Hereditary Influence in Thoracic Aortic Aneurysm and Dissection

Eric M. Isselbacher, MD, MSc; Christian Lacks Lino Cardenas, PharmD, MSc, PhD; Mark E. Lindsay, MD, PhD

Abstract—Thoracic aortic aneurysm is a potentially life-threatening condition in that it places patients at risk for aortic dissection or rupture. However, our modern understanding of the pathogenesis of thoracic aortic aneurysm is quite limited. A genetic predisposition to thoracic aortic aneurysm has been established, and gene discovery in affected families has identified several major categories of gene alterations. The first involves mutations in genes encoding various components of the transforming growth factor beta (TGF-β) signaling cascade (FBN1, TGFB1, TGFB2, TGFB3, SMAD2, SMAD3, and SKI), and these conditions are known collectively as the TGF-β vasculopathies. The second set of genes encode components of the smooth muscle contractile apparatus (ACTA2, MYH11, MYLK, and PRKG1), a group called the smooth muscle contraction vasculopathies. Mechanistic hypotheses based on these discoveries have shaped rational therapies, some of which are under clinical evaluation. This review discusses published data on genes involved in thoracic aortic aneurysm and attempts to explain divergent hypotheses of aneurysm origin. (Circulation. 2016;133:2516-2528. DOI: 10.1161/CIRCULATIONAHA.116.009762.)

Key Words: aorta ▪ aortic disease ▪ Marfan syndrome

Aortic aneurysm and dissection are associated with significant morbidity and mortality, accounting for >10,000 and contributing to >17,000 deaths annually in the United States. Numerous risk factors for aneurysms have been identified, although they differ, depending on the segment of the aorta involved. Aneurysmal disease in humans has been shown to involve the strong influence of hereditary predisposition, and genetic discovery has been progressing at an intensifying pace.

Genetic determinants are now understood to represent a major factor in determining aneurysmal risk for the individual patient, and genetic testing is a regular feature of clinical practice. In addition to clinical utility, genetic discovery has identified novel aspects of vascular biology that reveal cellular and tissue events contributing to aortic aneurysm, which may, in turn, offer new therapeutic targets. This review attempts to integrate our current understanding of human genetic discoveries, cellular and animal modeling of disease, and implications for aneurysm biology, with special focus on the emergence of thematic groups of genes implicated in aortic disease.

Epidemiology and Pathogenesis

Initial clues in the etiologic understanding of aneurysm came from clinical observations of repetitive anatomic patterns of disease. While studying the incidence trends of aortic aneurysm in the population of Olmstead County, Minnesota, researchers noted divergent incidence of thoracic aortic aneurysm (TAA) and abdominal aortic aneurysm, which was a startling finding at the time because it had previously been assumed that all aortic aneurysms had a similar pathogenesis. It is now well recognized that aortic root and ascending TAAs (ATAAs) are significantly different from abdominal aortic aneurysms in terms of risk factors, pathophysiology, and natural history, despite their common phenotypic manifestation. Abdominal aortic aneurysm is a disease driven primarily by atherosclerosis. As a result, abdominal aortic aneurysm shares many risk factors with coronary artery disease, including cigarette smoking, hypertension, diabetes mellitus, and male sex.

In contrast, genetic influences play a more prominent, if not dominant, role in TAA expression. As many as 20% of classically affected individuals have a first-degree relative with a dilated thoracic aorta. In keeping with this idea, the standardized incidence rate among sibling pairs for TAA is 2-fold higher than that for abdominal aortic aneurysm. Mendelian pedigrees demonstrate autosomal dominant inheritance, suggesting large influences of single genes segregating...
with thoracic aortic disease. Less well understood are the inherent differences between ATAAs and descending TAAs. Genetically triggered thoracic aortic disease invariably involves the ascending aorta, whereas no Mendelian pedigree has yet been described with isolated descending TAA segregation. This seems to imply that single-gene disorders may uniquely associate with ATAAs. Indeed, clinical observations suggest that descending TAAs are more often associated with atherosclerosis, age, and hypertension than are ATAAs, even in the absence of syndromic association.

Syndromic Versus Nonsyndromic TAAs

When TAAs appear to be familial, clinicians tend to describe them as being either syndromic or nonsyndromic. Specific syndromic aortic aneurysm conditions include Marfan syndrome (MFS; Online Mendelian Inheritance in Man [OMIM] No. 154700), Loeys-Dietz syndrome (LDS; OMIM No. 609192), and vascular Ehlers-Danlos syndrome (OMIM No. 103050), among others (Table 1). Classically, well-described external physical features associated with connective tissue disorders (CTDs) have included findings (and sometimes dysfunction) in the integumental, musculoskeletal, ocular, craniofacial, and cardiovascular systems. It should be understood that although some familial aortic conditions are correctly classified as CTDs, the presence of these external CTD features alone does not indicate cardiovascular risk. Although associations with CTD and aortic disease were once exclusive, improved phenotyping has now described extracardiac phenotypes in some non-CTD aortic conditions, signs of which may be less familiar to cardiologists who encounter aortic disease patients. Perhaps unsurprisingly, investigation in such families has discovered deficits in genes with utility specific to the cardiovascular system such as functions specific to the smooth muscle cells of the aortic media. When present, external phenotypic features may be difficult to detect without a high degree of suspicion; for example, livedo reticularis, iris flocculi, mydriasis, and peripheral vascular malformation have been described in such patients, in some cases with high penetrance.

In nonsyndromic TAAs, abnormalities are limited to the cardiovascular system. The majority of these conditions demonstrate autosomal dominant inheritance, but affected individuals do not exhibit external features of CTD or any other recurrent phenotype. Conditions that are usually considered nonsyndromic include familial thoracic aneurysms and dissections (also known as familial TAA), as well as TAA associated with bicuspid aortic valve (BAV).

Table 1. Syndromic and Nonsyndromic Aneurysm Conditions

<table>
<thead>
<tr>
<th>Syndromic Aneurysm Conditions</th>
<th>Nonsyndromic Aneurysm Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>MFS</td>
<td>FTAAD</td>
</tr>
<tr>
<td>LDS</td>
<td>Familial TAA</td>
</tr>
<tr>
<td>Vascular Ehlers-Danlos syndrome</td>
<td></td>
</tr>
<tr>
<td>Shprintzen-Goldberg syndrome</td>
<td></td>
</tr>
<tr>
<td>Aneurysms-osteoarthritis syndrome</td>
<td></td>
</tr>
<tr>
<td>Cutis laxa with aneurysm</td>
<td>BAV with aneurysm</td>
</tr>
</tbody>
</table>

BAV indicates bicuspid aortic valve; FTAAD, familial thoracic aortic aneurysm and dissections; LDS, Loeys-Dietz syndrome; MFS, Marfan syndrome; and TAA, thoracic aortic aneurysm.

Marfan Syndrome

The study of genetically triggered aortic disease has often been focused on MFS, but the work has advanced the understanding of TAA more broadly. Key accomplishments of MFS research include establishing the link between progressive aortic growth and aortic dissection (AoD), demonstrating the benefits of prophylactic aortic root surgery to prevent AoD, and most recently, introducing medical therapies directed at pathological signaling events within the aortic wall. However, one
must be careful not to be complacent in concluding that AoD arises purely from progressive aneurysmal growth of the aorta. Indeed, sporadic type A and B AoDs often occur at aortic dimensions not usually considered aneurysmal.47,48

MFS is a monogenic disorder caused by recurrent heterozygous mutations in the gene \textit{FBN1} encoding the protein fibrillin-1. Fibrillin-1 is a large extracellular matrix (ECM) protein that forms polymers called microfibrils, which closely associate with elastic fibers. Patients with MFS manifest abnormalities in multiple organs, especially the ocular, skeletal, and cardiovascular systems. Ectopia lentis (dislocation of the lens) is a highly specific manifestation of MFS. Typical skeletal manifestations include overgrowth of long bones, resulting in tall stature, arachnodactyly, pectus deformities of the chest, and characteristic facial features (Figure 1). The cardiovascular features of MFS were first described systematically by McKusick in \textit{Circulation} in 1955,40 and in the intervening decades, the cardiovascular phenotype of MFS has

### Table 2. Human Hereditary Aneurysm Conditions

<table>
<thead>
<tr>
<th>Gene (Protein)</th>
<th>Human Aneurysmal Syndrome</th>
<th>OMIM No.</th>
<th>Inclusion on Available Clinical Panel Testing</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ECM proteins</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>\textit{FBN1} (fibrillin-1)</td>
<td>MFS\textsuperscript{11}</td>
<td>154700</td>
<td>+++</td>
</tr>
<tr>
<td>\textit{EFEMP2} (fibrillin-4)</td>
<td>Cutis laxa, autosomal recessive, type IB\textsuperscript{12}</td>
<td>614437</td>
<td>+</td>
</tr>
<tr>
<td>\textit{ELN} (elastin)</td>
<td>Cutis laxa, autosomal dominant\textsuperscript{13}</td>
<td>123700</td>
<td>+</td>
</tr>
<tr>
<td>\textit{COL3A1} (Collagen 3 (\alpha-1))</td>
<td>Ehlers-Danlos syndrome, type 4\textsuperscript{14}</td>
<td>130050</td>
<td>+++</td>
</tr>
<tr>
<td>\textit{COL4A1} (Collagen 4 (\alpha-1))</td>
<td>HANAC\textsuperscript{15}</td>
<td>611773</td>
<td>-</td>
</tr>
<tr>
<td>\textit{COL4A5} (Collagen 4 (\alpha-5))</td>
<td>X-linked Alport syndrome\textsuperscript{16}</td>
<td>301050</td>
<td>-</td>
</tr>
<tr>
<td>\textit{PLD1} (lysyl hydroxylase 1)</td>
<td>Ehlers-Danlos syndrome, type 6\textsuperscript{17}</td>
<td>225400</td>
<td>+</td>
</tr>
<tr>
<td>\textit{PLD3} (lysyl hydroxylase 3)</td>
<td>Bone fragility with contractures, arterial rupture, and deafness\textsuperscript{18}</td>
<td>612394</td>
<td>+</td>
</tr>
<tr>
<td>\textit{LOX} (lysyl oxidase)</td>
<td>TAA and dissection\textsuperscript{19}</td>
<td>Unassigned</td>
<td>-</td>
</tr>
<tr>
<td>\textit{MFAP5} (microfibrillar associated protein 5)</td>
<td>Familial TAA, AAT\textsuperscript{20}</td>
<td>616166</td>
<td>+</td>
</tr>
<tr>
<td><strong>TGF-(\beta) pathway</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>\textit{TGFB1} (transforming growth factor-(\beta) receptor 1)</td>
<td>Familial TAA, AAT/LDS 1\textsuperscript{21,22}</td>
<td>609192</td>
<td>+++</td>
</tr>
<tr>
<td>\textit{TGFB2} (transforming growth factor-(\beta) receptor 2)</td>
<td>Familial TAA, AAT/LDS 2\textsuperscript{21–23}</td>
<td>610168</td>
<td>+++</td>
</tr>
<tr>
<td>\textit{TGFB2} (transforming growth factor-(\beta)2)</td>
<td>LDS 4\textsuperscript{24,25}</td>
<td>614816</td>
<td>+++</td>
</tr>
<tr>
<td>\textit{TGFB3} (transforming growth factor-(\beta)3)</td>
<td>LDS 5\textsuperscript{26}</td>
<td>615582</td>
<td>+</td>
</tr>
<tr>
<td>\textit{SMAD2} (SMAD family member 2)</td>
<td>Aortic and peripheral arterial aneurysm and dissection\textsuperscript{27}</td>
<td>Unassigned</td>
<td>-</td>
</tr>
<tr>
<td>\textit{SMAD3} (SMAD family member 3)</td>
<td>Aneurysms-osteoaorthritis syndrome/LDS 3\textsuperscript{28}</td>
<td>613795</td>
<td>+++</td>
</tr>
<tr>
<td>\textit{SMAD4} (SMAD family member 4)</td>
<td>JP/HHT syndrome\textsuperscript{29}</td>
<td>175050</td>
<td>++</td>
</tr>
<tr>
<td>\textit{SKI} (v-SKI sarcoma oncogene homolog)</td>
<td>Shprintzen-Goldberg syndrome\textsuperscript{30}</td>
<td>182212</td>
<td>+++</td>
</tr>
<tr>
<td><strong>Cytoskeletal/ smooth muscle contraction apparatus proteins</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>\textit{ACTA2} ((\alpha)-smooth muscle actin)</td>
<td>Familial TAA, AAT\textsuperscript{63}</td>
<td>611788</td>
<td>+++</td>
</tr>
<tr>
<td>\textit{MYH11} (smooth muscle myosin)</td>
<td>Familial TAA, AAT\textsuperscript{42,33}</td>
<td>132900</td>
<td>+++</td>
</tr>
<tr>
<td>\textit{FLNA} (filamin A)</td>
<td>Periventricular nodular heterotopia\textsuperscript{24}</td>
<td>300049</td>
<td>++</td>
</tr>
<tr>
<td>\textit{MYLK} (myosin light chain kinase)</td>
<td>Familial TAA, AAT\textsuperscript{75}</td>
<td>613780</td>
<td>+++</td>
</tr>
<tr>
<td>\textit{PRKG1} (protein kinase, cGMP-dependent, type I)</td>
<td>Familial TAA, AAT\textsuperscript{36}</td>
<td>615436</td>
<td>++</td>
</tr>
<tr>
<td><strong>Neural crest migration</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>\textit{NOTCH1} (notch1)</td>
<td>BAV with aneurysm\textsuperscript{37}</td>
<td>109730</td>
<td>++</td>
</tr>
<tr>
<td><strong>Unknown</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>\textit{SLC2A10} (glucose transporter 10)</td>
<td>Arterial tortuosity syndrome\textsuperscript{38}</td>
<td>208050</td>
<td>+++</td>
</tr>
<tr>
<td>\textit{MAT2A} (methionine adenosyltransferase II, (\alpha))</td>
<td>FTAAD\textsuperscript{39}</td>
<td>Unassigned</td>
<td>+</td>
</tr>
<tr>
<td>\textit{FOXE3} (forkhead box 3)</td>
<td>FTAAD\textsuperscript{39}</td>
<td>Unassigned</td>
<td>-</td>
</tr>
</tbody>
</table>

Presence of gene in academic and commercial next-generation sequencing aortopathy panel tests: +, inclusion in 0% to 25% of censored aortopathy panels; +++, inclusion in 34% to 66% of censored aortopathy panels; and ++++, inclusion in 67% to 100% of censored aortopathy panels (Table I in the online-only Data Supplement). BAV indicates bicuspid aortic valve; ECM, extracellular matrix; FTAAD, familial thoracic aortic aneurysm and dissections; HANAC, hereditary angiopathy with nephropathy, aneurysms, and muscle cramps; JP/HHT, juvenile polyposis/hereditary hemorrhagic telangiectasia syndrome; LDS, Loeys-Dietz syndrome; MFS, Marfan syndrome; OMIM, Online Mendelian Inheritance in Man; TAA, thoracic aortic aneurysm; and TGF-\(\beta\), transforming growth factor-\(\beta\).
been more clearly defined. The primary cardiovascular abnormality is an aneurysm of the aortic root, which often extends into the proximal portion of the tubular ascending thoracic aorta to create a pear-shaped aortic dilatation that is sometimes referred to as annuloaortic ectasia. Affected patients are at risk of AoD, and the risk increases with aortic diameter. However, medial degeneration is diffuse, and all large and medium arteries are at risk for dissection.

MFS is caused by a variety of genetic alterations in the FBN1 gene. Although heterozygous missense mutations are most commonly observed, nonsense and whole-gene deletions have been routinely described. Clinical genetic sequencing currently identifies ≈90% of classically affected probands, raising the possibility of mutations outside the coding region that affect the expression of fibrillin-1 such as intronic mutations that create abnormal splicing and premature termination. Therefore, both human genetic observations and experiments in animal models have confirmed that loss of function of the FBN1 gene is the causal mechanism in MFS.

At the protein level, loss of fibrillin-1 function was initially thought to affect the nature and composition of the ECM by affecting structural function. Early models of fibrillin-1 function emphasized the structural aspects of microfibrils and hypothesized that a lack of polymeric fibrillins reduced tissue strength and integrity, most manifest in elastin-rich tissues such as the aorta. In addition to their structural role, fibrillins perform a regulatory role by influencing cell-signaling events; this regulatory action is dominated by the interaction between fibrillins and the class of cell signaling molecules known as transforming growth factor-β (TGF-β). TGF-β signaling pathway occurs in the aorta. Conversely, ectopia lentis, a finding highly specific to dysfunction of the fibrillin-1 protein, is common in MFS but is not observed in LDS.

There is a much wider phenotypic variation in the extra-cardiovascular manifestations of LDS than in MFS. The variation is so great, in fact, that nonsyndromic multigenerational families have been prospectively diagnosed with familial thoracic aeurysms and dissections without connective tissue features and discovered to have mutations in TGFBR1 and TGFBR2. Such observations have led to controversy concerning the diagnostic nomenclature of patients with mutations in the TGF-β signaling pathway.

Mutations causing vascular disease in TGFBR1 and TGFBR2 have been exclusively missense and commonly, although not exclusively, occur in the kinase domain of the receptors. Whole-gene deletions or nonsense mutations that would be predicted to cause nonsense-mediated decay have not been identified. In fact, genomic deletions in TGFBR1 do not cause vascular disease at all and are instead associated with Ferguson-Smith disease, a disorder of self-healing squamous epitheliomas. Such genetic observations have led to predictions that missense mutations work through either a dominant-negative or complex gain-of-function mechanism. Experiments in cell culture have documented a loss of function for signaling competency for many mutations; however, TGF-β signaling, as assayed by phosphorylation of smad2 protein, is paradoxically increased in tissues from patients with LDS.

Both human genetic discoveries and experiments in animal models of LDS have reaffirmed these paradoxical observations. Mutations in SMAD3 were identified to cause a syndrome of TAA and systemic findings, including widely spaced eyes, bifid uvula, and early-onset osteoarthritis called aneurysms-osteoarthritis syndrome. Mutations in the TGFBR2 gene (encoding isoform 2 of TGF-β) were described as causal in a human TAA condition sharing significant phenotypic overlap with LDS. More recently, mutations in the closely related TGFBR3 gene have been shown to extend the spectrum and to cause syndromic TAA. Patients shared features of aortic root aneurysm, arterial tortuosity, congenital
talipes equinovarus, and pectus excavatum. Proteins encoded by TGFβ2, TGFβ3, and SMAD3 are unequivocal positive regulators of canonical TGF-β signaling, and reported mutations suggest loss of function of the cognate protein. Furthermore, mice harboring deletion mutations in either Tgfb2 or Smad3 demonstrate an independent aneurysm phenotype, and exacerbation of the aneurysm is observed in Fbn1<sup>C1039G</sup> mice when combined with haploinsufficiency for the cosmad Smad4. These data suggest that loss of canonical TGF-β signaling potency correlates with TAA.

Further work on receptor mutations causing LDS has reinforced these findings. An allelic series of mutations introduced into mice demonstrated that germline deletion of TGFBR1 or TGFBR2 is unable to produce vascular phenotypes, whereas introduction of a heterozygous mutation in the native locus mimicking a human LDS mutation induced aortic root aneurysm, arterial tortuosity, and extracardiac phenotypes. Vascular disease also developed in animals in which a mutant receptor was ectopically overexpressed. These observations superficially support a dominant-negative mechanism; however, when signaling potency was directly studied in cells isolated from mutant embryos, there was a 50% decrease in TGF-β signaling potential on activation with ligand. Interestingly, haploinsufficient cells showed no decrease in signaling potential except at supraphysiological doses of ligand, perhaps accounting for the lack of phenotype from deletion mutations. This result is consistent with studies demonstrating that individual TGF-β receptor subunit pairs signal independently within the tetrameric TGF-β receptor complex, obviating a classic dominant-negative mechanism of action. These data indicate that loss of canonical TGF-β signaling, either by haploinsufficient expression of SMAD3 or TGFBR2 or by missense mutations in TGFBR1 or TGFBR2 with resultant decrease in signaling potential, can cause TAA and systemic signs consistent with LDS (Figure 2A).

Sporadic de novo mutations in the gene SKI encoding a TGF-β repressor were found to cause Shprintzen-Goldberg syndrome (OMIM No. 182212), which is associated with TAA. In contrast to LDS, aneurysmal disease in Shprintzen-Goldberg syndrome seems not to be as aggressive, and dissections have not been reported. Additionally, experiments in cells of patients with Shprintzen-Goldberg syndrome show clear upregulation of TGF-β–mediated signaling events, in contrast to LDS cells, which generally show partial loss of signaling potency at the cellular level. The results of these cellular experiments would largely be predicted by the known function of SKI as a potent inhibitor of SMAD protein function. SKI binds directly to the MH2 domains of SMAD2 and SMAD3, displacing the transcriptional activator p300 and recruiting mSin3A and histone deacetylases. SKI-SMAD complexes are still competent to bind SMAD-binding DNA elements, thereby converting the local chromatin environment to a repressive state and inhibiting TGF-β–mediated transcription. Described mutations in SKI are clustered in the N-terminal SMAD2/3–binding domain and the Dachshund-homology domain, responsible for binding SMADs and other cofactors, predicting loss of function for the SKI protein in agreement with cellular experiments.

Analysis of TGF-β signaling in vascular tissues has not yet been published in Shprintzen-Goldberg syndrome models or patient samples. It therefore remains unclear how mutations that superficially produce opposite biochemical effects on the same pathway produce human syndromic conditions with such a high degree of phenotypic overlap. These data argue for the involvement of counterregulatory or compensatory signaling events, a contention that has been emphasized in pathogenic models of TGF-β vasculopathies.

**Smooth Muscle Contraction Vasculopathies**

In contrast to syndromic forms of TAA, some families exhibit autosomal-dominant inheritance of aneurysmal disease with few outward physical manifestations. Syndromic aortic conditions tend to be caused by genes with wide expression patterns in multiple organ systems. In contrast, gene defects causing familial thoracic aneurysms and dissections represent defects in proteins with functionality specific to the aorta, in particular to vascular smooth muscle cells (VSMCs). The first reported gene defect in the smooth muscle contraction vasculopathy gene family was MYH11, encoding a smooth muscle–specific myosin isoform. Smooth muscle myosin heavy chain is expressed in definitive VSMCs and uterine and enteric smooth muscle. Reported patients with smooth muscle myosin heavy chain mutations exhibit typical ascending TAA with a high penetrance of patent ductus arteriosus. Penetrance is incomplete, but individuals carrying the abnormal MYH11 alleles demonstrate evidence of increased arterial stiffness, a hallmark of aneurysmal tissue.

The next gene identified as causal in familial thoracic aneurysms and dissections was ACTA2, the locus encoding the smooth muscle–specific isoform of actin (α-smooth muscle actin). α-Smooth muscle actin is a protein that is a well-described component of smooth muscle cells, but it is also expressed widely in cells during inflammation and is a known transcriptional target of TGF-β signaling. Patients with mutations in ACTA2 exhibit a diverse vasculopathy characterized primarily by ATAA. Other cardiovascular abnormalities include cerebral aneurysm, myocardial infarction, and a neurovascular malformation resembling moyamoya disease.

In ACTA2-associated vasculopathy, there appear to be distinct allele-specific differences in disease severity. In particular, missense mutations at arginine 179 produce a severe syndrome associated with multiple congenital anomalies, early-onset aortic aneurysm and dissection, and congenital mydriasis (Figure 1). Severe aortic disease, however, is not limited to the R179 mutation; childhood AoD has also been described in mutations at different positions. So far, described mutations in ACTA2 have been missense in nature, and vascular disease has not been associated with large-gene deletions or nonsense mutations that would invoke simple haploinsufficiency as a mechanism. The Acta2<sup>−/−</sup> knockout mouse is viable with a normal life span, and explanted Acta2<sup>−/−</sup> VSMCs are hyperproliferative. The human mutational repertoire in ACTA2 is more consistent with a dominant-negative effect (individual missense mutations spread throughout the protein); however, pathogenetic clarity will likely require more advanced gene modeling experiments in small animal models to formally exclude a gain-of-function mechanism for these alleles.
Subsequent reports have reinforced the importance of the actin-myosin interaction in genetically triggered TAA. Putative loss-of-function mutations in \textit{MYLK} were reported to cause TAA in a large family with autosomal-dominant inheritance.\textsuperscript{35} \textit{MYLK} encodes myosin light chain kinase, a positive regulator of the actin-myosin interaction, and mutations predicted loss
of function of this regulator. Conversely, a recurrent mutation in PRKG1 (c.530G>A, p.Arg177Gln) was recently described as causing TAA. The mutation appears to disinhibit activity of the type 1 cGMP-dependent protein kinase. The type 1 cGMP-dependent protein kinase inhibits myosin light chain phosphatase, thereby functioning as a negative regulator of actin-myosin interaction. Consequently, increased PRK-1 activity results in decreased phosphorylation of myosin light chain and inhibition of actin-myosin interaction. In summary, genetic perturbations that tend to decrease actin-myosin interaction, through decreased function of either the actin-myosin pair (ACTA2 or MYH11) or their regulators (MYLK or PRKG1), within VSMCs tend to cause TAA (Figure 2A).

BAV/TAA

BAV is the most common developmental malformation of the heart, affecting between 0.5% and 1% of the general population. In ~40% to 50% of those with BAV, there is an associated dilatation of the ascending thoracic aorta (or aortic root). Therefore, BAV-associated TAA (BAV/TAA) is likely the most common type of aneurysm affecting humans. Familial predisposition in BAV/TAA is a well established; indeed, it is so common that screening of first-degree relatives is recommended in routine clinical practice. Some forms of monogenic TAA have a high predisposition for BAV such as LDS, ELN-related cutis laxa, and TAA associated with LOX mutations; however, these conditions likely represent only a very small fraction of human BAV disease. The most common forms of familial BAV appear to occur in an autosomal-dominant pattern but with incomplete penetrance. Often cosegregating in families with BAV are other forms of left-sided heart obstruction, including coarctation of the aorta, mitral stenosis, and in severe cases, hypoplastic left heart syndrome. Although they have been associated with a large pedigree segregating with BAV, coarctation of the aorta, and tetralogy of Fallot, mutations in NOTCH1 are thought to be rare among the general population of BAV patients. BAV with or without aortic dilatation is also a common feature of Turner syndrome, which is caused by monosomy of the X chromosome.

Available data suggest that typical BAV/TAA is genetically complex. Although pedigrees with apparent autosomal inheritance with incomplete penetrance are common, causative single loci have proved elusive. The inability to identify simple mendelian loci with whole-exome approaches suggests that many pedigrees may be attributable to polygenic influence rather than simply incomplete penetrance, which has been the commonly cited explanation. Unraveling the complex genetic architecture of BAV/TAA will likely require large-scale collections combined with deep-sequencing approaches.

Pathogenic Models of Genetically Triggered Aortic Disease

Research in experimental aneurysm has repetitively revealed overactivity of the TGF-β pathway in TAA. Furthermore, the additional evidence of human mutations in genes encoding effectors of canonical TGF-β signaling has led to the hypothesis that aberrant TGF-β signaling drives aneurysm progression. Although postnatal observations of increased TGF-β signaling are robust, genetic perturbations tend to result in loss of TGF-β signaling potency, illustrating the complexity of the signaling perturbation. How then do these mutations induce increased TGF-β signaling? Loss-of-function mutations have been proposed to cause disease through upregulation of counterregulatory pathways that may directly drive aneurysm. An example is upregulation of the mitogen-activated protein kinase pathway noted in Fbn1<sup>C<sub>1002K</sub></sup> mice, with inhibition of this noncanonical pathway retarding aneurysm progression. It should be noted that human aortic samples available for examination often represent late-stage disease, and it remains possible that at an earlier stage of development low TGF-β signaling precedes upregulation. Other pathogenic models posit dysfunction of the smooth muscle contractile apparatus as the fundamental anomaly in TAA on the basis of multiple loss-of-function mutations discovered in components and regulators of smooth muscle contraction. This hypothesis emphasizes the importance of the actin-myosin unit in aortic homeostasis. Failed contractile structure leads to focal adhesion complex and other cell surface receptor rearrangement. As a result, the VSMC fails to sense and attach properly to the surrounding ECM. These VSMCs undergo phenotypic change and secrete matrix-degrading enzymes with nonproductive remodeling of the aortic media. Importantly, this model naturally incorporates hypertension, which is thought to be a clinical risk factor for AoD.

Although these models have clear and compelling supporting evidence from the genetic literature and from experimental aneurysm studies, it is difficult to reconcile the two. Is increased TGF-β signaling a final common pathway for genetic perturbations causing aneurysm? Dysregulation of TGF-β is believed to induce VSMC phenotypic change and the secretion of matrix-degrading enzymes such as matrix metalloproteinases (MMPs) and would therefore represent a candidate final common pathway. Increased phosphorylation of the TGF-β effector SMAD2 has been demonstrated in tissue from patients with smooth muscle contraction vasculopathies, leading to the concept that increased TGF-β signaling could be a commonality across genetic perturbations causing aneurysm. However, dependence of aneurysm pathology on TGF-β has yet to be demonstrated in any smooth muscle contraction vasculopathy disease model, leading to continued questions of correlation and causality. Alternatively, there is a lack of evidence that increased TGF-β signaling, which has been repeatedly observed in patient samples, drives decreased contraction of smooth muscle cells. In contrast, TGF-β is a signal associated with transcriptional activation of contractile gene expression through SMAD3 and myocardin. Although increased contractile protein expression has recently been described in aneurysm samples taken from patients with MFS, the exact opposite has been observed in patients with mutations in TGFBR2 and sporadic TAA. These conflicting observations may indicate that relative upregulation or downregulation is less important than disruption per se of proper contractile protein homeostasis. This would also be consistent with observations that both loss of function and duplications in the contractile gene MYH11 are associated with TAA. These observations are consistent with disruption of cellular...
structures that are highly dependent on a regular stoichiometry. The polymeric actinomyosin cytoskeleton would certainly qualify in this respect. (discussed below).

**ECM Genes and the Relationship to Matrix-Independent Gene Groups**

Conduit arteries such as the aorta have 1 primary function: to accept the output of ventricular systole and carry blood to target organs. The engineering requirements of this function involve primarily components of the ECM: collagens (primarily I and III), elastin, and microfibrils necessary to provide structure and strength. Pathological observations of aortic tissue have consistently noted striking abnormalities of the ECM of the aortic media with elastin fiber fragmentation and VSMC disarray. It therefore comes as no surprise that defects in members of the ECM itself cause aneurysms when dysfunctional. In humans, examples include genetic variation in genes encoding ECM proteins such as fibrillins (FBN1), collagens (COL3A1), elastin (ELN), matrix-stabilizing enzymes such as fibrulin-4 (EFEMP2), and lysyl oxidase (LOX), a copper-containing oxidase responsible for cross-linking of collagens and elastin. Recently, defects in the ECM component microfibrillar-associated protein 5, encoded by MFAP5, have been shown to cause TAA. EFEMP2 and LOX mutations in particular illuminate the importance of elastogenesis to TAA pathogenesis. Fibulin-4 is a critical factor required for the recruitment of lysyl oxidase to tropoelastin (the building block of amorphous elastin fibers) in vivo, and inactivation of either fibrulin-4 or lysyl oxidase causes aneurysm in both humans and mice.

Although a model of TAA induced by improper developmental elastogenesis is experimentally validated, how do we account mechanistically for alteration in cell-autonomous gene products such as members of the TGF-β signaling cascade or members of the smooth muscle contraction apparatus? Available data have widely implicated failure of ECM homeostasis (with elastin destruction) through upregulation of matrix-degrading enzymes or decreased activity of inhibitor proteins. Upregulation and altered activation of MMPs, specifically MMP-2 and MMP-9, and other matrix-degrading enzymes are commonly observed in TAA. MMP upregulation has been associated with upregulation of various signaling pathways observed to be dysregulated in aneurysmal VSMCs, including insulin-like growth factor-1 receptor, TGF-β receptor, platelet-derived growth factor, and angiotensin II type 1 receptor signaling (Figure 2B). There are many connections between angiotensin II signaling and TGF-β signaling. Angiotensin II signaling is known to upregulate autocrine production of TGF-β signaling in cell culture, and both TGF-β signaling and angiotensin II signaling can directly activate SMAD proteins and activate MMPs. In addition to degradation of ECM, MMPs can release and activate latent matrix-associated signaling molecules, notably including TGF-β. ECM destruction by MMPs can lead to aortic medial weakening and aneurysm owing to the limited ability of the mature VSMCs to generate new elastin fibers. Enzymatic activation in genetically triggered human TAA is mediated by VSMCs (rather than inflammatory cells) and linked to changes in cellular phenotypes. Ascending aortic aneurysms demonstrate phenotypic modulation of VSMCs to a “synthetic” phenotype involving loss of stress fibers, endothelial reticular hypertrophy, and secretion of matrix-degrading enzymes (Figure 2B).

Loss of stress fibers and deficiency of the filamentous actin cytoskeleton are particularly notable aspects of TAA pathology (Figure 3). Interestingly, several classes of cell-autonomous TAA gene defects have close associations with the actin cytoskeleton. Actin reorganization is a hallmark of migratory mesenchymal cells induced by canonical TGF-β signaling, and underdeveloped stress fibers result when the canonical TGF-β pathway is inhibited. Mutations resulting in disruption of the VSMC contractile apparatus more directly influence cytoskeletal dynamics. Fibroblasts from patients with ACTA2 mutations demonstrate underdevelopment of stress fibers and fail to fully express contractile proteins when stimulated with TGF-β. Perturbation of actin dynamics through mutation in the FLNA gene, encoding filamin A, directly implicates the filamentous cytoskeleton in TAA pathogenesis. Women with X-linked periventricular nodular heterotopia associated with mutations in FLNA have a risk of TAA caused by filamin A deficiency. Filamin A is a broadly expressed integrator of cell signaling events (RhoA and SMADs) and mechanical forces with the actin cytoskeleton, necessary for orthogonal branching of actin and filament linkage to multiple extracellular receptors. Cells deficient in filamin A show defective TGF-β signaling, as assessed by SMAD2 activation, providing a link between TGF-β signaling and proper actin assembly. Genetic evidence from patients with cervical artery dissection, a phenotype seen in genetically triggered aortopathy, further supports the association of

![Figure 3. Loss of cytoskeletal structure in thoracic aortic aneurysm (TAA) tissue. TAA stained with Verhoeff-van Gieson (VVG) stain demonstrates familiar elastin fiber paucity and fragmentation (top). Staining of aortic tissue for filamentous actin (F-Actin) shows a nearly complete loss of intracellular organized cytoskeletal architecture in TAA (bottom).](Image)
vascular fragility with cytoskeletal dynamics. Sporadic cervical artery dissection has recently been linked to common variation at the PHACTR1 locus. PHACTR1 binds G-actin through its 4 RPEL domains and directs assembly of stress fibers and cellular motility. How could disturbance in actin assembly mediate aneurysmal phenotypes? Interestingly, regulation of filamentous actin assembly is intimately linked to both cellular morphology and MMP expression, cellular phenotypes displayed within TAA tissue. For instance, depolymerization of actin cytoskeleton with cytochalasin D, but not the microtubulin-destabilizing toxin nocodazole, has been shown to directly induce the activation of MMPs, including MMP-2. In this way, TAA cells have features in common with cancer cells that have achieved anchorage-independent growth. In fact, M2 melanoma cells deficient for filamin A exhibit constitutive secretion of active MMP-9, an activity that is downregulated nosensing (FLNA or PHACTR1), through altered mechanosensing (TGF-β), 6 actin assembly is intimately linked to both cellular morphological disruptions of the cytoskeleton are difficult to separate experimentally. Whether effects are mediated through direct alteration of actin dynamics (FLNA or PHACTR1), through altered mechanosensing (ACTA2, MYH11), or as a downstream target of disordered TGF-β signaling (TGFBR1, TGFBR2, SMAD3), disruption of the filamentous cytoskeleton is an emerging theme of genetic discovery in aneurysm.

Implications for Therapy
Just as we have come to recognize over the years that the thoracic aorta behaves differently from the abdominal aorta and, further still, that the ascending thoracic aorta behaves differently from the descending thoracic aorta, so too have we come to appreciate that TAAAs of different origins can behave quite differently. Indeed, because BAV-associate TAAs are genetically mediated and demonstrate medial degeneration histologically, for many years, experts thought that such aneurysms were especially vulnerable to AoD, just as in MFS. However, a recent study found that in patients presenting with an acute AoD, the mean aortic diameter among those with an underlying BAV was actually significantly smaller (ie, not smaller) than among those with tricuspid aortic valves (6±15 vs 56±11 mm, respectively; P=0.0001), refuting the notion that a genetic underpinning predicts more virulent disease. Conversely, many patients with MFS suffer type B AoD at aortic diameters that are quite normal.

Even more provocative is recent evidence that among those with MFS, the underlying gene mutation may significantly affect outcome. In a registry of patients with MFS and FBN1 mutations, patients with haploinsufficient mutations had a 2.5-fold increased risk for cardiovascular death and a 1.6-fold increased risk for any aortic complication compared with patients with a dominant-negative mutation. Although the clinical trials of losartan to slow aortic root growth in MFS have been by and large disappointing, a related study by the same investigators discovered that therapy with losartan significantly reduced the rate of aortic growth in haploinsufficient MFS patients but not in dominant-negative patients. Collectively, these findings highlight both the challenges and opportunities that face us in determining optimal treatment strategies for TAAs. We simply cannot expect to see the same aneurysms, even when they involve the same aortic segment, will behave similarly. The underlying cause and even the underlying genetic mutation appear to affect both risk and response to therapy. Therefore, efforts to better define individual patients’ specific genetic defects may inform future research efforts and define the groups in which specific therapies may be most efficacious.

Future Directions
There are many questions to address in the field of hereditary aneurysm, the answers to which may inform therapeutic approaches. What are the processes by which cells within the aortic media undergo phenotypic change in response to mutations in VSMC contractile or the canonical TGF-β signaling pathway? Cellular phenotypic changes are typically accompanied by large-scale epigenetic chromatin remodeling. In fact, extensive epigenetic changes have been described at the SMAD2 promoter in cells from TAA. Modulation of these and similar pathways may useful to improve aortic performance.

In addition to epigenetic investigation, next-generation sequencing approaches have much to offer in the analysis of the genetics of aortic disease. Although great progress has been made in the identification of rare mendelian forms of aortic disease, very little is known about more common genetic variation conferring susceptibility to TAA or to AoD. Sporadic TAA patients tend to be older and to have more comorbidities than patients with mendelian forms of TAA and AoD, suggesting etiologic diversity. Considering the rarity of identifiable genetic causality in described TAA and AoD cohorts, it is clear that the large majority (80%–90%) of patients with these diseases have poorly understood genetic predisposition. A better understanding of the genetic landscape of TAA with these approaches will no doubt identify new targets for therapeutic intervention.

A single genome-wide association study performed on nonsyndromic individuals with TAA identified a susceptibility locus at 15q21.1 overlapping the gene FBN1; indeed, the redundant identification of genes involved in both monogenic and sporadic forms of the same disease has become a theme in the investigation of cardiovascular disorders. Although this important study cemented the association of fibrillin-1 with forms of nonsyndromic TAA, only this 1 association reached genome-wide significance, likely as a consequence of limited power. Research groups studying myocardial infarction, hyperlipidemia, atrial fibrillation, stroke, and other cardiovascular conditions have made more significant progress through the establishment of large-scale genetic collections. Considering the progress made by the combination of
both common and rare human variation in these conditions, expanding future efforts in aortic research more aggressively into large-scale populations seems worthwhile.

Sources of Funding
M.E.L. is supported by a grant from the National Institutes of Health (14GRNT18420018), and by the Toomey Fund for Aortic Dissection Research and the Fredman Fellowship.

Disclosures
None.

References
11. Ohkawa T, Wessels MW, Loeys BL. Mutations in a TGF-


44. Tran-Fadulu V, Punnah H, Kim DH, Vick GW 3rd, Lonsford CM, Lafont AL, Boccalandro C, Smart S, Peterson KL, Hain JZ, Willing MC, Coselli JS, LeMarie SA, Aah C, Byers PH, Milewicz DM. Analysis of multigenic families with thoracic aortic aneurysms and dissections due to...


76. Isseibacher et al. Genetics of Aortopathy and Aneurysm. 2527

77. Isselbacher et al. Genetics of Aortopathy and Aneurysm. 2527


Hereditary Influence in Thoracic Aortic Aneurysm and Dissection
Eric M. Isselbacher, Christian Lacks Lino Cardenas and Mark E. Lindsay

Circulation. 2016;133:2516-2528
doi: 10.1161/CIRCULATIONAHA.116.009762
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2016 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://circ.ahajournals.org/content/133/24/2516

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published
in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial
Office. Once the online version of the published article for which permission is being requested is located,
click Request Permissions in the middle column of the Web page under Services. Further information about
this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org//subscriptions/