)-like domains (Fig. 1B), which include 8 and 6 cysteine residues that are involved in 4 and 3 intramodule disulfide bond formation, respectively. Of the 47 EGF-like domains, 43 contain a consensus sequence for calcium binding (cb-EGF) that is typically present as multiple tandem repeats and play a crucial role in microfibril stability and assembly [2].

Although there are no obvious mutational hot spots and the precise molecular mechanism for how mutations affect fibrillin-1 structure and function remains largely elusive, the relationships between the location or type of *FBN1* mutations and phenotypes have been extensively reported to search for better methods for risk stratification and personalized treatment strategies [6]. It has

Table 2
The revised Ghent nosology for Marfan syndrome [3].

In the absence of family history:
(1) Aortic aneurysm ($Z \geq 2$) AND ectopia lentis
(2) Aortic aneurysm ($Z \geq 2$) AND *FBN1* mutation
(3) Aortic aneurysm ($Z \geq 2$) AND systemic score ($\geq$7 pts)
(4) Ectopia lentis AND *FBN1* mutation with known aortic aneurysm
- Ectopia lentis syndrome: Ectopia lentis with or without systemic score AND with an *FBN1* mutation not known with aortic aneurysm or no *FBN1* mutation
- MASS: Aortic aneurysm ($Z < 2$) AND systemic score ($5 \geq$ with at least one skeletal feature) without ectopia lentis
- Mitral valve prolapse syndrome: mitral valve prolapse AND aortic aneurysm ($Z < 2$) AND systemic score (<5) without ectopia lentis
In the presence of family history:
(5) Ectopia lentis AND family history of MFS (as defined above)
(6) Systemic score ($\geq$7 pts) AND family history of MFS (as defined above)
(7) Aortic aneurysm ($Z \geq 2$ above 20 years old, $\geq$3 below 20 years) AND family history of MFS (as defined above)
Scoring of systemic features:
Maximum total: 20 points; score $\geq$7 indicates systemic involvement
Wrist AND thumb sign – 3 (wrist OR thumb sign – 1)
Pectus carinatum deformity – 2 (pectus excavatum or chest asymmetry – 1)
Hind foot deformity – 2 (plain pes planus – 1)
Pneumothorax – 2
Dural ectasia – 2
Protrusio acetabuli – 2
Reduced US/LS AND increased arm/height AND no severe scoliosis – 1
Scoliosis or thoracolumbar kyphosis – 1
Reduced elbow extension – 1
Facial features (3/5) – 1 (dolichocephaly, enophthalmos, down-slanting palpebral fissures, malar hypoplasia, retrognathia)
Skin striae – 1
Myopia $>$3 diopters – 1
Mitral valve prolapse (all types) – 1

MFS, Marfan syndrome; MASS, myopia, mitral valve prolapse, borderline ($Z < 2$) aortic root dilatation, striae, skeletal findings phenotype; US/LS, upper segment/lower segment ratio; and *Z*, *Z*-score.

long been known that a higher probability of ectopia lentis is found in patients with a missense variant affecting a cysteine residue particularly in the first 16 exons, and exons 25–33 including a central stretch of cb-EGF domains are recognized as a critical region for severe form of neonatal MFS, which is characterized by severe mitral and/or tricuspid valvular insufficiency and pulmonary emphysema. In addition, recent reports have shown significantly increased risk of aortic events in patients carrying a truncating variant or a variant with a haploinsufficient (HI)-type effect, typically comprising nonsense and splice site variant, or small insertions/deletions leading to out-of-frame effects, compared to patients carrying a variant with dominant negative (DN)-type effect, typically comprising missense variants or small insertions/deletions leading to in-frame effects (Fig. 2) [6].

To elucidate the relationship between *FBN1* genotype and severe aortopathy (aortic root replacement, type A dissections, and related death) in Japanese patients with MFS, we have recently evaluated 248 patients with pathogenic or likely pathogenic *FBN1* variants [7]. Only *FBN1* variants fulfilling the 2015 American College of Medical Genetics and Genomics-Association for Molecular Pathology criteria for pathogenicity ($\geq$class 4) were included. Variants were simply subcategorized into 2 groups (HI and DN) according to the predicted effects on protein structure and function; however, most splice site variants were classified into DN variants in this study, because most exons in *FBN1* have lengths that are multiples of 3 bp and the defective proteins presumably exert in-frame exon skipping effects. Prophylactic aortic root replacement had been recommended for those having an aortic diameter $\geq$45 mm at our institute.

The main results from our Japanese patients were consistent with previous data from non-Japanese patients that show HI patients had a 2.1-fold higher risk of severe aortic events compared with DN patients, and male patients had an increased risk: the median event-free survival period from birth for men and women was 31.0 and 41.0 years, respectively, in HI group and 41.0 and 55.0 years, respectively, in DN group [7]. The increased risk in men is attributed not only to the absolute threshold for aortic surgery independent of body size and the larger overall body surface area of men, but also to rapid growth in men. In addition, we newly identified an early-onset genetic subgroup within the slow-onset DN group, showing that patients with variants affecting or creating Cysteine residues and in-frame Deletion (DN-CD) variants in the central tandem cb-EGF domains (exons 26–37, c.3083–4582 and 44–50, c.5297–6163) were as deleterious as HI patients and were at

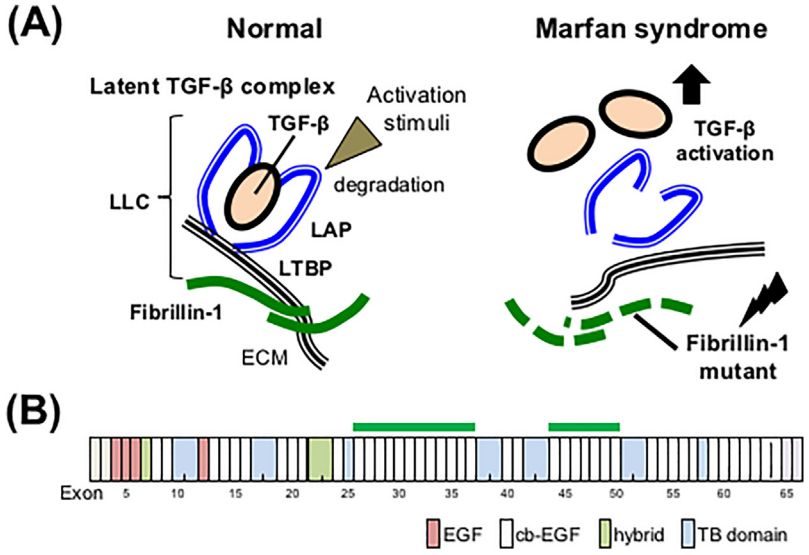

Fig. 1. Mutations in the *FBN1* gene encoding fibrillin-1 cause dysregulation of transforming growth factor (TGF)-β bioavailability. (A) (Left side) TGF-β is secreted in an inactivated latent form that requires proteolysis for activation. (Right side) Mutated fibrillin-1 in Marfan syndrome leads to failed sequestration of latent TGF-β in the extracellular matrix (ECM) and subsequent activation of TGF-β signaling cascades. (B) Domain structure of fibrillin-1.
LLC, large latent complexes; LAP, latency-associated peptide; LTBP, latent TGF-β binding protein; EGF, epidermal growth factor-like domains; cb-EGF, EGF-like domains with a calcium binding domain; TB, TGF-β binding protein-like domains; Hybrid, hybrid domain containing features of both TB and cb-EGF domains.

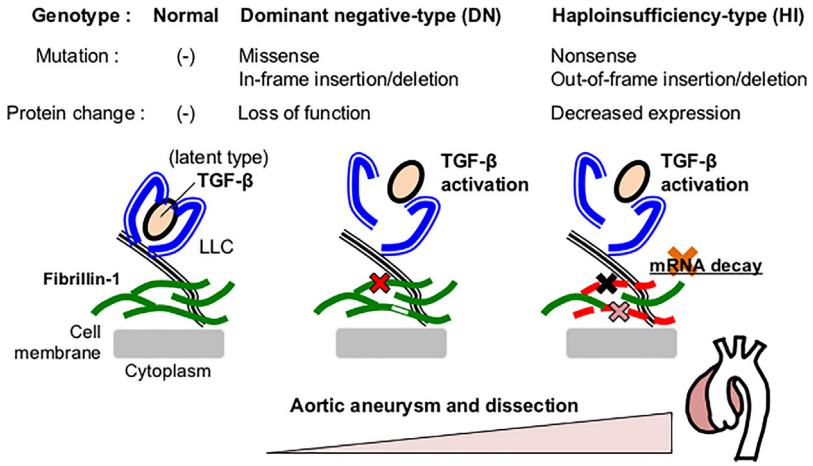

Fig. 2. *FBN1* genotypes and aortopathy in Marfan syndrome. Haploinsufficient-type variants, such as nonsense and out-of-frame variants that presumably cause nonsense-mediated mRNA decay, result in more severe aortic phenotypes than those with dominant negative-type variants, such as missense and in-frame variants that are expected to exert loss-of-function effects [12].
TGF, transforming growth factor.

a 6.3-fold higher risk than those with other types of DN variants (DN-nonCD) (Fig. 3) [7]. Because the underlying mechanisms are unknown and variability in phenotypes has been reported not only across families with the same *FBN1* genotype but also within families, MFS patients should not be monitored based on genotype alone. However, we believe that our simple genetic classification would provide valuable directives and perspectives to patients and relatives with tremendous amount of anxiety during their long-term follow-up period. Further investigations are expected to reveal the relationship between *FBN1* genotype and other manifestations influencing QOL, such as kyphoscoliosis and myocardial function as well as potential differences in drug response.

Loeys–Dietz syndrome

LDS is a recently established autosomal dominant heritable MFS-like syndrome, caused by pathogenic variants in TGF-β signal-related genes, and LDS patients were classified according to the mutated genes in the 2014 revised nosology for LDS diagnosis: *TGFBR1* (LDS1), *TGFBR2* (LDS2), *SMAD3* (LDS3), and *TGFB2* (LDS4) [8]. *TGFB3* (LDS5) and *SMAD2* (LDS6) have been also recently reported to be causing genes for MFS-like syndromic HDAAD, but are still assigned to category “uncertain” for the clinical validity in 2018 (Table 1) [1], pending additional evidence showing the severity of associated aortic disease and risk of progression. Estimated incidence of LDS is approximately 5%–10% among patients with suspected MFS, and the frequency of mutation in the *TGFBR2* seems to be more frequent (55%–60%) than other gene mutations (*TGFBR1*, 20%–25%; *SMAD3*, 5%–10%, *TGFB2*, 5%–10%; *TGFB3*, 1%–5%; and *SMAD2*, 1%–5%) [9].

Vascular and skeletal features in LDS demonstrate overlap with those of MFS, and 20% LDS patients have systemic score of ≥7 points of the 2010 revised Ghent criteria for MFS (Table 2). However, LDS patients do not show ectopia lentis and the majority do not have prominent overgrowth of the long bones (tall height and long arms and legs), and approximately 10%–20% of patients have congenital heart defects, such as patent ductus arteriosus (PDA), atrial septal defect, bicuspid aortic valve (BAV), and ventricular septal defect (VSD) [10]. Further characteristically, LDS patients have a triad of clinical features: hypertelorism (widely spaced eyes), cleft palate or bifid (split) uvula, and aortic/arterial

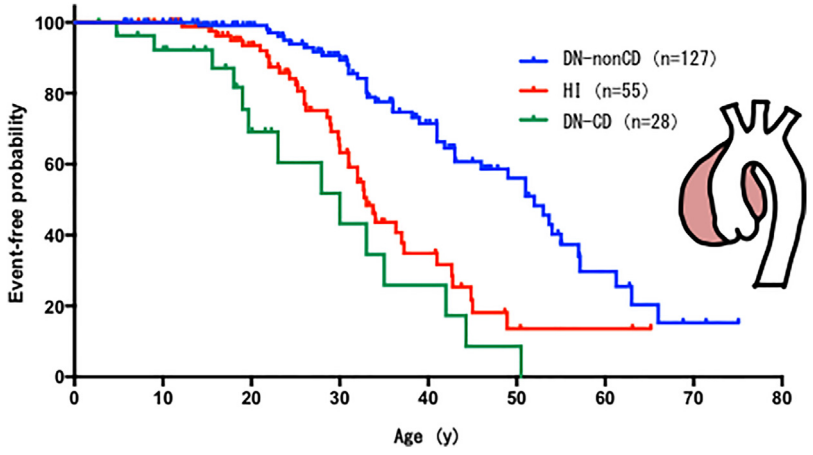

Fig. 3. Kaplan–Meier estimates comparing the probability of the first severe aortic event between genotypes. Severe aortic event is defined as aortic root replacement, type A dissections, and related death. Cumulative aortic event-free probability of 28 dominant negative (DN)-CD Japanese patients (green), 93 haploinsufficient-type (HI) patients (red), and 127 DN-nonCD patients (blue) are shown [7]. DN-CDs are variants affecting or creating Cysteine residues or in-frame Deletion (DN-CD) variants in the central tandem cb-EGF domains (exons 26–37 and 44–50, shown in green line in Fig. 1B).

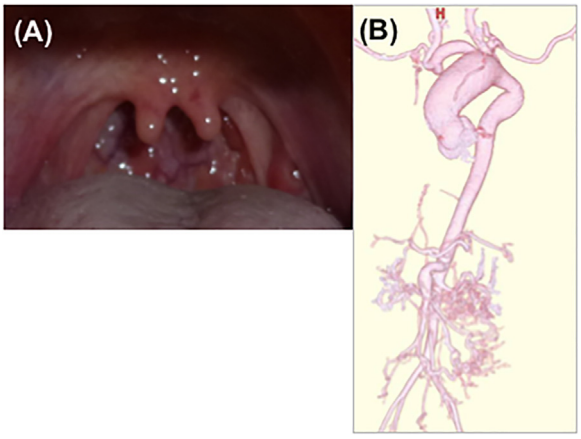

Fig. 4. Loeys–Dietz syndrome. (A) Bifid (split) uvula in a patient with a *TGFBR2* pathogenic variant. (B) Ascending thoracic aortic aneurysm and arterial tortuosity and aneurysms in a patient aged 4 years with a *TGFBR1* pathogenic variant.

tortuosity and aneurysms (Fig. 4), and other LDS-specific features can include cervical spine malformation and/or instability, translucent skin with easy bruising, and dystrophic scars. These indicate that TGF-β signaling pathways play crucial roles in the development and maintenance of various tissues, including arteries and craniofacial growth and patterning. Pathogenic variants in LDS are predicted to lead to loss of protein function; however, aortic tissues from affected patients and LDS knock-in mice showed overactivity of TGF-β signaling cascades, and the molecular mechanisms for the TGF-β paradox have been actively investigated [11,12].

Pathogenic variants in *TGFBR1* and *TGFBR2* are mostly missense and detected within the serine/threonine kinase (STK)-encoding regions of the receptors. Although TGFBR2 forms heteromeric complex with TGFBR1 and cooperatively initiates signaling after binding TGF-β ligands, clinical features associated with a pathogenic variant in *TGFBR1* slightly differ from that in *TGFBR2* [10]; to date, patients with LDS1 and LDS2 had been recognized to rapidly develop progressive aortic/arterial aneurysms, resulting in ruptures at an early age and at smaller dimensions, and surgical repair of the aortic root has been recommended earlier than MFS, potentially at a diameter of ≥40 mm in Japan, and indeed even children <10 years of age have a higher risk for developing aortic dissections (Fig. 4B) [10,13]. However, recent international registry data gathered by the Montalcino Aortic Consortium (MAC 2016) revealed that the survival of patients with a *TGFBR1* or a *TGFBR2* pathogenic variant is much better than initially reported [14] with approximately 75% survival at 75 years of age [10]. In addition, *TGFBR1* males have a greater aortic risk than females, whereas there is no similar sex effect among *TGFBR2* patients, and *TGFBR2* females are prone to develop type A aortic dissection at a diameter ≤45 mm. Furthermore, aortic tortuosity, hypertelorism, and translucent skin are associated with an increased aortic dissection risk, suggesting that syndromic patients with these LDS phenotypic features should be managed more aggressively than patients lacking them [10]. Valve-sparing aortic root replacement is recommended in LDS1/2 adults with dimension >40 mm in the 2014 revised nosology [8]; however, the MAC recommends prophylactic surgery when the diameter reaches 45 mm, and the threshold could be lowered toward 40 mm in *TGFBR2* females with low body surface area and presenting with extra-aortic features [10]. A less aggressive surgical treatment strategy could be pursued in the absence of any familial event or rapid increase in aortic diameter, particularly in *TGFBR1* females [10].

With respect to LDS3–6, the reported number of patients with *SMAD3*, *TGFB2*, *TGFB3*, or *SMAD2* pathogenic variants may be inadequate to draw any conclusions on the phenotypic spectrum. In 2018, a multi-country survey on *SMAD2/3* and *TGFB2/3* variants summarized and compared the clinical features of LDS3–6 patients with 45 new pathogenic variants as well as 74 previously reported ones [15]: The aortic phenotype in LDS3 patients is similar to that of LDS1/2 patients and LDS4/5 represents mild cardiovascular features. Accordantly, the recommended threshold diameter for prophylactic surgery in LDS3 and LDS4 is 40 and 50 mm in the 2014 revised nosology, respectively [8].

In MAC study, there seem not to be increased risks of the occurrence of uterine rupture and pregnancy-related aortic dissections in LDS1/2 compared to MFS patients that were initially emphasized in 2006 [14], and osteoarthritis could be observed in all LDS types that was initially described in aneurysms-osteoarthritis syndrome caused by pathogenic variants in *SMAD3* [16]. Further investigations on relationship between LDS genotype and phenotype are needed to detect high-risk subgroups warranting improved risk stratification and management.

Vascular Ehlers–Danlos syndrome

EDS is a clinically and genetically heterogeneous group of hereditary connective tissue disorders characterized by skin hyperextensibility, joint hypermobility, and tissue fragility, which is caused by pathogenic variants of genes encoding collagen or collagen-modifying enzymes [17]. The different EDS types had been classified into 6 main subtypes according to the Villefranche nosology since 1997 [18], and have been recently re-classified in a system of 13 subtypes based on clinical findings, inheritance pattern and molecular defects, by the International EDS Consortium in 2017.

Hemorrhagic manifestations, such as gum bleeding, menometrorrhagia, postnatal or peri-operative hemorrhage, are observed

Table 3
Diagnostic criteria for vascular Ehlers–Danlos syndrome [17].

Major criteria:
(1) Family history of vEDS with documented causative variant in *COL3A1*
(2) Arterial rupture at a young age
(3) Spontaneous sigmoid colon perforation in the absence of known diverticular disease or other bowel pathology
(4) Uterine rupture during the third trimester in the absence of previous C-section and/or severe peripartum perineum tears
(5) Carotid-cavernous sinus fistula formation in the absence of trauma
Minor criteria:
(1) Bruising unrelated to identified trauma and/or in unusual sites such as cheeks and back
(2) Thin, translucent skin with increased venous visibility
(3) Characteristic facial appearance
(4) Spontaneous pneumothorax
(5) Acrogeria
(6) Talipes equinovarus
(7) Congenital hip dislocation
(8) Hypermobility of small joints
(9) Tendon and muscle rupture
(10) Keratoconus
(11) Gingival recession and gingival fragility
(12) Early-onset varicose veins (under age 30 and nulliparous if female)
Minimal criteria suggestive for vEDS:
- A family history of the disorder, arterial rupture, or dissection in individuals <40 years of age, unexplained sigmoid colon rupture, or spontaneous pneumothorax in the presence of other features consistent with vEDS should all lead to diagnostic studies to determine if the individual has vEDS.
- Heterozygous mutation in the *COL3A1* gene
- Three heterozygous *COL1A1* mutations leading to a arginine-to-cysteine substitution: c.934C>T, p.Arg312Cys; c.1720C>T, p.Arg574Cys and c.3277C>T, p.Arg1093Cys

vEDS, vascular Ehlers–Danlos syndrome.

in varying EDS subtypes [19], but vEDS (previously known as EDS type IV) is considered the most serious form of EDS owing to the possibility of arterial dissection and rupture, intestinal perforation, or uterine rupture (Table 3). Pepin et al. reviewed clinical records in 1231 individuals with vEDS (630 index cases and 601 relatives), the median survival is 46 ± 1.8 years for males and 54 ± 2.5 years for females, with an increased number of deaths in young males [20]. vEDS patients often present with easy bruising, characteristic facial appearance, and thin skin with increased venous visibility; however, it is often difficult to make a diagnosis until serious complications occur.

The estimated prevalence of vEDS ranges from 1/50,000 to 1/200,000 and >95% of patients carry a heterozygous mutation in the *COL3A1* gene, encoding pro-alpha1 chain of type III collagen [21]. Three identical pro-alpha1(III) chains, consisting of a central collagenous domain characterized by the repeated amino acid motif [Glycine (Gly)-Xaa-Yaa]$_{343}$, where Xaa and Yaa can be any amino acid, and two flanking N- and C-terminal noncollagenous domains, are assembled into type III pro-collagen homotrimers (Fig. 5). Noncollagenous domains are proteolytically removed and triple helices formed associate laterally to form collagen fibrils with a characteristic banded pattern.

More than 700 unique *COL3A1* pathogenic variants have been identified and approximately 50% of cases are sporadic. The relationship of *COL3A1* genotype to vascular phenotype has been also analyzed [20,22,23]: The majority (~2/3) are missense mutations affecting the glycine residue of Gly-Xaa-Yaa triplets. The mutated residues were mostly either valine (Val), glutamate (Glu), or aspartate (Asp), and this selection bias is positively correlated to the triple helix destabilizing effects [24]. Most of the remaining pathogenic variants are located within potential splice-sites leading to an in-frame exon skipping and generation of a shorted translated product. These 2 genotypes are at high risks for arterial complications and survival. In contrast, patients with nonsense and small insertion/deletion variants leading to HI-type effects, and non-glycine sequence and N- and C-terminal missense variants, are reported to have milder clinical features and/or present at older age. These might indicate that DN-type variants profoundly disrupting and/or destabilizing the collagen triple helical structure are associated with a more severe phenotype, and it is also important to suspect that non-syndromic-appearing patients with milder arterial features may have the milder types of *COL3A1* variants, even though some variants will require biochemical studies to reveal their causal role on type III collagen alteration of assembly and/or production [23].

The 2017 revised EDS criteria [17] classified some arginine-to-cysteine substitution variants in *COL1A1* (p.Arg312Cys, p. Arg574Cys, and p.Arg1093Cys) [25] as causal for vEDS that are associated with rupture of medium-sized arteries in adult age as well as classic EDS presentation.

Familial non-syndromic thoracic aortic aneurysm and dissection

Familial non-syndromic TAADs could be caused by pathogenic variants in genes coding proteins with functionality specific to the aorta, and the defects cause only TAADs with few outward physical manifestations; surgical repair is typically recommended when the ascending aortic size is 4.5–5.0 cm. The ClinGen Aortopathy Expert Panel classified 5 causative genes for HTAAD as category A2 genes primarily causing isolated TAAD [1]: *ACTA2*, *MYH11*, *MYLK*, and *PRKG1* encode components of the contractile apparatus of the vascular smooth muscle cells (SMC), and *LOX* encodes an extracellular copper enzyme that catalyzes the cross-linking of collagens and elastin. However, the genetic etiology is substantially heterogeneous and approximately 80% of all cases of familial non-syndromic TAADs cannot be explained by pathogenic variants in any of these genes [26]. The natural history and clinical events of non-syndromic TAADs has not been well established; therefore, the diagnosis of familial non-syndromic TAADs is difficult. The management of family members should be carefully and appropriately performed in compliance with ethical guidelines for genetic testing.

ACTA2 is the major responsible gene for familial non-syndromic TAADs (14%–21%) and encodes aortic α-smooth muscle actin protein [26,27], a transcriptional target of the TGF-β signaling. All mutations identified to date are either missense or in-frame insertion-deletion (indel) mutations with DN-type effect, and currently, there is no evidence to support that the HI variants predispose to TAADs [28]. Although the penetrance is approximately 50%, patients frequently develop other vascular features and/or functional disorders of SMCs, such as cerebral aneurysm, neurovascular malformation resembling moyamoya disease, coronary artery disease, PDA, livedo reticularis, and iris floccule [5,26,27].

Other causative genes contribute remarkably less to the genetic etiology of non-syndromic TAADs (≤1%). *MYH11* encodes SM myosin heavy chain (Myosin 11), and a considerable number of genetic variants of unknown significance (UVS) are found throughout the gene; however, most pathogenic variants responsible for TAADs are either missense or in-frame indel mutations within the coiled-coil domain and interfere with protein interactions [26,29]. One missense variant (p. Arg712Gln) is located in the actin-binding and ATP-binding

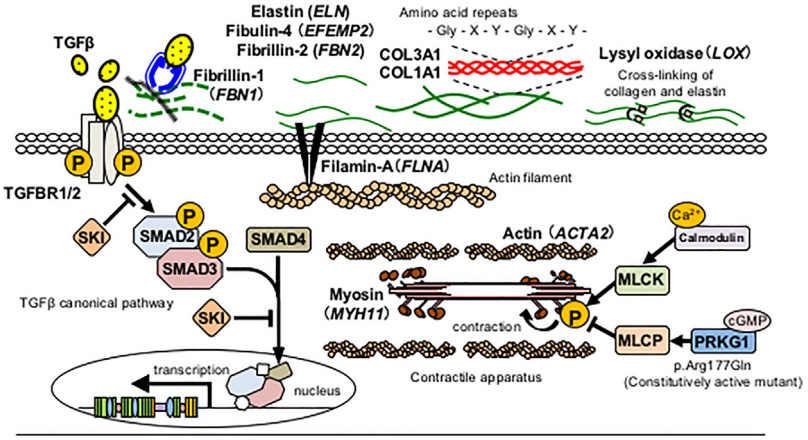

Fig. 5. Signaling pathways and factors causing hereditary thoracic aneurysm and dissection (HTAAD).
TGF, transforming growth factor.

head region [28]. Although the penetrance is incomplete, most pathogenic variants in *MYH11* have been found in patients with TAAD exhibiting PDA.

SMC contractile forces are generated by cyclic interactions between SM myosin heads and the actin filaments and regulated by the reversible phosphorylation of myosin light chain (MLC); Ca^{2+}/calmodulin (CaM)-dependent MLC kinase (MYLK) phosphorylates MLC and increases myosin ATPase activity, creating actin–myosin cross-bridges for contraction, whereas MLC phosphatase (MLCP) dephosphorylates MLC to inhibit the cross-bridge formation resulting in the SMC relaxation, and the activity is increased by cGMP-dependent protein kinase-1 (PKG-1) (encoded by *PRKG1*). Consequently, mutations in *MYLK* and *PRKG1* have been reported to cause TAADs [30,31], by rendering the aorta less competent to withstand biomechanical forces from pulsatile blood flow.

MYLK produces 3 isoforms and the only 130-kDa short form (amino acids 923–1914) is expressed in human aorta, which contains the catalytic and CaM domain required for activation and kinase activity, and heterozygous *MYLK* mutations cause either haploinsufficiency or destroy the CaM binding domain [30]. Importantly, in the majority of patients, little or even no aortic dilatation occurred before dissection [26,30], and thus the management of their relatives should be particularly careful. In the *PRKG1* gene, a recurrent gain-of-function mutation (c.530G>A; p.Arg177Gln) causes TAADs with complete penetrance. The p.Arg177Gln mutation disrupts binding to the high-affinity cGMP binding site; however, the altered PKG-1 is constitutively active even in the absence of cGMP and hypertension is present in several patients [31].

LOX encodes lysyl oxidase (LOX), also known as protein-lysine 6-oxidase, plays a critical role in extracellular matrix maturation by cross-linking collagen and elastin. The heterozygous loss-of-function mutation, particularly variants that disrupt the catalytic activity or lead to haploinsufficiency, predisposes to TAADs involving both aortic root and ascending aorta [32]. Patients exhibit some overlapping syndromic features, such as pectus deformities and striae, but do not fulfill the diagnostic criteria for MFS, and mutation carriers frequently present with BAV (~15%) [32].

Conclusion

We reviewed here the genetic basis of HTAADs particularly caused by mutations in category A genes defined by the ClinGen Aortopathy Expert Panel [1]. Recent progress in sequencing technology would facilitate further discovery of new causative genes, and targeted multi-gene panel testing for HTAADs makes it possible to screen multiple candidate genes simultaneously in a clinical setting, including category non-A genes, such as *SKI* for Shprintzen–Goldberg syndrome (SGS) and *FBN2* for congenital contractural arachnodactyly (also known as Beals syndrome). These can facilitate an easier and more efficient diagnosis; however, doctors and/or genetic counselors must remember to explain the benefits, risks, and limitations in detail before testing. Genetic testing can reveal information about family members, which can still result in genetic discrimination in employment or insurance. In addition, the negative test result of the proband for some known TAADs-causing genes does not suggest that family members do not have an increased risk of developing TAADs in the future. Furthermore, at the present, genetic testing does not provide adequate information on how the disorder will progress over time. Further genetic investigations are required to put precision medicine into practice, with the appropriate genetic counseling.

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Conflict of interest

The authors declare that there is no conflict of interest.

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References

[1] Renard M, Francis C, Ghosh R, Scott AF, Witmer PD, Ades LC, et al. Clinical validity of genes for heritable thoracic aortic aneurysm and dissection. J Am Coll Cardiol 2018;72:605–15.
[2] Takeda N, Yagi H, Hara H, Fujiwara T, Fujita D, Nawata K, et al. Pathophysiology and management of cardiovascular manifestations in Marfan and Loeys–Dietz syndromes. Int Heart J 2016;57:271–7.
[3] Loeys BL, Dietz HC, Braverman AC, Callewaert BL, De Backer J, Devereux RB, et al. The revised Ghent nosology for the Marfan syndrome. J Med Genet 2010;47:476–85.
[4] Faivre L, Collod-Beroud G, Loeys BL, Child A, Binquet C, Gautier E, et al. Effect of mutation type and location on clinical outcome in 1,013 probands with Marfan syndrome or related phenotypes and FBN1 mutations: an international study. Am J Hum Genet 2007;81:454–66.
[5] Morisaki T, Morisaki H. Genetics of hereditary large vessel diseases. J Hum Genet 2016;61:21–6.
[6] De Backer J, Campens L, Muino Mosquera L. Looking for the missing links: challenges in the search for genotype-phenotype correlation in Marfan syndrome. Circ Genomic Precis Med 2018;11:e002185.
[7] Takeda N, Inuzuka R, Maemura S, Morita H, Nawata K, Fujita D, et al. Impact of pathogenic FBN1 variant types on the progression of aortic disease in patients with Marfan syndrome. Circ Genomic Precis Med 2018;11:e002058.
[8] MacCarrick G, Black 3rd JH, Bowdin S, El-Hamamsy I, Frischmeyer-Guerrerio PA, Guerrerio AL, et al. Loeys–Dietz syndrome: a primer for diagnosis and management. Genet Med 2014;16:576–87.
[9] Meester JAN, Verstraeten A, Schepers D, Alaerts M, Van Laer L, Loeys BL. Differences in manifestations of Marfan syndrome, Ehlers–Danlos syndrome, and Loeys–Dietz syndrome. Ann Cardiothorac Surg 2017;6:582–94.
[10] Jondeau G, Ropers J, Regalado E, Braverman A, Evangelista A, Teixedo G, et al. International registry of patients carrying TGFBR1 or TGFBR2 mutations: results of the MAC (Montalcino Aortic Consortium). Circ Cardiovasc Genet 2016;9:548–58.
[11] Akhurst RJ. The paradoxical TGF-beta vasculopathies. Nat Genet 2012;44:838–9.
[12] Takeda N, Hara H, Fujiwara T, Kanaya T, Maemura S, Komuro I. TGF-beta signaling-related genes and thoracic aortic aneurysms and dissections. Int J Mol Sci 2018;19. pii:E2125.
[13] Patel ND, Alejo D, Crawford T, Hibino N, Dietz HC, Cameron DE, et al. Aortic root replacement for children with Loeys–Dietz syndrome. Ann Thorac Surg 2017;103:1513–8.
[14] Loeys BL, Schwarze U, Holm T, Callewaert BL, Thomas GH, Pannu H, et al. Aneurysm syndromes caused by mutations in the TGF-beta receptor. N Engl J Med 2006;355:788–98.
[15] Schepers D, Tortora G, Morisaki H, MacCarrick G, Lindsay M, Liang D, et al. A mutation update on the LDS-associated genes TGFB2/3 and SMAD2/3. Hum Mutat 2018;39:621–34.
[16] van de Laar IM, Oldenburg RA, Pals G, Roos-Hesselink JW, de Graaf BM, Verhagen JM, et al. Mutations in SMAD3 cause a syndromic form of aortic aneurysms and dissections with early-onset osteoarthritis. Nat Genet 2011;43:121–6.
[17] Malfait F, Francomano C, Byers P, Belmont J, Berglund B, Black J, et al. The 2017 international classification of the Ehlers–Danlos syndromes. Am J Med Genet C: Semin Med Genet 2017;175:8–26.
[18] Beighton P, De Paepe A, Steinmann B, Tsipouras P, Wenstrup RJ. Ehlers–Danlos syndromes: revised nosology, Villefranche, 1997. Ehlers-Danlos National Foundation (USA) and Ehlers-Danlos Support Group (UK). Am J Med Genet 1998;77:31–7.
[19] Malfait F. Vascular aspects of the Ehlers–Danlos syndromes. Matrix Biol 2018;71–72:380–95.
[20] Pepin MG, Schwarze U, Rice KM, Liu M, Leistritz D, Byers PH. Survival is affected by mutation type and molecular mechanism in vascular Ehlers–Danlos syndrome (EDS type IV). Genet Med 2014;16:881–8.
[21] Schwarze U, Schievink WI, Petty E, Jaff MR, Babovic-Vuksanovic D, Cherry KJ, et al. Haploinsufficiency for one COL3A1 allele of type III procollagen results in

a phenotype similar to the vascular form of Ehlers–Danlos syndrome, Ehlers–Danlos syndrome type IV. Am J Hum Genet 2001;69:989–1001.
[22] Pepin MG, Murray ML, Byers PH. Vascular Ehlers–Danlos syndrome. In: Adam MP, Ardinger HH, Pagon RA, Wallace SE, Bean LJH, Stephens K, et al., editors. GeneReviews((R)). Seattle, WA: University of Washington; 1993.
[23] Frank M, Albuisson J, Ranque B, Golmard L, Mazzella JM, Bal-Theoleyre L, et al. The type of variants at the COL3A1 gene associates with the phenotype and severity of vascular Ehlers–Danlos syndrome. Eur J Hum Genet 2015;23:1657–64.
[24] Beck K, Chan VC, Shenoy N, Kirkpatrick A, Ramshaw JA, Brodsky B. Destabilization of osteogenesis imperfecta collagen-like model peptides correlates with the identity of the residue replacing glycine. Proc Natl Acad Sci U S A 2000;97:4273–8.
[25] Malfait F, Symoens S, De Backer J, Hermanns-Le T, Sakalihasan N, Lapiere CM, et al. Three arginine to cysteine substitutions in the pro-alpha (I)-collagen chain cause Ehlers–Danlos syndrome with a propensity to arterial rupture in early adulthood. Hum Mutat 2007;28:387–95.
[26] Verstraeten A, Luyckx I, Loeys B. Aetiology and management of hereditary aortopathy. Nat Rev Cardiol 2017;14:197–208.
[27] Guo DC, Pannu H, Tran-Fadulu V, Papke CL, Yu RK, Avidan N, et al. Mutations in smooth muscle alpha-actin (ACTA2) lead to thoracic aortic aneurysms and dissections. Nat Genet 2007;39:1488–93.
[28] Milewicz DM, Trybus KM, Guo DC, Sweeney HL, Regalado E, Kamm K, et al. Altered smooth muscle cell force generation as a driver of thoracic aortic aneurysms and dissections. Arterioscler Thromb Vasc Biol 2017;37:26–34.
[29] Zhu L, Vranckx R, Khau Van Kien P, Lalande A, Boisset N, Mathieu F, et al. Mutations in myosin heavy chain 11 cause a syndrome associating thoracic aortic aneurysm/aortic dissection and patent ductus arteriosus. Nat Genet 2006;38:343–9.
[30] Wang L, Guo DC, Cao J, Gong L, Kamm KE, Regalado E, et al. Mutations in myosin light chain kinase cause familial aortic dissections. Am J Hum Genet 2010;87:701–7.
[31] Guo DC, Regalado E, Casteel DE, Santos-Cortez RL, Gong L, Kim JJ, et al. Recurrent gain-of-function mutation in PRKG1 causes thoracic aortic aneurysms and acute aortic dissections. Am J Hum Genet 2013;93:398–404.
[32] Guo DC, Regalado ES, Gong L, Duan X, Santos-Cortez RL, Arnaud P, et al. LOX mutations predispose to thoracic aortic aneurysms and dissections. Circ Res 2016;118:928–34.