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Genetics of the Extracellular Matrix in Aortic Aneurysmal Diseases

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Abstract

Aortic aneurysms are morbid conditions that can lead to rupture or dissection and are categorized as thoracic (TAA) or abdominal aortic aneurysms (AAA) depending on their location. While AAA shares overlapping risk factors with atherosclerotic cardiovascular disease, TAA exhibits strong heritability. Human genetic studies in the past two decades have successfully identified numerous genes involved in both familial and sporadic forms of aortic aneurysm. In this review we will discuss the genetic basis of aortic aneurysm, focusing on the extracellular matrix and how insights from these studies have informed our understanding of human biology and disease pathogenesis.

INTRODUCTION

Aortic aneurysms comprise a heterogeneous set of morbid conditions that can lead to vessel rupture or dissection, contributing to > 16,000 deaths annually in the United States [1] and typically occur in either the abdominal aorta (AAA) or thoracic aorta (TAA). This important dichotomy not only distinguishes the anatomic location where these aneurysms occur but also provides mechanistic distinctions, as the molecular causes and pathobiology of these conditions differ.

AAA, the more common of these two forms, shares many risk factors with atherosclerotic cardiovascular disease (ASCVD), such as male sex, older age, elevated plasma cholesterol, and smoking [2]. Beyond a significant overlapping risk profile with ASCVD, inherited factors also play a role in the development of AAA. For example, a positive family history

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doubles the risk of developing AAA [3]. By contrast, TAA is not associated with typical ASCVD risk factors, instead exhibiting much stronger heritability, where risk of disease increases with the number of affected relatives [4]. Many patients with TAA exhibit a pattern of Mendelian inheritance [5], suggesting the presence of a single gene mutation conferring risk of disease in individual pedigrees. Indeed, some of these familial TAA patients harbor established monogenic forms of disease such as Marfan or Loeys-Dietz syndromes. However, up to 20% of individuals with thoracic disease do not have a syndrome but do have related family members with disease (termed Familial Thoracic Aortic Aneurysm and Dissection or FTAAD).

Human genetic studies of individuals and families presenting with aortic aneurysmal disease have been successful in mapping multiple genes underlying these conditions. Subsequent model system and in vitro studies have provided mechanistic insights into how these mutated genes contribute to disease pathogenesis. By identifying the molecular etiology and mechanism of aortic aneurysmal diseases, these studies have highlighted smooth muscle function and components of the extracellular matrix (ECM), including elastic fibers, collagen fibers, and members of the TGF- β signaling pathway that are critical for normal development and structural integrity of the vessel wall. In the following, we discuss syndromic and non-syndromic manifestations of aortic aneurysm, highlighting the genes involved in these processes (with a particular focus on the ECM) and how they have informed our understanding of human biology and disease pathogenesis.

STRUCTURE AND FUNCTION OF THE AORTIC ECM

The aorta is composed of three layers: a thin inner layer or tunica intima, a thick middle layer mostly composed of smooth muscle cells (tunica media), and an outer layer called tunica adventitia (Figure 1). The tunica intima consists of a single layer of endothelial cells with an associated sub-endothelial matrix. The tunica media is a thick muscular layer consisting of smooth muscle cells and elastic lamellae, interspersed by collagen fibers and proteoglycan-rich extracellular matrix. The tunica media is bound by the internal elastic lamella (IEL) in the luminal border and external elastic lamella (EEL) in the abluminal border. The outermost layer, tunica adventitia, resides external to the EEL. The tunica adventitia is composed of a collagen-rich ECM and is populated by fibroblast cells. Small arterioles and venules supplying the aorta (vasa vasorum) as well as nerve bundles also reside in this layer.

The aorta is an elastic reservoir that absorbs the systolic pulsatile blood flow from the heart before distributing that force forward during diastole via the Windkessel effect. The mechanical properties of the arterial wall are mediated by collagen and elastic fibers. These two major constituents of the ECM reside in different yet overlapping anatomic locations and serve distinct functions. The elastic fibers, distensible with a low tensile strength, are woven in an interconnected lamellar network in the tunica media throughout the vessel wall [6]. Collagens are circumferentially aligned fibers that provide tensile strength at higher pressures [6]. The distribution of collagen in the aorta exhibits spatial specificity: collagen types I, III and IV colocalize in the intimal and medial layers in the ascending aorta, while types I and IV do so in the descending segments (type III shows variability in this region [7]).

Type III collagen is heavily expressed in the adventitial layer of the entire aorta where they are organized in arrays of fibers [7]. The most common histological finding in TAA, irrespective of its specific etiology, is cystic medial degeneration, characterized by fragmentation and loss of elastic tissue, depletion of vascular smooth muscle cells, defects in collagen microarchitecture, and increased extracellular mucin deposition[8–10], suggesting a final common mechanism centered on ECM disruption.

GENES ENCODING COMPONENTS OF THE AORTIC ECM

The elastic lamellae consist of elastic fibers, formed by polymerized elastin and microfibrils. *ELN* encodes tropoelastin, characterized by hydrophobic sequences alternating with lysine-containing, cross-linking modules [6]. The lysine residues of tropoelastin are cross-linked by lysyl oxidases [11], forming the molecular basis of its physical elastic properties.

Tropoelastin self-assembles into polymer networks of elastin, which then deposits onto microfibrils to form elastic fibers, a process facilitated by fibrillin proteins [12]. Microfibrils are filamentous structures 10–15 nm in diameter, composed of polymerized fibrillins [13]. Fibrillins are large, cysteine-rich glycoproteins that have calcium-binding epidermal growth factor-like (cb-EGF) and 8-cysteine-rich domains. Three fibrillin genes (*FBN1*, *FBN2*, and *FBN3*) exist in the human genome, whereas *Fbn3* appears to have been inactivated in the mouse via chromosomal fission [14]. Expression of *Fbn2* is seen early in development during early elastogenesis and persists for a shorter period of time while *Fbn1* is expressed later, corresponding to the formation of organ structures in a pattern similar to elastin [15]. *Eln* expression begins during development and increases until early postnatal period, then decreases sharply to low levels in adulthood [16].

Microfibrils can associate with other ECM components, such as fibulins, latent transforming growth factor (TGF)- β -binding proteins (LTBPs), and microfibril-associated glycoproteins (MAGPs). The fibulin family consists of 7 ECM proteins that also contain cb-EGF and unique carboxy-terminal modules. These proteins, evolutionarily conserved as early as *C. elegans*, have a multitude of protein ligands [17], and hence have been hypothesized to function as molecular connections that stabilize the ECM structures, such as elastic fibers and basement membranes [18]. Four LTBPs exist in the human genome. They, similar to fibrillins, have cb-EGF and 8-cystein domains [19]. While fibrillins are evolutionarily conserved as far back as *C. elegans*, LTBPs are conserved to deuterostomes, suggesting LTBPs evolved later than fibrillins [20]. LTBPs can bind propeptides of TGF- β , thus helping the assembly and secretion of TGF- β molecules [21] and modulating extracellular TGF- β signaling [22]. MAGP-1 and MAGP-2 are extracellular proteins that can interact with TGF- β factors, NOTCH and their ligands, as well as elastic fiber proteins. They interact with fibrillin to modulate microfibril function [6].

Collagens have a triple helical domain of repeats with glycine occupying every third amino acid residue (Gly-X-Y). Types I, III, IV, V, and VI collagens, encoded by the genes *COL1A1*, *COL1A2*, *COL3A1*, *COL3A1*, *COL4A1*, *COL4A2*, *COL5A1*, *COL5A2*, and *COL5A3*, are expressed in the human aorta, with type I constituting two thirds of the total collagen [23]. Spatial heterogeneity is present in the expression pattern of collagen. Types I, III and V collagen are present in all layers of the aorta with type I most predominant in the

intima and adventitia, type III slightly more abundant in the media, and type V greater in the intima. Types IV and VI are found mostly in the intima [23]. The expression pattern of collagen in the aorta might underlie the different phenotypes observed in patients and animal models of different collagen mutations. Collagens type I, II, III and V are fibril-forming collagens that form fibers within tissues to impart structural support. Types IV, VIII and X collagens are network-forming proteins. Type IV collagen is a major structural protein of the basement membrane. As these ECM molecules collectively provide tensile and structural strength to the vessel wall, it is perhaps not surprising that mutations in the genes encoding them may result in aneurysmal diseases.

INHERITED BASIS OF AORTIC ANEURYSM

As more genes whose mutations are involved in genetic aneurysmal diseases are identified, a theme emerges whereby genes involved in elastic fibers, collagen fibers, TGF- β signaling, and smooth muscle function are critical in the formation of aortic aneurysm (Table 1).

Mutations affecting the elastic fiber

Mutations in *ELN*—Mutations in *ELN* can cause autosomal dominant and autosomal recessive forms of cutis laxa, a systemic disorder of connective tissues that manifest as progressive loosening of the skin, as well as disorders of organs containing elastic tissue, such as the pulmonary and cardiovascular systems [24, 25]. Patients with the autosomal dominant form have a normal lifespan, whereas those with the autosomal recessive form are at risk for early morbidity and mortality due to cardiopulmonary conditions, such as emphysema or thoracic aortic aneurysm [25]. Individuals with autosomal dominant cutis laxa may also manifest aortic aneurysm as reported by Szabo et al. who found a dominantly inherited 25-bp *ELN* deletion and a de novo 1-bp *ELN* deletion (both of which shifted the open reading frame and induced premature termination codons) in individuals with cutis laxa and aortic aneurysm [26]. Histological analysis of aneurysmal aortas from affected individuals revealed a paucity of elastic fibers and classic characteristics of medial degeneration.

Microdeletions in chromosome 7q11.23, which encompasses a region up to 1.5–1.8 Mb containing 26–28 genes including *ELN* [27], causes Williams-Beuren syndrome, also known as Williams syndrome. Williams syndrome is a multi-organ disorder including mental retardation, ebullient personality, elf-like facies, failure to thrive, endocrine abnormalities, and supravalvular aortic stenosis (SVAS) [27]. Non-syndromic forms of SVAS have been described and mapped to disruption in *ELN* [28, 29]. Stenotic changes, found in 79–90% of Williams syndrome patients, may less commonly involve other medium and large arteries, such as pulmonary, intracranial, coronary, mesenteric, and renal arteries [27]. These obstructive lesions are characterized histologically by atherosclerosis-independent medial hypertrophy due to excessive smooth muscle proliferation, as well as fragmentation of elastin in the tunica media [30]. Cardiovascular events are the most common cause of death in Williams syndrome patients [27]. Beyond stenotic lesions, aneurysmal changes of the coronary artery and aorta also have been reported in patients with Williams syndrome [31, 32]. It is interesting to note that these conditions, attributed to alterations in the same locus,

manifest non-overlapping symptomatology. A corollary can be found in the blood pressure phenotype in *Eln* heterozygous mice, which varies with genetic background [33]. It is possible that other genetic modifiers, especially loci involved in smooth muscle function, may interact with elastin to modulate aortic stenosis or aneurysm. Further studies are needed to provide additional insights into the phenotypes of *ELN* gene alterations.

Mutations in *LOX*—Lysyl oxidase (*LOX*) and its related gene family members are a group of copper-dependent oxidodeaminases that cross-link lysyl residues on these structural proteins in the process of forming proper elastic lamellae and collagen fibers. *Lox* knockout mice die perinatally due to aortic aneurysm associated with aortic regurgitation [34]. However, not until recently did whole genome [35] and exome [36] sequencing identify mutations in *LOX* as a cause of FTAAD in humans. In one family, a missense mutation in *LOX* (c.893T > G, p.Met298Arg) was found to segregate with disease. This mutation was “knocked-in” at the orthologous position in the mouse genome using CRISPR/Cas9 technology. Mice homozygous for the mutant allele had markedly reduced *Lox* activity, discontinuous elastic lamellae in the aortic wall, and died perinatally due to aortic aneurysm and dissection, recapitulating the human phenotype [35]. Pathogenic mutations in *LOX* appear to result in loss of function [35, 36], supporting a model of haploinsufficiency in humans. Interestingly, the phenotype was not compensated by other *Lox* family members (*Lox11*, *Lox12*, *Lox13*, and *Lox14*), supporting the functional non-redundancy of *Lox* family proteins.

Mutations in *EFEMP2/FBLN4*—Autosomal recessive mutations of *EFEMP2*, also known as *FBLN4*, which encodes fibulin-4, have been found in human patients with autosomal recessive cutis laxa, arachnodactyly, ascending aortic aneurysm, aortic tortuosity, and hypoplasia of the pulmonary arteries [37–40]. Pathogenic mutations, typically missense or frameshift alterations [37, 40, 41], tend to occur in the cb-EGF domain [41]. Immunohistochemistry of aortic tissue from carriers demonstrated increased TGF- β protein expression and increased downstream pSMAD2 staining [40]. Animal models of fibulin-4 deficiency exhibit phenotypes similar to humans. Mice homozygous with a knocked-in *Fbln4* allele corresponding to a recurrent human missense mutation (c.169G > A, p.Glu57Lys) recapitulated findings in autosomal recessive cutis laxa, such as loose skin, aortic aneurysm, arterial tortuosity, emphysema, and skeletal changes [42]. Conditional deletion of *Fbln4* in smooth muscle cells caused disorganized thick elastic laminae with aberrant deposition of elastin, increasing arterial stiffness [43]. *Fbln4* null mice are not viable and were found to have irregular elastin aggregates instead of intact elastic fibers in the aorta [44]. Functionally, *Fbln4* interacts with the pro-peptide of *Lox*, promoting the assembly of *Lox* onto tropoelastin during elastogenesis [43]. Therefore, fibulin-4 appears to provide a link between the structural components of the elastic fiber and their crosslinking enzymes.

In contrast to the established role of *FBLN4*, the role of *FBLN5* in aortic aneurysm has not been clearly defined. The expression of fibulin-5 was noted to be decreased in human aneurysmal abdominal aorta [45], but a study of common polymorphisms in *FBLN5* failed to find an association with aortic aneurysm [46]. Patients with *FBLN5* mutations can present

with autosomal recessive or autosomal dominant cutis laxa, typically caused by missense mutations or duplications in the cb-EGF domains [47–49]. While SVAS may be present, aortic aneurysm or dissection has not been reported in patients with *FBLN5* mutations [47]. In contrast to the lethality in *Fbln4* null mice, *Fbln5* null mice enjoy a normal lifespan without evidence of aortic aneurysm or dissection [50, 51]. They develop elastic fiber defects such as an elongated aorta with tortuosity, emphysema, and pelvic organ prolapse [50, 52]. While sharing binding partners in elastogenesis, *Fbln4* exhibits strong binding with *Lox*, whereas *Fbln5* binds *Lox* with less affinity [53]. *Fbln5*, in contrast, recruits *Lox11* to elastic fibers [54]. The lethal phenotypes in *Fbln4* and *Lox* mutants, compared to the milder elastogenic defects in *Fbln5* and *Lox11* mutants, suggest that these phenotypic differences may be explained by their differential affinity to binding partners.

Mutations in MAGP-2—Missense and nonsense mutations in *MFAP5* which encodes MAGP-2 have been identified in French families with FTAAD [55]. The aortic tissue of affected individuals had elevated pSmad2/3 expression, attributed to reduced TGF- β sequestration. Mis-regulation of *mfap2* expression in zebrafish via either morpholino knockdown or overexpression both leads to dilated blood vessels, indicative of a critical dose of *mfap2* in blood vessel formation [56]. Mice lacking either *Mfap2* (encoding MAGP-1) or *Mfap5* exhibit minimal vascular phenotypes, whereas those lacking both exhibit blood vessel dilation [57]. In contrast, humans with FTAAD caused by *MFAP5* do not also harbor mutations in *MFAP2*.

Mutations affecting the collagen fiber

Mutations in type I collagen—Type I collagen is an abundant protein in many tissues including bone, skin, ligaments, tendons, and blood vessel walls. It consists of a heterotrimer with 2 pro- α 1(I)- and 1 pro- α 2(I)-collagen chains, encoded by the *COL1A1* and *COL1A2* genes. The central triple helical domain of these pro- α -chains contains repeating Gly-X-Y triplet amino acids, with hydroxyproline and arginine being the most common amino acids in the Y position. Mutations in type I collagen genes, especially missense mutations affecting the glycine residues, cause osteogenesis imperfecta. Rare non-glycine substitutions may result in connective tissue disorders, such as osteogenesis imperfecta, classic Ehlers-Danlos syndrome, or infantile cortical hyperostosis. Heterozygous missense mutations in *COL1A1* or *COL1A2* causing substitutions of arginine in the Y position to cysteine or glycine have been described in patients with aneurysms of the aorta, femoral artery, or renal artery [58, 59]. These Arg-to-Cys substitutions lead to a disturbance in collagen fibrillogenesis, resulting in collagen fibrils with variable diameters and irregular interfibrillar spaces [58]. A mouse model of a homozygous 1.3-kb deletion in the first intron of *Col1a1* had reduced expression of the type I α 1 chain, decreased collagen content in the aorta and developed aortic aneurysm, subsequently dying from aortic dissection [60].

Mutations in type III collagen—Ehlers-Danlos syndrome (EDS) type IV (also known as vascular EDS), is one of the most severe forms of connective tissue disease. Patients with EDS type IV, transmitted in an autosomal dominant fashion, develop easy bruising, skin with visible veins, characteristic facial features, and rupture of arteries, uterus, or intestines [61]. These patients suffer from reduced survival compared to the general population due to

arterial dissection or rupture [61]. EDS type IV was first reported due to a deletion in the *COL3A1* locus [62] in 2000, the first single-gene linked to aneurysmal disorders [63]. Additional reported pathogenic mutations include exonic deletions, intronic deletions, missense mutations, and splice-site mutations. The type of mutation may associate with disease severity as null mutations seem to portend a more favorable outcome compared with missense substitutions at glycine residues or mutations at the canonical sites of pre-mRNA splicing [61]. Murine mutant alleles of *Col3a1* have been generated and most mice with homozygous *Col3a1* mutations die perinatally; those surviving die from ruptured large blood vessels [64]. The mutant mice have decreased number of type I collagen fibrils with variable diameter. Type III collagen therefore have an important regulatory role in type I collagen fibrillogenesis.

Mutations in type IV collagen—Mutations in *COL4A1* have been identified in patients with Hereditary Angiopathy with Neuropathy, Aneurysms, and Muscle Cramps (HANAC) syndrome, an autosomal dominant disease whose clinical manifestation includes aneurysms of the internal carotid arteries and cerebral arteries [65]. HANAC syndrome arises from missense mutations that cause substitutions of glycine in the Gly-X-Y repeats, although other glycine-substituting missense mutations of *COL4A1* were found in patients with small vessel disease involving retinal vessels and cerebral hemorrhage, but not aneurysm [66]. Therefore, mutations in *COL4A1* may have multiple phenotypic consequences. In mice, deficiency of *Col4a1* causes mid-gestation embryonic lethality associated with structural deficiencies in the basement membrane [67]. By contrast, mice carrying the heterozygous *Col4a1^{Raw}* (retinal arteriolar wiring) allele harbor detachment of endothelium from the tunica media in the descending aorta. Their vascular smooth muscle cells exhibit reduced contractile strength and their endothelial cells have reduced reaction to NO-mediated vasodilation [68]. These findings may underlie the vascular pathogenesis of HANAC syndrome.

The X-linked Alport Syndrome, characterized by progressive renal dysfunction and abnormalities of the ears and eyes, arise from mutations in *COL4A5*, which encodes the $\alpha 5(\text{IV})$ chain. A small number of cases of TAA and dissection have been reported in male patients with Alport syndrome [69]. A mutant mouse harboring an analogous human nonsense mutation (c.231G > T, p.Glu5X), however, was not found to have aortic aneurysm [70]. While renal dysfunction and chronic hypertension are risk factors for aortic disease in young patients [71], the findings of thoracic rather than abdominal disease, young age at disease onset (13–32 years of age), and short duration between end-stage renal disease and TAAD (3–12 years), suggest that renal disease and hypertension may be facilitators and not primary driving causes of TAAD in patients with Alport syndrome. Supporting this notion is the observation that in the International Registry of Aortic Dissection (IRAD), patients < 40 years of age were less likely to have traditional risk factors (e.g., hypertension) and more likely to have syndromic (e.g., Marfan syndrome) presentation [72]. Therefore, the mechanism and role of *COL4A5* in aneurysm remains elusive.

Mutations in lysyl hydroxylase 1—More than 20 mutations in lysyl hydroxylase, encoded by *PLOD1*, have been linked to the molecular genetics of Ehlers-Danlos syndrome

type VI [73]. In rare occurrences these patients have been reported to have spontaneous rupture of the intracranial, vertebral, intrathoracic, or femoral arteries [74]. It is not clear whether these ruptures involve aneurysmal dilation. A small portion of the *Plod1* mutant mice died suddenly which was attributed to aortic dissection [75]. While *Plod1* mutant mice have no change in the structure or thickness of their aortic walls, microstructural abnormalities including vacuolization and mitochondrial swelling have been observed in the mutant aortic wall smooth muscle cells, corresponding with the site of *Plod1* expression.

Mutations in *BGN*—Biglycan, encoded by *BGN*, belongs to the small leucine-rich proteoglycan (SLRP) family of secreted proteoglycans. Expressed in all layers of the aorta [76], biglycan interacts with many ECM components including collagen, elastin, and microfibrils [77] and is involved in collagen fibrillogenesis [78]. A recent study identified nonsense and missense *BGN* mutations (c.5G>A, p.Trp2*; c.908A>C, p.Gln303Pro) in patients with FTAAD who carried a clinical diagnosis of Marfan syndrome without an identifiable molecular cause [79]. Targeted sequencing in another cohort of FTAAD patients revealed additional alterations in *BGN* predicted to result in loss-of-function, including a splice-site mutation and exonic deletions. Collagen content was reduced in the aorta of some affected individuals, whereas elastin fibers appeared normal. Aortas from these subjects also had increased pSMAD2 staining. Therefore, *BGN* mutations appear to affect collagen homeostasis in the aortic wall, potentially through the TGF- β pathway. The function of *BGN* may be modified by other genetic factors. *Bgn*-null mice in the BALB/c background exhibit aortic rupture [80], whereas those in the C57BL6 background were not found to have a vascular phenotype [81]. The locus responsible for this genetic interaction remains unclear.

***FBN1*, Marfan syndrome, and the TGF- β pathway**—The Marfan syndrome is an autosomal dominant disease with a prevalence of 4–6 per 10,000 persons [82]. The classic syndrome involves abnormalities of the eye (ectopia lentis), aorta (ascending aortic aneurysm, aortic regurgitation), skeleton (long limbs, pectus deformity), and fingers (arachnodactyly, joint laxity). The revised Ghent nosology has been established to guide the clinical diagnosis of Marfan syndrome [83], placing an emphasis on findings of aortic dilation, ectopia lentis, and *FBN1* mutations. *FBN1* was identified as the causative gene for the Marfan syndrome more than 25 years ago [84]. Since then, more than 1800 causal point mutations have been identified [85]. The type and location of mutations appears to correlate with the phenotype as a higher proportion of patients with mutations in exons 24–32 develop ascending aortic aneurysm compared to those with mutations in other exons [86].

As fibrillin-1 associates with TGF- β -binding LTBP, the molecular pathogenesis of *FBN1* mutations has been attributed to upregulation of TGF- β signaling. Increased TGF- β signaling activity has been detected in the lung tissue in mice homozygous for a *Fbn1* hypomorphic allele using a GFP reporter [87]. A neutralizing antibody to TGF- β rescues the lung maturation phenotype in the *Fbn1* hypomorph mouse [87]. Mice harboring the *Fbn1*^{C1039G} allele, orthologous to a pathogenic human mutation, exhibit increased nuclear pSmad2, suggestive of increased TGF- β signaling. These mice develop aortic aneurysm, a phenotype rescued by TGF- β -neutralizing antibody or losartan [88], presumably due to TGF- β pathway inhibition. These findings formed the basis for the large Pediatric Heart

Network trial, which compared losartan versus atenolol, a β -blocker, in children and young adults with Marfan syndrome and aortic aneurysm [89]. This trial, however, did not demonstrate a superiority of losartan on inhibiting or ameliorating aortic growth compared to atenolol.

Other lines of genetic evidence add to the complexity of TGF- β signaling pathway in aneurysmal diseases. The Shprintzen-Goldberg syndrome (SGS) is characterized by mental retardation, skeletal muscle hypotonia, Marfanoid features, and aortic root aneurysm. SGS has been attributed to mutations in *SKI*, which encodes a negative regulator of TGF- β signaling [90]. Missense mutations or deletions in *SKI* disrupt the binding of SKI with SMAD proteins or transcriptional co-repressors, altering TGF- β signaling [91]. The Loeys-Dietz syndrome (LDS), described in 2005, manifests as arterial tortuosity along with aneurysms, craniofacial, neurocognitive and skeletal abnormalities [92]. LDS is caused by mutations in genes encoding proteins involved in the TGF- β pathway, including *TGFBR1* [92], *TGFBR2* [92], *SMAD3* [93], *TGFB2* [94], and *TGFB3* [95, 96]. Mice with heterozygous knocked-in alleles of pathogenic, missense mutations from either *Tgbr1* or *Tgbr2* recapitulate the LDS phenotype; their vascular smooth muscle cells and those from LDS patients exhibit reduced signaling response to TGF- β ligands, indicating these are loss of function mutations [97]. Similarly, loss of function of TGF- β ligands also leads to an aneurysmal phenotype [95, 96]. Furthermore, heterozygous loss of *Smad4* or *Tgfb2*, smooth muscle-specific deletion of *Tgfb2*, or administration of an TGF- β -neutralizing antibody worsen the aortic aneurysm phenotype in the MFS mouse model [94, 98–101]. Although these lines of evidence point to an aneurysmal phenotype from reduced TGF- β signaling, aortic tissues from patients or mice with various genetic etiologies of LDS, including *SMAD3*, *TGFB2*, *Tgbr1*, *Tgbr2* or *Tgfb2*, have increased expression of TGF- β ligand or pSmads [93, 94, 97]. Therefore, while it is clear that TGF- β pathway is genetically and mechanistically involved in the pathogenesis of thoracic aortic aneurysms, precisely how TGF- β signaling relates to the development of aneurysms remains unresolved [102].

How does one reconcile these seemingly contradictory findings? Some have proposed that TGF- β signaling may have dynamic and context-dependent effects on aneurysms where reduced TGF- β signaling contributes to the predisposition while increased TGF- β signaling promotes the progression [103]. Others point out the caveat of equating increased pSmad and TGF- β responsive genes as a pathognomonic signature of increased TGF- β signaling [102]. Finally, some suggest that TGF- β signaling is necessary for the proper development and differentiation of aortic smooth muscle cells and associated contractile-elastic units; failure of their appropriate development lead to altered mechanosensing, which in turn causes secondary, compensatory increases in TGF- β signaling [104]. It may be that this seeming contradiction stems from the conceptual disassociation of TGF- β and pSmad. At the ligand and receptor level, both genetic and non-genetic loss of TGF- β signaling causes an aneurysmal phenotype [92–101]. Finding increased expression of pSmad (or downstream genes) in aortic aneurysms does not prove causality [93, 94, 97]. Rather, it may be that increased pSmad is an adaptive, compensatory mechanism for aortic homeostasis in the face of aneurysm, instead of a pathogenic mechanism. Understanding precisely how the TGF- β pathway regulates aortic development, aging, and compensation for mechanical strength in a spatially and temporally controlled manner will require further research.

Mutations affecting the smooth muscle

As smooth muscle cells line the tunica media, it is not surprising that perturbation of smooth muscle cell function may be associated with altered blood vessel mechanics and dilation. A syndrome of TAAAD with patent ductus arteriosus (PDA) was mapped to chromosome 16p12.2-p13.13. These patients have low aortic mechanical compliance and distensibility [105]. Their smooth muscle cells do not have an increase in TGF- β or its downstream targets [106]. Among the > 60 genes in this region, *MYH11*, encoding smooth muscle myosin heavy chain, was found to harbor mutations that correlated with the clinical phenotype [105, 106]. Recently, a moyamoya-like vasculopathy with stenosis of intracranial arteries, aorta and renal arteries, as well as PDA, has been reported in a patient with a *MYH11* missense mutation (c.4604G > A, p.Arg1535Gln)[107]. In vitro expression of a mutant protein harboring a pathogenic allele showed co-immunoprecipitation with the tagged mutant protein, suggesting the mutants act in a dominant-negative fashion [105]. *Myh11* knockout mice exhibit perinatal lethality, developing PDA although aortic aneurysm has not been reported [108].

ACTA2 encodes smooth muscle α -actin and missense mutations in *ACTA2* have been identified as a cause of FTAAD [109]. The mutations affect actin filament assembly and decrease smooth muscle contraction. Intriguingly, patients with *ACTA2* mutations are also at increased risk for premature coronary artery disease, premature ischemic stroke, and a moyamoya-like phenotype [110]. Smooth muscle cells explanted from patients with *ACTA2* mutation exhibited increased proliferation in vitro [110]. In animal models, *Acta2*-null mice were viable but exhibited reduced vascular contractility and blood pressure, although it is not clear whether *Acta2* mutants developed aortic aneurysms [111]. These lines of evidence support a role of smooth muscle actin and smooth muscle myosin in occlusive vascular disease.

Vascular smooth muscle cells are known to play an important role in atherosclerosis, a form of occlusive vascular disease. Atherosclerotic plaques contain lipid-laden smooth muscle cells, which undergo phenotypic switching, reducing expression of smooth muscle cell markers (including MYH11 and ACTA2), and acquiring macrophage-associated markers [112]. By contrast, small arteries in *MYH11* and *ACTA2* mutations exhibit intimal thickening with strong expression of smooth muscle markers, medial fibrosis, and a paucity of lipids [106, 110, 113]. This distinct histopathology suggests that the pathogenic role of smooth muscle cells in patients with *MYH11* or *ACTA2* mutation may be different from those in atherosclerotic vascular diseases.

Bicuspid aortic valve and aortic aneurysm—Bicuspid aortic valve (BAV), the most common congenital cardiac defect, is found in 1–2% of the general population [1]. Bicuspid aortic valves are associated with ascending aortic aneurysms: about 50% of BAV patients younger than 30 years and about 90% older than 80 years have aortic dilation [114]. These patients have a risk of aortic dissection that is numerically low (3–45 per 10,000 patient-years) but higher than in the general population (8-fold increase in risk)[114]. BAV exhibits an autosomal dominant pattern of heritability with incomplete penetrance [115, 116]. Multiple genes and loci have been associated with BAV, including some in signaling

pathways (*NOTCH1* [117]) in addition to transcription factors (GATA family and NKX family transcription factors)[118]. Interestingly, mutations causing aortic aneurysmal diseases are also associated with increased risk for BAV. In a case-control study, bicuspid aortic valve was seen in 7% and 4% of patients with mutations in *TGFBR2* and *FBNI* respectively [119], a prevalence higher than the generally accepted 1–2% rate of BAV in the general population. Other loci involved in both conditions include *SMAD6* [120], *ACTA2*, chromosome 7q11.3 (encompassing *ELN*), and *COL3A1*.

Complex genetics of aortic aneurysmal disease—Beyond Mendelian family studies aimed at discovering single gene mutations of large effect, there have been efforts to identify genetic loci underlying complex forms of aneurysmal diseases. The first genome-wide association study (GWAS) in non-syndromic TAA identified 15q21.1, encompassing *FBNI*, as a susceptibility locus associated with disease at genome-wide significance [121], a finding which was subsequently validated in a separate cohort [122]. Further GWAS of non-familial sporadic thoracic aortic dissection identified *FBNI*, *LRPI*, and *ULK4* as loci associated with disease [123]. Parallel studies in AAA have now identified loci that are associated with disease at a genome-wide level of significance including *LRPI* [124], *DAB2IP* [125], *LDLR* [126], *SORT1* [127], and *CDKN2BAS1/ANRIL* [128] loci. Recent meta-analyses have revealed additional loci (*SMYD2*, *LINC00540*, *PCIFI1*, *ERG*) [129] and genetically-determined plasma lipids [130] that associate with AAA. These studies not only highlight the genetic differences between these disorders but interestingly also find overlapping loci for both TAA and AAA with coronary atherosclerosis, myocardial infarction, and intracranial aneurysm [127, 128, 131], implying that common mechanisms may underlie these disparate vascular diseases. These observations may be relevant for informing future mechanistic studies and therapeutic development.

Tying it all together: the extracellular matrix

As reviewed above, genes causing aortic aneurysmal diseases can be loosely categorized into three overlapping functional domains: 1) those providing structural integrity to the ECM; 2) those involved in smooth muscle function; and 3) those involved in the TGF- β pathway. The shared pathological features of ECM disruption across the various genetic forms of aneurysmal disease suggest a final common pathophysiologic mechanism converging on the ECM. How does this all tie together?

The interface between the ECM and smooth muscle cells is an emerging nexus that may provide a unifying mechanism of TAA formation. In the normal aortic media, smooth muscle exhibits a contractile phenotype, whereas those in the aneurysmal aorta show a synthetic phenotype, characterized by increased proliferation and expression of ECM components or matrix metalloproteinases (MMPs), and processes involved in the synthesis, maintenance, and homeostasis of the ECM [104, 132]. Aortic ECM constituents connect to smooth muscle cells and their intracellular contractile filaments via adaptor structures, forming a matrix-cell complex that transduces mechanical properties in the ECM to regulate smooth muscle phenotype [104]. Such a mechanism couples ECM homeostasis with smooth muscle phenotypes. Smooth muscle cells isolated from TAAD showed a synthetic phenotype [133, 134], with increased expression of elastin- and collagen-degrading MMPs enriched at

the site of dissection [135]. The smooth muscle phenotype switch precedes disruption of elastic fibers [136]. It is conceivable that mutations in ECM structural proteins also misregulate smooth muscle behavior via altered mechanics. Indeed, disrupted collagen or elastin in the ECM could promote smooth muscle cell proliferation and migration [137, 138]. *MYH11* and *ACTA2* encode proteins that form the contractile machinery of smooth muscle cells and are themselves markers of a contractile phenotype [132]. It is therefore plausible that mutations in these genes could lead to a synthetic phenotype. The TGF- β /Smad pathway is well-described to stimulate smooth muscle differentiation and maintain a contractile phenotype [132, 139]. TGF- β inhibits MMP expression and enhances the expression of tissue inhibitor of metalloproteinase (TIMP) proteins in smooth muscle [140]. Together, these lines of evidence support a critical role of a TGF- β signaling dependent, matrix-smooth muscle complex that regulates smooth muscle phenotype and the balance between MMP/TIMP, thereby regulating ECM homeostasis.

Conclusions and future perspectives

Research progress in the past few decades has yielded a plethora of genes involved in vascular aneurysmal diseases. The methodology has evolved from traditional Mendelian observations, linkage analysis, and site-directed cloning to high-throughput sequencing [118]. The next phase of human genetic studies and “big data” analyses are poised to identify additional causal genes and pathways for aortic aneurysmal disease, with the promise of providing further biological and clinical insights into these important disorders.

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Highlights

The extracellular matrix plays a critical role in determining the mechanical integrity of the aortic wall.

Mutations affecting the normal structure and function of the extracellular matrix can affect the stability of the aortic wall resulting in aneurysmal disease.

We provide an overview of the genes encoding the components of the aortic extracellular matrix and discuss syndromic and non-syndromic forms of aortic disease resulting from mutations in these genes.

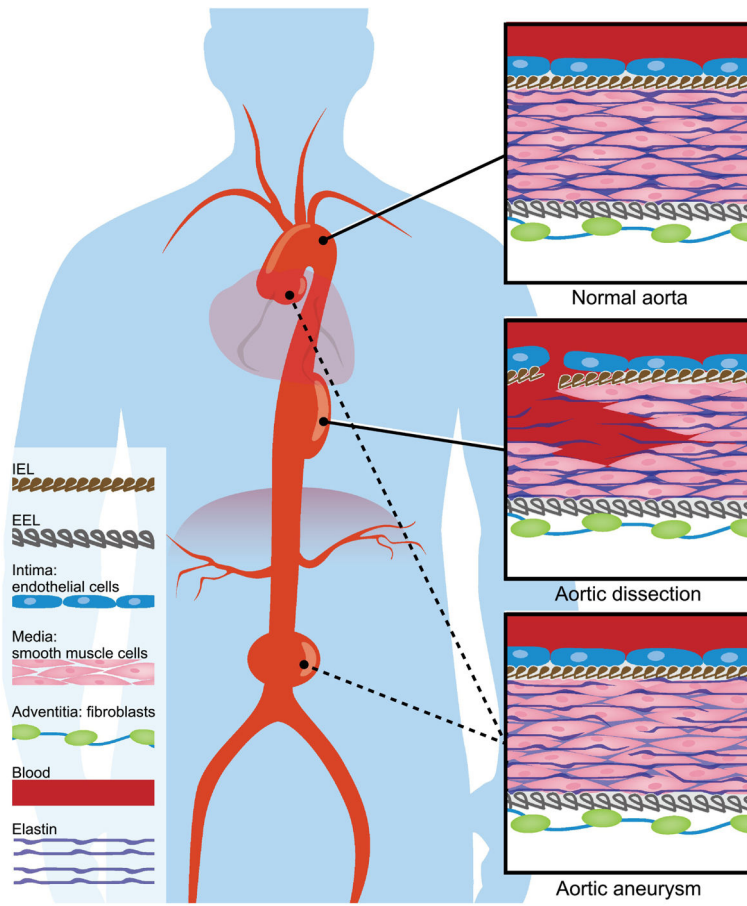


Figure 1.

Table 1

Inherited Basis Of Aortic Aneurysm

Gene	Location	Phenotype	Inheritance	OMIM	Ref
Mutations affecting the elastic fiber					
<i>ELN</i>	7q11.23	Cutis laxa/TAA	AD, AR	123700	[26]
<i>LOX</i>	5q23.1	FTAAD	AD	617168	[34–36]
<i>EFEEMP2/FBLN4</i>	11q13.1	Cutis laxa/TAA	AR	614437	[37–40, 42]
<i>MEFAP5</i>	12p13.31	FTAAD	AD	616166	[55, 57]
Mutations affecting the collagen fiber					
<i>COL1A1</i>	17q21.33	Classic EDS	AD	130000	[58–60]
<i>COL1A2</i>	7q21.3	EDS type 7B	AD	130060	[58, 59]
<i>COL3A1</i>	2q32.2	EDS type 4	AD	130050	[62, 63]
<i>COL4A1</i>	13q34	HANAC syndrome	AD	611773	[65]
<i>COL4A5</i>	Xp22.3	Alport syndrome	X-linked	301050	[69]
<i>PLOD1</i>	1p36.22	EDS type 6	AR	225400	[73–75]
<i>BGN</i>	Xq28	FTAAD	X-linked	300989	[79]
Mutations affecting the TGF-β signaling					
<i>FBNI</i>	15q21.1	Marfan syndrome, TAAAD, AAA, BAV	AD, sporadic	154700	[84, 85, 119, 121, 123]
<i>SKI</i>	1p36.33-32	SGS	AD	182212	[90]
<i>TGFBR1</i>	9q22.33	LDS type 1	AD	609192	[92]
<i>TGFBR2</i>	3p24.1	LDS type 2, BAV	AD, sporadic	610168	[92, 119]
<i>SMAD3</i>	15q22.33	LDS type 3	AD	613795	[93]
<i>SMAD6</i>	15q22.31	BAV	AD	614823	[120]
<i>TGFB2</i>	1q41	LDS type 4	AD	614816	[94]
<i>TGFB3</i>	14q24.3	LDS type 5	AD	615582	[95, 96]
Mutations affecting smooth muscle					
<i>MYH11</i>	16p13.11	FTAAD, PDA	AD	132900	[105, 106]
<i>ACTA2</i>	10q23.31	FTAAD, BAV	AD	611788	[109]
Other loci associated with aortic aneurysm					
<i>LRPI</i>	12q13.3	TAA, AAA	Sporadic		[123, 124]
<i>ULK4</i>	3p22.1	TAA	Sporadic		[123]

Gene	Location	Phenotype	Inheritance	OMIM	Ref
<i>DAB2IP</i>	9p33.2	AAA	Sporadic		[125]
<i>LDLR</i>	19p13.2	AAA	Sporadic		[126]
<i>SORT1</i>	1p13.3	AAA	Sporadic		[127]
<i>CDKN2BAS1/ANRIL</i>	9p21.3	AAA	Sporadic		[128]
<i>SMYD2</i>	1q32.3	AAA	Sporadic		[129]
<i>LINC00540</i>	13q12.11	AAA	Sporadic		[129]
<i>PCIF1</i>	20q13.12	AAA	Sporadic		[129]
<i>ERG</i>	21q22.2	AAA	Sporadic		[129]