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Genetic Contributions To Thoracic Aortic Disease

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Genetic Contributions to Thoracic Aortic Disease

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Abstract

Aims

Thoracic aortic aneurysms and dissections are significant yet under-recognized threats in cardiovascular health, often remaining undetected until catastrophic events occur. This study aims to explore the genetic landscape of thoracic aortic diseases, focusing on genetic mutations contributing to thoracic aortic aneurysm (TAA) and dissection. It also seeks to refine the size criteria for surgical intervention in TAA, aiding clinicians in decision-making and improving patient outcomes.

Methods

The study employed a comprehensive literature review, focusing on the genetic aspects of thoracic aortic diseases. In addition to a systematic MEDLINE search, the investigation used the Genomics England PanelApp to identify genes associated with TAA and dissection. This research builds on the work of Dr. John Elefteriades and the Yale Aortic Institute published in 2019 which incorporated updated size criteria for surgical interventions based on type of genetic mutation.

Results

The investigation expands the genetic landscape understanding of TAA, identifying 68 genes with different levels of association through the Genomics England PanelApp. Among these, thirty-three genes have a strong association ("green" designation), five are of moderate concern ("amber"), and thirty have a lesser-known impact ("red"). Additionally, the research proposes a shift in size criteria for surgical interventions,

specifically a “left shift” in thresholds, particularly for genes related to Loeys-Dietz syndrome and others.

Conclusions

This study highlights the evolving genetic complexity in thoracic aortic diseases. Several new genetic variants with strong associations to TAA have been identified, necessitating updates in genetic screening panels. The research also emphasizes the change in size criteria for surgical intervention, advocating for a more proactive approach in managing TAAs. Future research should continue exploring genetic contributors, reevaluating genes with medium or weak associations, and refining intervention criteria based on non-size factors. This advanced genetic understanding of TAA and dissection offers a nuanced perspective, paving the way for improved patient management and outcomes in thoracic aortic diseases.

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Introduction

Thoracic aortic aneurysms and thoracic aortic dissections remain "silent killers" in cardiovascular disease, and despite decades of research and advances in screening, often continue not discovered until their culminating, often lethal event. This section will provide an introduction to the aorta, aortic disease, and its treatment, as well as the efforts and techniques that currently exist for early detection of aortic aneurysm.

The Aorta

The aorta, the largest blood vessel in the body, serves as the conduit through which nutrient-rich blood exits the heart and starts its journey to perfuse the body and deliver oxygen to tissues, including the heart muscle itself. It originates at the aortic valve, connected to the left ventricle of the heart, and extends to its bifurcation into the iliac arteries.[1] The aorta is divided into four sections, which represent their different orientations and locations in the body: the ascending thoracic aorta, the aortic arch, the descending thoracic aorta, and the abdominal aorta. The ascending thoracic aorta exits the heart aiming slightly to the right and concludes at the aortic arch, which is demarcated by the branching of the right brachiocephalic artery. As the name "arch" indicates, the aorta takes a cane-shaped turn to the left and posteriorly to achieve this shape. The aortic arch ends, and the descending thoracic aorta begins, immediately distal to the origination of the left subclavian artery. The descending thoracic aorta continues until the aorta, drifting centrally as it moves inferiorly through the chest, passes through the opening in the diaphragm at the T12 level of the spine, or aortic hiatus, and becomes known as the abdominal aorta.[2]

The aorta itself is composed of layers of tissue that help it to achieve its function. The adventitial aorta, the outermost of three layers, is composed primarily of collagen. Although thin, it lends the aorta the majority of its tensile strength. The aortic media, or middle layer of the aorta, comprises up to 80% of its thickness and is formed by smooth muscle tissue, elastin, and collagen. The aortic intima, composed of a basement membrane made up by smooth muscle and connective tissue, hosts endothelial cells that come in direct contact with the blood passing through the aorta. It is the thinnest layer of the three, and its fragile composition makes it more susceptible to damage as a result.[3]

The development of the aorta begins in the third week of gestation, when the vascular islands that have been emerging independently integrate into paired aortae with a ventral and dorsal component. The ventral components of this primitive aorta form the aortic sac, while the dorsal aspects merge to form the descending thoracic aorta. Six paired aortic arches develop cranially to caudally, and eventually give rise to a number of important arteries. After the establishment of the sixth aortic arch in the fifth week of gestation, the connection between the dorsal component of the aorta and the pulmonary arteries regresses until the only remaining communication is the ductus arteriosus. This linkage remains patent in order to provide oxygenated blood entering the pulmonary artery to the aorta and thereby the primary circulation of the fetus; after birth the ductus closes and becomes the ligamentum arteriosum.[4]

Aortic disease

The aorta – while transporting over 50 million gallons of blood in the average lifetime – is susceptible to a variety of congenital and acquired disease processes that can

present in both an acute and chronic manner. The main categories of chronic aortic disease include thoracic aortic aneurysm (TAA) and abdominal aortic aneurysm (AAA), the traditional definition of which involves all the layers of the aorta exhibiting a localized dilatation of >50% in comparison to the same segment in a matched control population.[5]

The majority of people with aortic aneurysms are unaware of them, because aneurysms are largely asymptomatic. If symptomatic, the cardinal thoracic aortic aneurysm symptom is chest pain. The point at which a TAA becomes concerning for complication has been estimated around 4.0 cm or greater, according to a study that found a 4.0 to 4.5 cm aorta conferred an 89-fold higher risk of dissection compared to a reference group of > 3.4 cm.[6] In considering aortic size it is also important to normalize the measurement to a patient's overall height and body surface area, as a 4.5 cm aneurysm would functionally be much larger in a small woman compared to a large man.

When the aorta dilates it weakens, and the larger the aneurysm is, the more potential the aortic intima has to tear and create shearing in a mid-medial plane, creating an aortic dissection. There are several classifications to define aortic dissection; the most commonly used to describe the location of dissection is the Stanford classification system. A Stanford Type A dissection necessitates involvement of the ascending aorta, and a Type B dissection does not affect the ascending aorta but can include dissection of the descending aorta and can include the aortic arch. There is a third, type of dissection in this classification known as a non-A non-B dissection, confined only to the aortic arch, but it is exceedingly rare.

Acute aortic syndromes (AAS), including dissections, penetrating aortic ulcers, and intramural hematomas, are often lethal complications of aortic disease if left untreated – one study calculated that thoracic aortic dissections, which compose about 90% of AAS, accounted for 7.18% of all out-of-hospital cardiac arrests.[7] If patients become symptomatic, experiencing tearing chest pain that radiates to their back, or exhibit other signs of dissection, they may be able to reach the hospital, where further challenges await. Aortic dissection is notoriously difficult to diagnose, and is often missed even in the emergency room setting: a meta-analysis of studies investigating misdiagnosis of aortic dissection found the rate of incorrect diagnosis to be 33.8%.[8] Once diagnosed correctly, an investigation by Harris et al revealed that mortality for patients with Type A dissection managed medically was 23.7% at 48 hours, while patients with planned or completed surgical correction exhibiting mortality of 4.4% in the same time frame.[9] The total death toll in the United States as a result of aortic aneurysms in 2019 was 9,904, which is likely an underestimation due to lack of diagnosis (and attribution of sudden aortic related death to "heart attack"). These issues underline the necessity for improved early detection for this condition.[10]

Biomechanical and genetic underpinnings of aortic disease

The contributors to aortic disease are multifactorial in nature and include both mechanical and genetic underpinnings. A weakening of the aortic wall from any cause can result in aortic aneurysm formation, which dilates over its natural history and can predispose a patient to aortic dissection. From a biomechanical standpoint, increased pressure in the aorta such as in hypertension can precipitate this process, as and

aortopathy weakens the aortic wall and decreases its ability to withstand increased aortic blood pressure. It is important to note that even independently of aortic aneurysm, thoracic aortic dissection is possible, and is attributable to wall stress and inflammation.[11]

There are also complex genetic contributors to thoracic aortic disease, including aneurysms and dissections. Although genetic contributions to aneurysm formation exist in both thoracic and abdominal aortic aneurysms, research has shown a strong genetic history in the development of TAA. Within the genetic conditions, these can be divided into patterns of syndromic and non-syndromic disease.

Syndromic diseases associated with thoracic aortic disease have several manifestations that aid in their recognition, generally affecting not only the cardiovascular system but also the skeletal system and connective tissue. The best-known of these is Marfan syndrome, an autosomal dominant disease that causes a defect in FBN1 gene. Presentation of Marfan syndrome is variable, but includes elongated limbs, ocular manifestations such as lens subluxation, and various musculoskeletal signs.[12] Another syndrome commonly exhibiting thoracic aortic disease is Ehlers-Danlos, in which a mutation in COL5A1 or COL5A2, or related genes, predisposes those affected to aortic aneurysm and dissection while also affecting the connective tissue system and leaving patients with joint hypermobility and skin hyperextensibility.[13] Loeys-Dietz syndrome, which has more recently been discovered to affect, among others, the SMAD3 and TGFBR1/TGFBR2 genes, has a variable but similar presentation to Marfan and Ehlers-Danlos syndromes, all while predisposing patients to aortic aneurysm and eventual dissection.[14]

While syndromic diseases like those delineated above represent a large portion of the currently understood genetic contributors to aortic dissection, there is an increasing recognition in the literature of non-syndromic thoracic aortic disease—that is, genetic disorders limited in their impact to the thoracic aorta. The discovery of these genes has been guided by a recognition of a strong familial history of aortic aneurysm and dissection, prompting genetic sequencing of patients and their family trees to discover patterns of genetic disruption. The genes involved in familial thoracic aortic disease cause an array of pathophysiology that ultimately contribute to a disruption in the integrity of the aortic wall, and will be elaborated upon later in this thesis.

Treatment of aortic disease

The initial component of thoracic aortic disease treatment involves addressing systemic factors that may precipitate aneurysm formation, such as hypertension treatment with medication and lifestyle modifications. Since hypertension increases the risk of aortic dissection, a systolic blood pressure of less than 130 mmHg should be targeted.[15] Statins may also be used as part of the comprehensive treatment for TAA, as some evidence exists that they can protect against the vascular degeneration process in the natural history of aortic aneurysm (possibly descending and thoracoabdominal aneurysms versus thoracic only).[16] Smoking cessation is also highly encouraged, as tobacco use is another contributor to the formation of TAA.[17] Once medical therapy has been optimized, the latest guidelines released by the American College of Cardiology and American Heart Association in 2022 delineate several indications for surgical intervention of TAA. The first Class I recommendation for surgery is that patients with aortic root or ascending

aortic aneurysms who exhibit any symptoms should receive surgical intervention. The second Class I recommendation is that any patient with an aortic root or ascending aortic aneurysm with a measured diameter on computed tomography of 5.5 cm or greater should undergo surgery. Finally, it is a Class I recommendation that even with an aortic root or ascending aortic measurement of less than 5.5 cm, if the aorta has been noted on imaging to have grown more than 0.3 cm in two consecutive years or more than 0.5 cm in one year, surgery should be offered.[18]

Although the size criteria for intervention of TAA has historically hovered around the "hinge point" for aortic complications, thought to be around 6 cm where the aortic wall integrity decreases dramatically,[19] there is evidence to suggest that TAA rupture may occur at much smaller sizes than previously thought. Prompted by the findings in an International Registry of Acute Aortic Dissection study that saw 60% of patients experience aortic dissection at a diameter of 5.5 cm or less, Paruchuri et al described the "size paradox" of aortic dissection and calculated a 6,305-time relative risk of a > 4.5 cm aneurysm rupture compared to an aorta measured at 3.5 cm. There has been discussion in the literature that a "left shift", or further reduced size threshold for surgical intervention in TAA, is warranted.[6] The 2022 guidelines made a Class II recommendation that patients with an aortic diameter of 5.0 cm or greater could be considered for surgical repair at centers of excellence, where the procedures are done commonly and with limited risk of morbidity and mortality. A study by Perez et al used available imaging of the thoracic aorta to assess 407 patients' aortic diameter at the time of their Type A or Type B dissections; the authors discovered that 29.3% of aortic dissections could have been prevented with a surgical intervention criteria of 5.0 cm instead of 5.5 cm.[20]

Options for surgical correction vary depending on the location of the aneurysm. For a root aneurysm, both valve-sparing and valve-involving root replacement techniques exist; one might opt for a valve-sparing approach such as the David procedure in a younger patient to prolong the life of the patient's native valve.[21] In ascending aortic aneurysms, typically the dilated part of the aorta is removed and replaced, and if the zone up to the takeoff of the right brachiocephalic artery needs repairing, the resulting procedure is called a hemiarch replacement. A total arch replacement can also occur in patients with dilation that extends further along the arch. Repair techniques for Type B dissections are outside the scope of this thesis.

Early detection efforts in thoracic aortic aneurysm

The practice of screening patients for TAA is crucial, as many patients are unaware of their condition while asymptomatic. The methods of screening involve screening for the size of an aneurysm, genetic screening to determine a predisposition to developing aneurysms in the future, and a variety of other proposed factors.

The imaging of TAAs can be performed with echocardiography, computed tomography angiography (CTA) or magnetic resonance imaging (MRI). CTA is thought to provide a more accurate measurement of the aorta compared to echocardiography, and can evaluate the complete thoracic aorta while echocardiography only shows the lower ascending aorta. MRI, on the other hand, may be a suitable imaging option for patients who want to avoid exposure to contrast.[19] The most important principle in imaging TAAs is to strive for consistency in the measuring tool to allow for the most precise possible comparison between time points.

One opportunity to capitalize on engaging patients in aortic surveillance is the realm of incidental detection, in which patients undergoing imaging for a variety of other conditions are discovered to have indolent aneurysms. A study by Weininger et al found that among 261 TAAs discovered incidentally on CTA, only 6.9% of patients were referred to a cardiac surgeon for further evaluation. Additionally, only 37.4% of patients had a follow-up CT scan within one year. In the investigation, aneurysm size and family history were found to be significantly associated with referral to a cardiac surgeon, which underscores the importance of inquiring about family history during workup for TAA. Whether the finding is incidental or discovered due to an imaging study after a discussion of family history, TAA should be monitored at regular intervals and surveillance should be arranged at the time of the incidental finding. A typical screening schedule would consist of reimaging after one to two years, but is flexible depending on the patient's aortic diameter and the rate of change when compared to previous scans.

Another factor of increasing importance in screening for TAA is genetic screening. As discussed earlier in this introduction, syndromic thoracic aortic disease is important to recognize, and diagnosis in a patient should invite genetic screening for as many family members as possible in order to identify who may need regular surveillance for development of TAA or who already bears the condition. Even in non-syndromic thoracic aortic disease, at least 13 to 20% of afflicted patients have first-degree relatives who are also affected.[22] A study by Robertson et al found that 1 in 6 patients undergoing aortic surgery had genetic features suggestive of familial aortic disease. The authors thereby argued that first-degree relatives of patients undergoing surgery for TAA should also undergo genetic screening.[23]

Even with aortic surveillance using imaging and genetic screening, thoracic aortic dissection remains an unpredictable event. In studying a large database of patients with TAA, Elefteriades et al pinpointed 14 non-size criteria that could be used to determine appropriate surgical intervention. The list included chest pain, length and/or tortuosity of the aneurysm, genes, family history, bicuspid aortic valve, diabetes, biomarkers ("RNA Signature"), aortic stress due to factors like exercise and blood pressure, root location of dilatation, inflammation seen on positron emission tomography (PET) imaging, a specific genetic variant known as KIF6 (Kinesin family member 6), female sex, a prescription for fluoroquinolone, and finally age.[24] The authors investigated each of these criteria in detail and determined that the majority of the criteria could be considered to increase a patient's risk of aneurysm rupture, and therefore may warrant more prompt intervention especially when occurring in tandem. As our knowledge grows, so will the official criteria for surgical intervention of TAA.

Statement of Purpose

The purpose of this investigation is to describe the landscape of genetic mutations involved in thoracic aortic disease. Given that aortic aneurysm and dissection remain incredibly difficult to predict, it is important to concentrate the literature and distill the importance of the almost 70 genes that are currently thought to contribute to TAA and dissection. Furthermore, another aim of this project is to assign size criteria for surgical intervention in thoracic aortic aneurysm. This information will help clinicians understand when to refer patients for surgical intervention based on existing evidence according to the specific gene involved, and allow surgeons to better risk stratify patients with thoracic aortic disease when offering surgery.

Methods

Student Contributions

The conceptualization of the thesis was performed by the author of this thesis and Dr. John Elefteriades, thesis advisor for this project. The data collection through literature review for this project was performed by the thesis author and another Yale School of Medicine MD student, Christina Waldron, and advised by YSM librarian Alyssa Grimshaw. Data synthesis and analysis was performed by the thesis author. Writing of all sections was performed by the thesis author. Review of the writing was performed by the thesis author and Dr. Elefteriades.

Ethics Statement

All research for this thesis was performed ethically and responsibly, adhering to the guidelines for professional integrity in research delineated by the Yale School of Medicine's Code of Conduct.

Human Subjects Research

This study was approved by the Institutional Review Board requested by the Aortic Institute at Yale School of Medicine. The study received an exempt status from a waiver of consent. No special focus was placed on patients from historically vulnerable populations.

Methods Description

A comprehensive literature review was performed to accurately describe the landscape of genes involved in thoracic aortic aneurysm and dissection as well as possible intervention criteria based on gene. All genes involved in thoracic aortic aneurysm were identified by using the comprehensive Genomics England PanelApp, which lists 68 genes that have a strong, medium, or weak association with TAA formation and dissection. The database MEDLINE was used to perform systematic searches of the medical literature using keywords related to TAA, dissection, and the gene in question. This study also builds upon the work of Dr. Elefteriades' research group in 2019, in which a size diagram was built to demonstrate the suggested time of surgical intervention for each gene.[25] New genes are discovered regularly, so updating our recommended surgical intervention criteria every several years is essential.

The evidence collected by the various committees associated with the PanelApp was included in this analysis, and augmented by an Ovid literature review to identify any other evidence as well as available size criteria to guide surgical intervention.

Statistical Methods

No statistical methods were used to analyze this descriptive study.

Results

There have been several efforts made to describe the genetic landscape of TAA since a genetic basis to thoracic aortic disease was first suspected decades ago. Most pertinent to this thesis is the work of Dr. John Elefteriades and lab members, who first created a comprehensive list of genes that contribute to TAA and aortic dissection and delineated a size threshold warranting surgical intervention for each gene.[26] The list of genes was updated by Dr. Elefteriades' group at the Yale Aortic Institute and updated size intervention criteria proposed in 2019 by Vinholo et al, and included a list of 37 genes with known associations to thoracic aortic disease.

In the five years since this was published, the effort to describe genes associated with TAA has expanded tremendously. However, many individual institutions do not have access to genetic data on a scale to attribute causation in TAA and many other uncommon or rare diseases, which has hindered progress of genetic targets for many years. In order to encourage the standardization of gene panels on an international scale associated with diseases and provide a repository for information on all genes that are thought to be associated with a disease, a company called Genomics England partnered with the National Health Service (NHS) to create PanelApp.[27] The app takes an initial, broad list of genes that could be associated with a certain rare disease, and runs them through four databases. If found in three of four of the sources, the gene is labelled as "green", and it is deemed reasonable for those genes to be tested routinely on whole-exome or entire genome screening tests for patients. If found in two of the sources, the gene is deemed amber and caution is advised in testing, and if found in only one, an even lower level of confidence in the association between gene and disease is presumed. Furthermore, the

panel is refined by Genomics England curators who search routinely to establish modes of inheritance and discover additional evidence of gene-disease relationships. Crowdsourcing from experts within the scientific community is also encouraged, and those who wish to review genes may weigh in on the level of evidence sufficient for the gene to earn the "green", or disease-causing designation.

The complete list of genes suspected to relate to TAA is 68 entries in length, representing the largest existing repository of genetic contributors to the development of thoracic aortic aneurysm or dissection. As of 2024, thirty-three genes are designated green, five are amber, and thirty are red.[28]

Given the rapidly inflating landscape of genetic contributions to aortic disease, it is important to acknowledge additional efforts in delineating the genes with the strongest potential to cause harm to patients. One such attempt is a study conducted by Renard et al and published in the Journal of the American College of Cardiology, which aimed to identify the genes that should be selected to test for in a screening capacity that may predispose to hereditary thoracic aortic aneurysm and dissection. The authors employed the semiquantitative Clinical Genome Resource (ClinGen) framework to interrogate the strength of association between genes and diseases, and sorted 53 candidate genes into categories of definitive, strong, moderate, limited, and no reported evidence. The nine genes in the "definitive" category and the two in the "strong" category were classified as genes that predispose to heritable presentations of thoracic aortic aneurysm and dissection (HTAAD): ACTA2, COL3A1, FBN1, MYH11, SMAD3, TGFB2, TGFBR1, TGFBR2, MYLK, LOX, and PRKG.[29] As a result, the 2022 ACC/AHA guidelines

regarding aortic aneurysm were updated to recommend testing for these pathogenic variants in cases of TAA (Class I).

Acknowledging these different sources of legitimacy in contributors to HTAAD, these results will superimpose the Genomics England PanelApp, ClinGen, and Aortic Institute work to provide a robust background of genes that contribute to HTAAD.

Genes with a strong association

We begin our review by surveying the genes deemed to have a strong association with TAA from the Genomics England PanelApp, which also includes the 11 ClinGen genes. These genes have a variety of impacts on the aorta, from vascular smooth muscle cells, the extracellular matrix of layers of the aorta, the transforming-growth factor beta pathway, and other mechanisms of contribution. Table 1 lists all the genes that have earned the green designation on PanelApp. The 11 ClinGen genes are completely overlapping with this list, and are also included in the table. Those denoted in red were not previously included in the Aortic Institute's list from 2019, and as they are new to our group's work, they will be discussed below.

ABL1 is traditionally known for it its part in the fusion gene BCR-ABL found in leukemia cancer cells with the Philadelphia chromosome, but has not been observed to have germline mutations of its own.[30] A study by Wang et al demonstrated, however, ABL1 variants in patients with an autosomal-dominant pattern of a constellation of signs and symptoms, such as congenital heart disease, skeletal malformations, and failure to thrive. The CHD took the form of atrial and ventricular septal defects, coarctation of the

aorta, as well as aortic root dilation. The authors also reproduced the mutations in mice, and found the important role played by ABL1 in the development of organisms.[31]

Gene	Protein	Animal model?	Syndromic TAAD	Non-syndromic TAAD	Associated disease or syndrome	Associated vascular characteristics	Mode of inheritance	OMIM
ABL1	Tyrosine kinase	Yes[31]	+	-	Syndrome with CHD, skeletal anomalies, FTT	ARD, coarctation of the aorta	AD	189980
ACTA2	Smooth muscle alpha-actin	Yes[40]	+	+	AAT, multisystemic smooth muscle dysfunction + MYMY5	TAAD, early aortic dissection, CAD, stroke (moyamoya disease), pulmonary artery dilation, BAV[32,33]	AD	11788 613834 614042
ASPH	alpha-ketoglutarate-dependent hydroxylase	No	+	-	Traboulsi syndrome[38]	ARD, aortic regurgitation	AR	600582
BGN	Biglycan	Yes[41]	+	-	Meester-Loeys syndrome	ARD, TAAD, pulmonary artery aneurysm, IA, arterial tortuosity[34]	X-linked	300989
CBS	Cystathionine beta-synthase	Yes[42]	+	-	Homocystinuria[39]	ARD	AR	613381
COL3A1	Collagen 3 alpha-1 chain	Yes	+	-	EDS, vascular type (IV)	TAAD, early aortic dissection, visceral arterial dissection, vessel fragility, IA	AD	130050
COL5A1	Collagen 5 alpha-1 chain	No	+	-	EDS, classic type I	ARD, rupture/dissection of medium-sized arteries[35–37]	AD	130000
COL5A2	Collagen 5 alpha-2 chain	Partially	+	-	EDS, classic type II	ARD	AD	130000

Gene	Protein	Animal model?	Syndromic TAAD	Non-syndromic TAAD	Associated disease or syndrome	Associated vascular characteristics	Mode of inheritance	OMIM
EFEMP2	Fibulin 4	Yes[52,53]	+	-	Cutis laxa, AR Type Ib	Ascending aortic aneurysms, other arterial aneurysms, arterial tortuosity, stenosis	AR	614437
ELN	Elastin	No	+	-	Cutis laxa, AD	ARD, ascending aortic aneurysm and dissection, BAV, IA, SVAS[43,44,45]	AD	123700 185500
FBLN5	Fibulin 5	Yes[54]	+	-	Cutis laxa, AD Cutis laxa, AR Type I	AAT, SVAS	AD, AR	604580
FBN1	Fibrillin 1	Yes[55,56,57,58,59]	+	+	Marfan syndrome	ARD, TAAD, AAA, arterial aneurysms, pulmonary artery dilatation, tortuosity[46]	AD	154700
FBN2	Fibrillin 2	No	+	-	Contractural arachnodactyly	Rare ARD and aortic dissection, BAV, PDA[47]	AD	121050
FKBP14	FKBP prolyl isomerase 14	No	+	-	EDS VIA[50] Ullrich congenital muscular dystrophy[51]	AAT, aortic rupture, ARD	AR	614505
FLNA	Filamin A	Yes[60,61]	+	-	Periventricular nodular heterotopia and otopalatodigital syndrome	Aortic dilatation/aneurysms, peripheral arterial dilatation, PDA, BAV, IA[48,49]	XLD	300049
IPO8	Importin 8	Yes[62]	+	-	LDS, Shprintzen-Goldberg syndrome	AAT, ARD	AR	605600
LOX	Lysyl oxidase	Yes[63,64,65,66]	-	+	AAT	TAAD, AAA, hepatic artery aneurysm, BAV, CAD	AD	617168

Gene	Protein	Animal model?	Syndromic TAAD	Non-syndromic TAAD	Associated disease or syndrome	Associated vascular characteristics	Mode of inheritance	OMIM
MFAP5	Microfibril-associated glycoprotein 2	Partially[71]	-	+	AAT	ARD, TAAD	AD	616166
MYH11	Smooth muscle myosin heavy chain	Partially[72]	-	+	AAT	TAAD, early aortic dissection, PDA, CAD, peripheral vascular occlusive disease, carotid IA	AD	132900
MYLK	Myosin light chain kinase	No[73]	-	+	AAT	TAAD, early aortic dissections	AD	613780
NOTCH1	NOTCH1	Partially	-	+	AOVD	BAV, TAAD[67,68]	AD	109730
PLOD1	Lysyl hydroxylase 1	No	+	-	EDS VIA Nevo syndrome	AAT, aortic rupture, ARD	AR	153454
PRKG1	Type I cGMP-dependent protein kinase	No	-	+	AAT	TAAD, early aortic dissection, AAA, coronary artery aneurysm, dissection, aortic tortuosity, small	AD	615436
SKI	Sloan Kettering proto-oncoprotein	No	+	-	Shprintzen-Goldberg syndrome	ARD, arterial tortuosity, pulmonary artery dilation, splenic arterial aneurysms[69]	AD	182212
SLC2A10	Glucose transporter 10	No	+	-	Arterial tortuosity syndrome	ARD, ascending aortic aneurysm, other arterial aneurysms, arterial tortuosity, elongated arteries, aortic/pulmonary artery stenosis[70]	AR	208050

Gene	Protein	Animal model?	Syndromic TAAD	Non-syndromic TAAD	Associated disease or syndrome	Associated vascular characteristics	Mode of inheritance	OMIM
SMAD2	SMAD2	No	+	-	Unidentified CTD with arterial aneurysm and dissection	ARD, ascending aortic aneurysm, vertebral/carotid aneurysms and dissections, AAA[74]	AD	601366
SMAD3	SMAD3	Partially[82]	+	+	LDS Type III	ARD, TAAD, early aortic dissection, AAA, arterial tortuosity, other arterial aneurysms and dissections, IA, BAV[75,76]	AD	613795
SMAD4	SMAD4	Yes[83]	+	-	JP/HHT syndrome	ARD, TAAD, AVM, IA[77,78]	AD	175050
SMAD6	SMAD6	No	-	+	AOVD	BAV/TAA	AD	602931
TGFB2	TGF-beta 2	Yes[84]	+	+	LDS Type IV	ARD, TAAD,, arterial tortuosity, other arterial aneurysms, BAV[79]	AD	614816
TGFB3	TGF-beta 3	No	+	-	LDS Type V	ARD, TAAD, AAA/dissection, other arterial aneurysms, IA and dissection[80]	AD	615582
TGFBR1	TGF-beta receptor type 1	Yes[85]	+	+	LDS Type I + AAT	TAAD, early aortic dissection, AAA, arterial tortuosity, other arterial aneurysms/dissection, IA, PDA, BAV[81]	AD	609192
TGFBR2	TGF-beta receptor type 2	Yes[85]	+	+	LDS Type II + AAT	TAAD, early aortic dissection, AAA, arterial tortuosity, other arterial aneurysms/dissection, IA, PDA, BAV[81]	AD	610168

Table 1. All genes strongly associated with TAAD in the gene panel by PanelApp. Text in black represents the previous version of the Aortic Institute's list. Genes that were not on the 2019 version of the Aortic Institute's list are denoted in blue, and ClinGen genes are denoted in purple.

Table abbreviations: AAA, abdominal aortic aneurysm; AAT, aortic aneurysm, familial thoracic; AD, autosomal dominant; AOVD, aortic valve disease; AR, autosomal recessive; ARD, aortic root dilatation; AVM, arteriovenous malformation; BAV, bicuspid aortic valve; CAD, coronary artery disease; CTD, connective tissue disease; CVD, cerebrovascular disease; EDS, Ehlers-Danlos syndrome; FTAA, familial thoracic aortic aneurysm; FTAAD, familial thoracic aortic aneurysm and/or dissection; HHT, hereditary hemorrhagic telangiectasia; IA, intracranial aneurysm; JP, juvenile polyposis; LDS, Loeys-Dietz syndrome; MYMY, moyamoya disease; OMIM, Online Mendelian Inheritance in Man; PDA, patent ductus arteriosus; SVAS, supravalvular aortic stenosis; TGF, transforming growth factor; TAAD, thoracic aortic aneurysm and/or dissection; TGFBR, TGF- receptor; XLD, X-linked dominant.

Traboulsi syndrome is known to be caused by mutations in ASPH and inherited in an autosomal recessive fashion. The ASPH protein plays an important role in the extracellular matrix composition of the aorta; it is responsible for the hydroxylation of asparagine and aspartate residues in proteins containing an epidermal growth factor domain, including FBN1. Consequently, the syndrome presents with some overlapping features when compared to Marfan syndrome, including ocular findings like lens dislocation, and some distinct signs such as a flattened malar region of the face. A study in the European Journal of Medical Genetics, published in 2022, presented seven patients from six different families with the syndrome, and five of the patients had aortic root dilation with early-onset dilation in some.[38]

Cystathionine beta-synthase deficiency, resulting from missense mutations in the CBS gene, causes homocystinuria. The defective version of the autosomal recessive gene gives rise to a host of features, such as optic lens dislocation and developmental delay.[39] Gaustadnes et al found that in a cohort of 36 Australian patients with CBS deficiency, one

exhibited aortic root dilation. A study by Narayanan et al proposed a mechanism for the observation of aortic aneurysm in patients with hyperhomocysteinemia, and hypothesized its involvement in the methylation of genes involved in the extracellular matrix of the aorta could be the culprit.[42]

The FBLN5 gene codes for the protein fibulin-5, and an autosomal-recessive homozygous missense mutation is responsible for human AR cutis laxa type I. Patients with cutis laxa are typically observed to have connective tissue anomalies such as loose skin and various systemic symptoms. A study by Loeys et al demonstrated cutis laxa with ascending aortic aneurysm and supravalvular aortic stenosis in a large, cosanguinous Turkish family, illustrating the role of FBLN5 in typical elastogenesis.[86]

Many genetic variants are now known to result in Ehlers-Danlos syndrome, characterized by features such as joint hypermobility and hyperelastic skin. A homozygous frameshift mutation in FKBP14 has been demonstrated to lead to the kyphoscoliotic form of EDS (EDS VIA), caused by a disturbance in protein folding in the endoplasmic reticulum and subsequent extracellular matrix disruptions. A linkage analysis study performed by Baumann et al in a large family found two affected individuals, exhibiting aortic root dilation, ascending aortic aneurysm, and aortic rupture.[51]

Another gene, IPO8, exhibits its effect in the TGF-beta signaling pathways that help to compose the aorta and other structures. In a study published in 2021, bi-allelic loss-of-function mutations in 12 patients resulted in a syndrome resembling Loeys-Dietz Syndrome (LDS) and Shprintzen-Goldberg syndrome, and the authors created a zebrafish model to demonstrate the role in IPO8 of TGF-beta dependent SMAD trafficking to the

nucleus. The initial patients exhibited joint hyperlaxity, dysmorphic features, and cardiovascular features including aortic root dilation and early-onset TAA.[62]

The final new gene on the list with a strong association to TAA is PLOD1, which codes for lysyl hydroxylase. Giunta et al found that patients with an autosomal recessive variant in PLOD1 and subsequent lysyl hydroxylase deficiency had developed what they referred to as Nevo syndrome, with similar clinical features to EDS VIA. Both homozygous nonsense mutations and deletion mutations caused the clinical syndrome. Like in EDS VIA, patients exhibited kyphosis, joint laxity, and hypotonia of the musculature, and also demonstrated increased length at birth. From a cardiovascular standpoint, the patients in the cohort demonstrated aortic root dilation and ascending aortic aneurysm with potential for aortic rupture, similar again to EDS VIA.

Amber and red genes

In addition to the 33 genes that made the strong association list on PanelApp, there are an additional 35 that were classified as either "Amber" or "Red" depending on their level of evidence. Upon review of these genes and their associated evidence, it seems that many of the clinical features caused by variants in the Amber/Red genes may resemble phenotypic connective tissue disorders such as EDS and EDS, as well as related conditions like Ribbing disease and Stickler syndrome. It is possible that with more evidence, variations in these genes will turn out to be disease-causing, but they do not currently meet that standard.

	Existing evidence
ARIH1	Fly model demonstrates ARIH1 mutation implication in aortic aneurysms, with one case report of a child harboring aortic and other aneurysms[87,88]
FOXE3	One study showed FOXE3 mutations in patients with TAAD who were part of a large family, accompanying mouse model which showed impaired smooth muscle density[89]
LTBP3	Two studies showing LTBP3 mutation in patients with syndromic thoracic aortic disease[90]
PMEPA1	Eight families in one study described to have PMEPA1 mutations and aortic aneurysm as well as syndromic skeletal deformities[91]
THSD4	Mouse model with THSD4 variants resulted in TAAD pathogenesis, aortic sample from a patient with THSD4 variant showed aortic degeneration[92]
ABCC6	Homozygosity mapping in families with ABCC6 deficiency resulting in pseudoxanthoma elasticum demonstrated aortic elastic fiber fragmentation, but no aortopathy observed
ACVR1	In a mouse model, ACVR1 knockout resulted in congenital defects including double outlet right ventricle and common arterial trunk. No evidence of aortopathy[93]
ADAMTS2	Mutations in ADAMTS2 are responsible for EDS Type VIIC, which has no demonstrated cardiac phenotype[94]
ALDH18A1	Autosomal-recessive cutis laxa type 3, caused by mutations in ALDH18A1, shares a similar phenotype to other types of cutis laxa but without evidence of aortopathy[95]
ATP6V0A2	Mutations in ATP6V0A2 result in autosomal recessive cutis laxa type 2, no evidence of aortopathy[96]
ATP7A	In the "blotchy" mouse, created by a mutation in ATP7A, the rodents routinely develop ascending aortic aneurysms; in humans an ATP7A mutation causes Menkes disease and occipital horn syndrome, but despite evidence of congenital heart disease there has been no recorded aortopathy[97,98]
B4GALT7	Mutation causes EDS, specifically progeroid-like phenotype, but no evidence of aortopathy[99]
CHST14	Mutation causes a musculocontractual type of EDS (MC-EDS), causes valve abnormalities and congenital heart disease but no aortopathy
COL11A1	Associated with Stickler syndrome and Marshall syndrome but no aortopathy[100,101]
COL11A2	Mutations in COL11A2 have been linked to Kawasaki disease and development of coronary artery lesions, also implicated in fibrochondrogenesis 2, otospondylomegaepiphyseal dysplasia but no evidence of aortopathy[102,103]
COL1A1	Gene is implicated in both EDS arthrochalasia type 1 and osteogenesis imperfecta, no aortopathy[104,105]

COL1A2	COL1A2 mutations have been described in a cardiac valvular form of EDS, osteogenesis imperfecta, etc but no aortopathy[106]
COL2A1	Mutations in gene cause Stickler syndrome, but minimal aortic involvement observed[107]
COL4A1	Mutation associated with arterial abnormalities and aneurysms elsewhere in the body, but not thoracic aortic pathology[108]
COL9A1	Mutations associated with multiple epiphysesal dysplasia and Stickler syndrome, type IV, but no aortopathy compoment[29]
COL9A2	Mutation causes Stickler syndrome without aortopathy[109]
COL9A3	Mutation causes Ribbing disease/hereditary multiple diaphyseal sclerosis, one study reported 15 patients in three families with the condition and 3 developed TAA or AAA[110]
EMILIN1	Mutations in EMILIN1 responsible for connective tissue disease but no evidence of aortopathy[111]
FLCN	Mutation in FLCN responsible for Birt-Hogg-Dubé syndrome; one patient with BHD developed aortic dissection at age 76 but relationship to BHD is unknown[112,113]
HEY2	A family analysis revealed that HEY2 mutations lead to congenital heart disease and TAA, but effect limited by variable expressivity as well as incomplete penetrance[114]
HNRNPK	Au-Kline syndrome (connective tissue disease) caused by mutation in HNRNPK, one case series includes patients with aortic root dilation[115]
KCNN1	Nothing for KCNN1. KCNN2 identified as potentially important in development of coronary artery aneurysms in Kawasaki disease, no TAA[116]
LTBP2	Weill-Marchesani syndrome 3 caused by LTBP2 mutation, no aortopathy observed despite marfanoid features[117]
MAT2A	One study showed large family affected by MAT2A mutations with thoracic aortopathy, zebrafish model demonstrated effect of MAT2A knockout on cardiovascular development[118]
MED12	Mutations in MED12 lead to Lujan syndrome, study with mouse model and human tissues showed impact of MED12[119,120]
MYLK2	MYLK is associated with aortopathy, nothing about MYLK2 in the literature
PKD1	PKD1 mutations cause polycystic kidney disease -- one mouse model showed mechanism for PKD1 in the structural integrity of vessels. Aortopathy not observed in patients[121]
PKD2	PKD2 mutations observed to cause cardiac defects in mice, no aortopathy observed in humans[122]
SLC39A13	An autosomal-recessive form of EDS (spondylocheiro dysplastic) is caused by SLC39A13 mutation, but no aortopathy observed[123]

TNXB	Mutation causes EDS, tenascin-X deficient type with no associated aortopathy[124]
ZNF469	ZNF469 mutation causes brittle cornea syndrome, patients exhibit some marfanoid features, but no aortopathy noted[125]

Table 2. Amber and red genes from Genomics England PanelApp with associated evidence of TAA causation.

Uncertain categorization

There are a small number of genes from the Aortic Institute's 2019 paper that were not listed as part of Genomics England's TAA gene panel. These genes include HCN4, LTBP1, ROBO4, TIMP1, and TIMP3. All five genes were included on the list following retrospective studies of symptomatic or syndromic patients, with evidence demonstrating the effect of gene variations using gene-based burden tests, tissue models, or animal models.[126,127,128,129] In addition, five genes from the Aortic Institute's report were part of Genomics England's panel, but assigned them medium ("Amber") or weak ("Red") associations with TAA. These genes include ARIH1, COL1A2, FOXE3, LTPB3, and MAT2A. All of these genes have been associated with aortopathy in the literature, and all except COL1A2 have observed aortic pathology in affected patient cohorts (for COL1A2, the aortic root dilation was borderline in both patients with the mutations). The systems for selecting the genes to include in Genomics England's PanelApp differed from the method of inclusion of disease-causing genes in the Aortic Institute report, so the incongruency remains, but the evidence for considering these 10 genes to have disease-causing potential is reasonable.

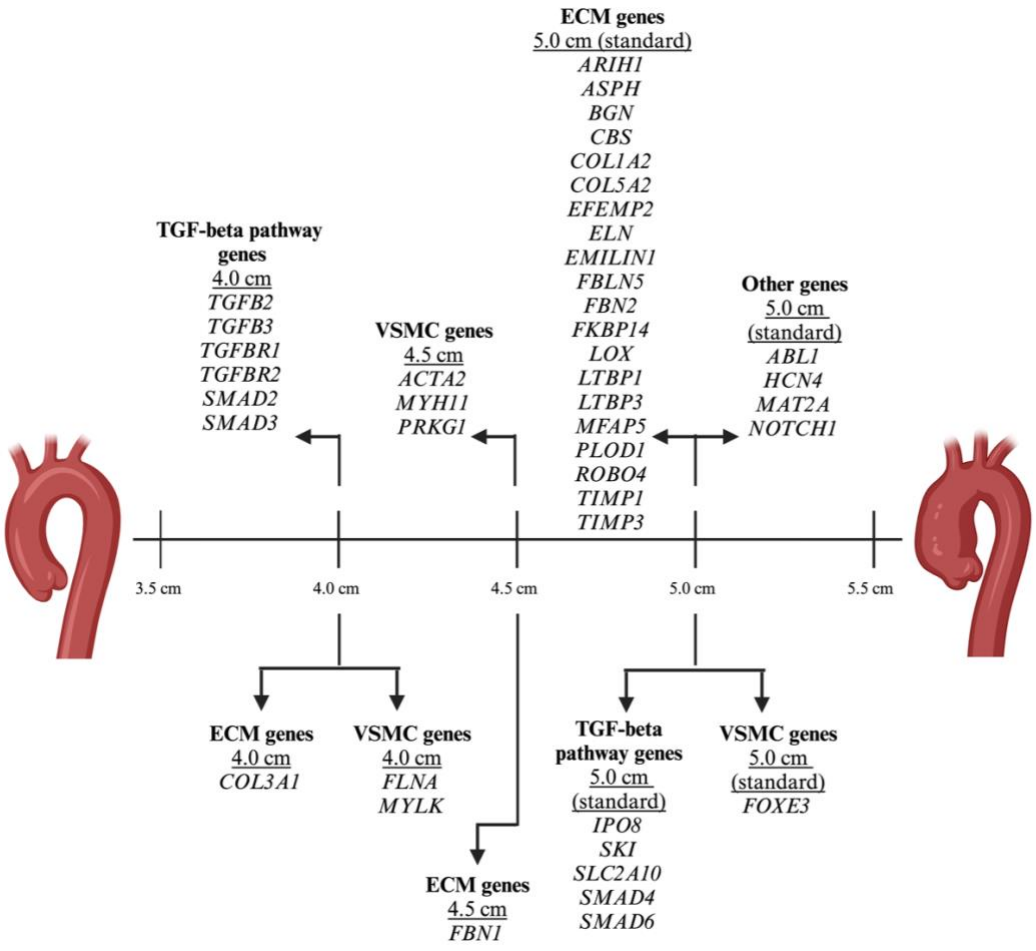

Figure 1. Size criteria for surgical intervention in TAA for all genes with a strong association in Genomics England's comprehensive gene panel and those included in the Aortic Institute's 2019 diagram. Abbreviations: ECM: extracellular smooth muscle, TGF: transforming growth factor beta, VSMC: vascular smooth muscle cells

Size criteria update

Another important component of the Aortic Institute's 2019 paper was the size diagram, which demonstrated the recommended point of intervention for TAA depending on its genetic association. The 2022 ACC/AHA guidelines recommend surgical intervention for all TAA at 5.0 cm, familial or not. A query of the literature in the MEDLINE database for updated guidelines or case reports with suggested updates to the

intervention criteria was initiated, and new data revealed potential earlier intervention could be beneficial for patients with several genetic variants.

The first group of genes with updated intervention criteria are those involved in Loeys-Dietz syndrome. In 2021, Velcvhev et al (including coauthors Harry Dietz and Bart Loeys) authored a book chapter named "Loeys-Dietz Syndrome" in Progress in Heritable Soft Connective Tissue Diseases. They named TGFBR1, TGFBR2, SMAD2, SMAD3, TGFB2 and TGFB3 as the disease-causing genes in LDS. In the section on surgical treatment, the authors recommend considering surgical intervention once the maximal diameter of the aorta passes 4.0 cm if a patient possesses a disease-causing variant in any of the above genes. They issue this recommendation because they acknowledge the multiple examples of patients experiencing thoracic aortic dissection at diameters below 4.5 cm while touting the safety of modern prophylactic repair.[130]

A study by Chen et al has suggested a lower threshold for surgical intervention in TAA associated with COL3A1 variants. In their cohort of 223 patients with TAA, the average ascending aortic dimension at the time of type A dissection at 4.0 cm. Previously, intervention was suggested at 4.5 cm, but similar to those with LDS, a substantial group of patients may be missed by that later intervention point.[131]

FLNA-associated TAA may also necessitate prophylactic surgical repair earlier than previously thought. In a case series of 114 patients with loss-of-function variants in the FLNA gene, 21 were found to have TAA. Two of those patients died of aortic rupture, one of them at a known aortic root diameter of 4.2 cm. Another patient had prophylactic aortic root replacement at a diameter of 4.2. The authors note that the characteristics of

their cohort might approximate patients with LDS, and in those cases surgical repair is now recommended once aortic diameter passes 4.0 cm.[132]

Finally, patients with MYLK mutations associated with TAA may also benefit from a lower threshold for prophylactic surgical repair. In 2019, Wallace et al published a case series of 33 patients with a variety of pathogenic variants in MYLK. They found that 20 patients (61%) in their cohort experienced aortic dissection. For the three patients with aortic root measurements, the median aortic root diameter at the time of type A dissection was 3.3 cm, and for the five patients with ascending aortic measurements, the median ascending aortic diameter at type A dissection was 4.25. With minimal to no aortic enlargement at the time of dissection, the authors suggest it may be prudent to consider surgical intervention at a lower threshold while taking into account other factors, such as age.[133]

Discussion

This review of the literature describing genes involved in thoracic aortic aneurysm has demonstrated the evolving understanding of this area of research and represents novel opportunities to screen patients for thoracic aortic aneurysm.

First, several new genetic variants have been identified in the literature that hold a strong association with TAA. Mutations in ABL1, ASPH, CBS, FBLN5, FKBP14, IPO8, and PLOD1 have been studied in patient cohorts and animal models; with this knowledge, genetic screening panels for TAA should be updated to include even more genes with definitive disease-causing components. In patients with any family history of TAA, aortic dissection, or sudden death, an increasing amount of genes to test for may help in the surveillance and prevention of life-threatening complications of TAA.

The literature review also revealed the sizeable number of genetic variants whose disease-causing influence in TAA pathogenesis that is currently unknown or not fully proven. Of the 68 genes currently part of the PanelApp by Genomics England, 35 had only earned “Amber” or “Red” designations.

Suggested size criteria for surgical intervention generally underwent a “left shift”, with expert recommendation and several analyses of thoracic aortic diameter at the time of dissection both contributing to a lower threshold of suggested intervention diameter. These changing best practices correspond with the overall thrust of the guidelines from the ACC/AHA to lower the general threshold for intervention in TAA generally (Left-Shift from 5.5 to 5.0 cm). Overall, the changes are symbolic of a recognition that surgical repair is an exceedingly safe option when compared to the ticking time bomb of TAA,

especially in patients with genetic variations that rebuke the traditional wisdom of TAA leading to dissection only at very large aortic diameters.

In addition, the importance of examining multiple sources to assess the genetic variants implicated in TAA formation cannot be overstated. In this review, we noted some discrepancies in both the list of gene variants included and the strength of the association with TAA between the PanelApp and the Aortic Institute's previous gene update. Although plenty of progress has been made in the identification of genes involved in TAA, there are likely many more genetic variants to be discovered. For example, in a concerted 5-year search for new TAA genes (via complex statistical search for "enrichment" causative genes in our TAA patients vs. controls). Inclusivity in generating these lists of genes will allow researchers to periodically revisit the evidence associated with each one and determine if subsequent research then warrants a change in association classification. We will continue the regular restructuring of our list of genes and the size "timelime" for intervention every 3 years, with interval publication in the journal *AORTA*. The process of searching for new genes is a difficult one, but the efforts of genome-wide association studies should persevere in the face of adversity to identify more genes implicated with TAA. The Aortic Institute has embarked on this work with the dissertation efforts of Dr. Bulat Ziganshin at Columbia University; simultaneously, the Institute has engaged in efforts to leverage the substantial amount of genetic data of their patients to determine the association of mutations in the CCN2 gene with TAA.[134] The Aortic Institute has additionally identified three other genes that cause thoracic aortic disease using whole-exome sequencing of 1429 patients, and the supporting data will be presented at an upcoming national meeting.[135] In a broader sense, repositories of genetic

data such as those possessed by the Aortic Institute or other multidisciplinary aortic teams should be leveraged to interrogate novel variants as well as those with weaker associations to TAA.

Finally, there has been an increasing consensus in the literature that size criteria have been an incredibly helpful tool in guiding intervention up to this point, but going forward surgical repair should be based on a variety of factors. Several studies, including one by Elefteriades et al, have discussed the importance of considering other methods for evaluating a patient with TAA's surgical candidacy.[24] A recurring theme in this review of an additional criterion to consider was the age of the patient, as it might may help anticipate the natural history of TAA in certain genetic variants such as MYLK, SMAD3, and more. Other criteria, such as sex, comorbidities, and family history, should be considered in nuanced discussions with patients in the context of multidisciplinary teams to afford patients with TAA all of the information they need to protect their health.

Challenges and Limitations

This study was limited by the paucity of existing studies on the genetic variants that contribute to the formation of thoracic aortic disease. There was very little literature available to appraise the influence of a genetic mutation on TAA formation, and the studies that do exist were largely confined to small sample sizes and reports of only a handful of families. Performing this review highlighted the utility of pooled databases for genetic research to create studies with larger sample sizes and greater statistical power.

From a process standpoint, this thesis was originally supposed to include an original investigation evaluating the influence of a variant in the CCN2 gene in TAA formation. The intention was to study the 1800 samples of genetic data from the Aortic Institute, identify the presence of CCN2 mutations in the cohort, and analyze the association of these variations with thoracic aortic disease. Due to delays in securing a sequencing facility and accessing the samples, the analysis could not be completed in time to include in the thesis. However, the Yale Center for Genome Analysis is now processing the samples, and the data is expected to be collected and analyzed within the next six weeks. This represents an exciting new frontier in the discovery of genes associated with thoracic aortic disease.

Conclusion

Despite advances in treatment and screening, thoracic aortic aneurysm and dissection remain deadly diseases. One tool that can be employed to reduce the burden of morbidity and mortality associated with thoracic aortic disease is genetic screening, as the scientific community has increasingly recognized the genetic underpinnings of thoracic aortic disease and consensus exists that the search for genetic variants is in a nascent stage. This study revealed several new variants that harbor strong associations with thoracic aortic aneurysm and dissection, and brought to light updated size criteria for intervention for several genetic variants. Future research should focus on continuing to identify genetic contributors to TAA, gathering more evidence to interrogate the genes with medium or weak associations to TAA, and identifying new criteria to determine the appropriate point for surgical intervention in patients with TAA.

Dissemination

The research presented in this thesis will be distributed as scholarly academic work. An update to the Aortic Institute's "Genes Involved in Thoracic Aortic Aneurysm and Dissection" 2019 paper that is suitable for journal publication will be prepared to submit to the journal AORTA.

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