



Published in final edited form as:

Circ Genom Precis Med. 2019 June ; 12(6): e002476. doi:10.1161/CIRCGEN.118.002476.

Clinical Implications of Identifying Pathogenic Variants in Individuals with Thoracic Aortic Dissection

Brooke N. Wolford, BS^{1,2,*}, Whitney E. Hornsby, PhD^{1,3,*}, Dongchuan Guo, PhD⁴, Wei Zhou, PhD^{5,6,7}, Maoxuan Lin, MS⁸, Linda Farhat, MS^{1,9}, Jennifer McNamara, MS^{1,3}, Anisa Driscoll, BS^{1,10}, Xiaoting Wu, PhD^{1,9}, Ellen M. Schmidt, PhD¹¹, Elizabeth L. Norton, MS¹², Michael R. Mathis, MD^{1,13}, Santhi K. Ganesh^{1,3,14}, Nicholas J. Douville, MD, PhD^{1,13}, Chad M. Brummett, MD^{1,13}, Jacob Kitzman, PhD^{1,4}, Y. Eugene Chen, MD, PhD^{1,3,15,16}, Karen Kim, MD^{1,9}, G. Michael Deeb, MD^{1,9}, Himanshu Patel, MD^{1,9}, Kim A. Eagle, MD^{1,3}, Dianna M. Milewicz, MD⁴, Cristen J. Willer, PhD^{1,2,3,14}, and Bo Yang, MD, PhD^{1,9}

¹Univ of Michigan, Michigan Medicine, Univ of Michigan, Ann Arbor, MI

²Dept of Computational Medicine & Bioinformatics, Univ of Michigan, Ann Arbor, MI

³Dept of Internal Medicine, Univ of Michigan, Ann Arbor, MI

⁴Dept of Internal Medicine, McGovern Medical School, Univ of Texas Health Science Center at Houston (UTHealth), Houston, TX

⁵Analytic & Translational Genetics Unit, Massachusetts General Hospital & Harvard Medical School, Boston

⁶Stanley Center for Psychiatric Research, Broad Institute of MIT & Harvard, Cambridge, MA

⁷Program in Medical & Population Genetics, Broad Institute of MIT & Harvard, Cambridge, MA

⁸Dept of Bioinformatics & Genomics, The Univ of North Carolina at Charlotte, Charlotte, NC

⁹Dept of Cardiac Surgery, Univ of Michigan, Ann Arbor, MI

¹⁰Office of Research - Precision Health, Ann Arbor, MI

¹¹Open Targets, Wellcome Sanger Institute, Cambridge, UK

¹²Creighton Univ School of Medicine, Omaha, NE

¹³Dept of Anesthesiology, Univ of Michigan, Ann Arbor, MI

¹⁴Dept of Human Genetics, Univ of Michigan, Ann Arbor, MI

¹⁵Dept of Pharmacology, Univ of Michigan, Ann Arbor, MI

¹⁶Dept of Molecular & Integrative Physiology, Univ of Michigan, Ann Arbor, MI

Correspondence: Cristen J. Willer, PhD, 5804 Medical Science II, 1241 E. Catherine St., Ann Arbor, MI, 48109-5618, Tel: 734.647.6018, Fax: 734.764.2255, cristen@umich.edu. Bo Yang, MD, PhD, 1500 East Medical Center Drive, 5155 Frankel Cardiovascular Center, Ann Arbor, MI, 48109, Tel: 734-647-9417, Fax: 734-764-2255, boya@med.umich.edu.

*contributed equally as co-first authors

Disclosures: Himanshu J. Patel (hjpatel@med.umich.edu) is a consultant for WL gore Edwards and Medtronic, and these efforts are "Modest". Cristen J Willer's spouse works at Regeneron Pharmaceuticals. Other Disclosures: none

Abstract

Background: Thoracic aortic dissection is an emergent life-threatening condition. Routine screening for genetic variants causing thoracic aortic dissection is not currently performed for patients or family members.

Methods: We performed whole exome sequencing of 240 patients with thoracic aortic dissection (n=235) or rupture (n=5) and 258 controls matched for age, sex, and ancestry. Blinded to case-control status, we annotated variants in 11 genes for pathogenicity.

Results: Twenty-four pathogenic variants in 6 genes (*COL3A1*, *FBN1*, *LOX*, *PRKG1*, *SMAD3*, *TGFBR2*) were identified in 26 individuals, representing 10.8% of aortic cases and 0% of controls. Among dissection cases, we compared those with pathogenic variants to those without and found that pathogenic variant carriers had significantly earlier onset of dissection (41 vs. 57 years), higher rates of root aneurysm (54% vs. 30%), less hypertension (15% vs. 57%), lower rates of smoking (19% vs. 45%), and greater incidence of aortic disease in family members. Multivariable logistic regression showed that pathogenic variant carrier status was significantly associated with age <50 [odds ratio (OR) = 5.5; 95% CI: 1.6-19.7], no history of hypertension (OR=5.6; 95% CI: 1.4-22.3) and family history of aortic disease (mother: OR=5.7; 95% CI: 1.4-22.3, siblings: OR=5.1; 95% CI 1.1-23.9, children: OR=6.0; 95% CI: 1.4-26.7).

Conclusions: Clinical genetic testing of known hereditary thoracic aortic dissection genes should be considered in patients with a thoracic aortic dissection, followed by cascade screening of family members, especially in patients with age-of-onset <50 years, family history of thoracic aortic disease, and no history of hypertension.

Journal Subject Terms:

Aortic Dissection; Genetics

Keywords

aortic dissection; rupture; genetics; diagnostics; pathogenic variants; gene sequencing

Introduction

Thoracic aortic dissection is a life-threatening condition, responsible for 15,000 deaths a year in the United States.^{1,2} Approximately 30% of patients presenting with a thoracic aortic aneurysm and dissection have an underlying genetic predisposition,³ which can be associated with syndromic features, such as Marfan syndrome or Loeys-Dietz syndrome, or not associated with syndromic features, as with *ACTA2*, *MYLK*, and *MYH11* mutations.⁴ Variants in many genes, including *FBN1*, *SMAD3*, and *ACTA2*, among others, can lead to either syndromic or non-syndromic thoracic aortic aneurysm and dissection.⁴⁻⁶ Recent advances in the field have shown definitive and strong evidence to support the role of pathogenic variants in *ACTA2*, *COL3A1*, *FBN1*, *MYH11*, *SMAD3*, *TGFBR2*, *TGFBR1*, *TGFBR2*, *MYLK*, *LOX*, and *PRKG1* as predisposing to hereditary thoracic aortic disease.⁷

These genetic findings play a critical role for the patient and family members, helping to guide clinical decision-making to prevent or lessen the likelihood of a catastrophic event. Aortic diameter is a central criterion when deciding prophylactic surgical intervention, and the recommended aortic diameter for surgical intervention differs for those with and without an underlying genetic predisposition. The American Heart Association/American College of Cardiology (AHA/ACC) guidelines⁸ recommend that patients with genetically mediated aneurysms undergo elective surgical repair at an ascending or aortic root diameter of 4.0–5.0 cm, depending on the condition. Whereas patients without a known genetic mutation may undergo elective surgical repair when the ascending or aortic root diameter is ≥ 5.5 cm, there are also established risk factors, such as an aortic diameter growth rate between > 3 to 5mm/year^{8,9} that may drive early surgical intervention. Recent work shows that different genes predisposing to hereditary thoracic aortic dissection have varying presentations and courses.^{10,11} For instance, patients with *ACTA2* mutations more often present with acute aortic dissections whereas patients with Marfan syndrome often present with skeletal and ocular features before thoracic aortic dilation is discovered.¹²

Despite the potential clinical impact of genetic findings, clinicians are usually not aware that a patient has an underlying pathogenic variant upon initial presentation with a thoracic aortic dissection. The identification of variants known to predispose to thoracic aortic dissection has the potential to improve clinical management and guide treatment strategies for patients and family members. The objective of this study was to evaluate trends in pathogenic variants carriers with a history of thoracic aortic dissection or thoracic aortic rupture, as well as to identify which patients and corresponding family members may benefit from clinic genetic testing. We examined all genes in the genome and none reached exome-wide significance for single variant tests or gene-based burden tests.

Methods

The full methods for this manuscript are available as supplemental material. In accordancy with the Transparency and Openness Promotion (TOP) guidelines the data that support the findings of this study are available from the corresponding author (CJW) upon reasonable request and approval from the institutional internal review board. The study was approved by the institutional review board at the University of Michigan and all subjects provided informed consent.

Results

Annotation of variants from research-level whole exome sequencing identifies pathogenic variants

A total of 240 cases with a clinical diagnosis of thoracic aortic dissection (type A or type B) or rupture with or without aortic aneurysm and 258 age-, sex-, and ancestry-matched controls had whole exome sequences available following quality control (see Methods for quality control failures). For the 498 samples passing quality control, 248 variants were annotated blind to the variant carrier's case or control status. 24 pathogenic variants in 6 genes (*COL3A1*, *FBN1*, *LOX*, *PRKG1*, *SMAD3*, *TGFBR2*) were identified, found exclusively in 26 cases (Table 1), representing 10.8% of cases and 0% of controls. Two

variants were seen twice in cases who were first degree relatives. There is a significant burden of pathogenic variants in *FBNI* in cases compared to controls ($N_{\text{cases}}=18$, $N_{\text{controls}}=0$; $p\text{-value}=2.5\times 10^{-5}$, Supplementary Table 1). These variants are predominantly found in calcium-binding epidermal growth factor domains of *FBNI* (Figure 1). We examined the proportion of pathogenic variants that were present in commonly used databases and found that of the 24, 11 were present in dbSNP¹³, 8 were listed as pathogenic in ClinVar¹⁴, and 2 were present in gnomAD¹⁵ (see Supplementary Note).

Validation targeted sequencing

Molecular Inversion Probe Sequencing was utilized to ensure the highest level of confidence in the whole exome sequencing variant calls and to protect against potential sample swaps. The carrier status of pathogenic variants identified through whole exome sequencing was verified by Molecular Inversion Probe Sequencing in all 26 samples (Supplementary Table 2).

Research-level whole exome sequencing and implications for precision health

For 17 of the 26 pathogenic variant carriers (hereon pathogenic carriers), the whole exome sequencing results aligned with the current clinical diagnoses in the electronic medical record, including 5 patients (5/17) in which clinical genetic testing previously identified the same pathogenic variant as whole exome sequencing (Table 2 and Supplementary Table 3). Whole exome sequencing results provided validation for 12 pathogenic carriers with a clinical diagnosis of Marfan syndrome based on the Revised Ghent Nosology.¹⁶ There were no genetic testing results for the above 12 patients other than the whole exome sequencing results from this study. For the 9 remaining pathogenic carriers, whole exome sequencing and annotation of pathogenic variants added diagnostic precision to the clinical diagnosis (Table 2). Specifically, 8 of these pathogenic carriers (8/9) lacked a specific clinical diagnosis, but whole exome sequencing and history of thoracic aortic dissection shifted the clinical diagnosis per guidelines to Marfan syndrome¹⁶ (*FBNI*, $n=4$), vascular Ehlers-Danlos syndrome⁸ (*COL3A1*, $n=1$), or familial thoracic aortic disease (*LOX*, *PRKGI*, and *SMAD3*, $n=3$). For 1 pathogenic carrier (1/9) there was an incorrect diagnosis of Marfan syndrome, which was amended to Loeys-Dietz syndrome based on a pathogenic variant identified in *TGFBR2* and history of an acute Type A aortic dissection. In addition, the whole exome sequencing results provide a basis for cascade screening for the family members of all 26 cases per AHA guidelines.⁸

Variants of unknown significance

86 of the 248 annotated variants in aortopathy genes were annotated as VUS. After excluding one of each first degree relative pair (see Methods) and cases with pathogenic variants, 58 of 213 cases (27.2%) and 51 of 258 controls (19.8%) had at least one VUS identified from whole exome sequencing, which was not significant ($p\text{-value}=0.072$, Supplementary Table 4). There is, however, a significant association between pathogenic variants and cases ($p\text{-value}=2.8\times 10^{-7}$, Supplementary Table 4). None of the 11 genes demonstrated association between carrier status for VUS and thoracic aortic dissection or rupture case/control status (Supplementary Table 1).

Clinical characteristics between pathogenic variant and non-pathogenic variant carriers

The pathogenic carriers were significantly younger with a median of 41 years (age range 18–61 years) versus 57 years (age range 17–89 years) of age. 77% of pathogenic carriers were < 50 years old while 72% of non-pathogenic carriers were >50 years old. Pathogenic carriers also had significantly more root aneurysms (54% vs. 30%), less hypertension (15% vs. 57%), and less history of smoking (19% vs. 45%) compared to the non-pathogenic carriers. Moreover, the pathogenic carriers had a greater incidence of thoracic aortic disease in parents, siblings, and children (all p-values<0.05) (Table 3). Pathogenic carriers presented with more type A than type B dissections although this comparison was not significant (69.2% vs. 58.9%, p=0.421). One pathogenic carrier had a bicuspid aortic valve compared to 17 non-pathogenic variant carriers with bicuspid aortic valves. Multivariable logistic regression showed that pathogenic carriers were significantly more likely to have dissection age < 50 years old, family history of thoracic aortic disease, and no history of hypertension (Table 4).

Discussion

The current study reports our initial experience with research-level whole exome sequencing in patients with thoracic aortic dissection or rupture with or without aneurysm. We tested 240 cases and 258 controls for pathogenic variants in 11 genes known to cause aortic dissection.⁷ By whole exome sequencing and validation targeted sequencing, we found pathogenic variants in 10.8% of cases and 0% of controls. 58 (27.2%) cases and 51 (19.8%) controls were identified as carriers of variants of unknown significance.

In the general population, the incidence of pathogenic variants in our 11 genes of interest is very low (1×10^{-7} %, see Supplementary Note). Our diagnostic yield of 10.8% parallels the 9.3% in previous work, which identified pathogenic variants in the same 11 genes based on research-level whole exome sequencing of 355 patients with sporadic aortic dissection and early onset (< 56 years of age).¹⁷ In contrast, the yield of whole exome sequencing in 102 thoracic aortic aneurysm and dissection patients was much lower, with only 3.9% of cases carrying a pathogenic variant in one of 21 genes of interest.¹⁸ Similarly, Weerakkody et al.¹⁹ performed targeted genetic analysis of 15 genes in a mixed cohort of 967 familial and sporadic thoracic aortic aneurysm or dissection cases, and identified 49 pathogenic or likely pathogenic variants in 47 patients which represents a diagnostic yield of 4.9%. We report a two-fold increased proportion of pathogenic variant carriers (10.8%) in a cohort with a more severe phenotype consisting of only thoracic aortic dissection or rupture cases, suggesting the utility of pursuing a clinical genetic diagnosis in this patient group specifically. The 89% of dissection cases that do not have a pathogenic variant may be due to a pathogenic variant currently annotated as a VUS, a pathogenic variant in a gene not yet identified, a high polygenic risk of aortopathy, and/or environmental risk factors. Additional studies of dissection cases may help identify novel genes underlying risk in remaining cases. Notably, the incidence of BAV in non-pathogenic variant carriers (17/216) is higher than that of the general population similar to other studies²⁰, indicating that BAV is a risk factor for aortic dissection even in the absence of a known pathogenic variant.

The significant risk factors for a pathogenic variant in patients with thoracic aortic dissection or rupture were young age (<50 years old), no history of hypertension, but strong family history of thoracic aortic aneurysm, dissection or rupture (Table 4). This is in agreement with a recent study in familial and sporadic cases of aneurysm or dissection of the thoracic aorta which demonstrated a significantly increased probability of harboring a pathogenic or likely pathogenic variant in cases which were syndromic, young (age <50), or with a known or probable family history.¹⁹ Patients with pathogenic variants in *TGFBR1/2* (Loeys-Dietz syndrome), *FBNI* (Marfan syndrome), and *MYH11* have a higher risk of aortic dissection and suffer more complications from aortic dissection, including death. Therefore AHA/ACC guideline recommends early and aggressive prophylactic operation to resect the abnormal thoracic aorta in patients with pathogenic variants.⁸ Our results support the clinical importance of obtaining clinical genetic testing of known hereditary thoracic aortic dissection genes for thoracic aortic dissection and rupture patients, especially those with onset < 50 years old, no history of hypertension, and a positive family history of thoracic aortic disease.

It is important to clarify that other circumstances may exist that would warrant similar or different recommendations based on our findings. For instance, if a patient had a positive family history of thoracic aortic disease, clinical genetic testing for the patient and family members especially the offspring would be recommended despite the patient's age at the time of dissection (< or > 50 years of age). If a patient had a negative family history and was < 50 years of age, clinical genetic testing for the patient would be recommended, but cascade screening for family members would only be recommended if a pathogenic variant was identified in the patient. Beyond clinical genetic testing, screening with a CT angiogram (CTA) or MRI would be recommended to rule out thoracic aortic disease among the patient's family members. If a patient had a negative family history and was > 50 years of age, clinical genetic testing for the patient or family members would not be recommended, although screening with a CTA or MRI would be recommended to rule out thoracic aortic disease among the patient's family members. Routine surveillance should be performed for all patients surviving a thoracic aortic dissection. Less frequent surveillance utilizing a CTA or MRI is recommended for family members without thoracic aortic disease at initial CTA or MRI since family members may have a higher risk of thoracic aortic dissection compared to the normal population.

We did not find a difference in the percentage of VUS in 11 dissection genes among cases compared to controls ($p = 0.07$). In contrast, a previous study found a significantly increased burden of VUS in hereditary thoracic aortic dissection genes in dissection cases < 56 years of age compared with public controls ($p = 2 \times 10^{-8}$).¹⁷ However, several differences in the two studies may contribute to the varied results. Whereas the sample size of the previous study's control group was substantially higher, we analyzed cases and controls from the same batch and performed all quality control and variant annotation blinded to case or control status. Additionally, a focus on younger onset dissection cases may identify higher rates of VUS that may actually be pathogenic.²¹ Although the 2015 American College of Medical Genetics guidelines²² state that "a variant of uncertain significance should not be used in clinical decision-making," we found evidence that VUS from clinical genetic testing resulted in the introduction of syndromic labels and diagnoses into the electronic medical

record. Specifically, a VUS in *TGFBR2* was subsequently described as a “novel change likely causing Loeys-Dietz syndrome.” The statistically similar rate of VUS in cases and controls demonstrates the need for a greater understanding of the high frequency of VUS in controls (15% in Guo et al¹⁷ and 20% in this study) and careful interpretation of VUS in clinical practice.

To address the limitation that our sample processing and whole exome sequencing was not performed in a CLIA-certified laboratory, we verified pathogenic variants using MIPS. Furthermore, we performed expert-annotation of variant pathogenicity blinded to case or control status. This, coupled with the absence of pathogenic variants in controls, provides increased confidence in the results. These precautions lend additional evidence that the research-level whole exome sequencing results are of high enough quality to return findings to patients, which will trigger verification by clinical genetic testing performed in a CLIA-certified laboratory and cascade screening for the same pathogenic variant in family members. Electronic medical record review of the cases with a pathogenic variant suggested an average of 4 (3.88) first degree relatives per patient that would now be candidates for cascade screening. We are also limited by the 1) retrospective review, 2) possibility of incomplete electronic medical records, especially if a patient was seen at an outside institution, and 3) potential for limited family history knowledge.

In conclusion, this work provides evidence that whole exome sequencing and annotation can accurately identify pathogenic variants in established genes for hereditary thoracic aortic dissection in patients with a thoracic aortic dissection or rupture. Moreover, the results highlight meaningful implications for precision health by providing clinical guidance on how to manage both patients and family members. We recommend clinical genetic testing of hereditary thoracic aortic dissection genes in patients who have suffered a thoracic aortic dissection, especially for those with an onset prior to 50 years old, a family history of thoracic aortic disease, and no history of hypertension. Clinical genetic testing may help to prevent catastrophic events, such as thoracic aortic dissections and death, for family members of pathogenic variant carriers who have a high risk but have yet to develop the phenotype.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments:

Sequencing/Genotyping services were provided through the RS&G Service by the Northwest Genomics Center at the University of Washington, Department of Genome Sciences, under U.S. Federal Government contract number HHSN268201100037C from the National Heart, Lung, and Blood Institute. The authors acknowledge the University of Michigan Medical School Central Biorepository for providing biospecimen storage, management, and distribution services in support of the research reported in this publication. We acknowledge the University of Michigan DNA Sequencing Core. We thank the clinicians, staff, and study participants from the CHIP Biorepository and Michigan Genomics Initiative.

Sources of Funding: National Institutes of Health (R01-HL127564, R35-HL135824, and R01-HL142023 to C.J.W., K08HL130614 and R01HL141891 to B.Y., R01HL109942 to D.M.M., and R01HL122684 and R01HL139672 to S.K.G.). National Science Foundation (DGE 1256260) to B.N.W. The Phil Jenkins and Darlene &

Stephen J. Szatmari Funds to B.Y. The Joe D. Morris Collegiate Professorship, the David Hamilton Fund, and the Phil Jenkins Breakthrough Fund in Cardiac Surgery to H.P.

References:

1. Kent KC, et al. Screening for abdominal aortic aneurysm: a consensus statement. *J Vasc Surg.* 2004;39:267–269. [PubMed: 14718853]
2. Clouse WD, et al. Acute aortic dissection: population-based incidence compared with degenerative aortic aneurysm rupture. *Mayo Clin Proc.* 2004;79:176–180. [PubMed: 14959911]
3. Milewicz DM, et al. Heritable Thoracic Aortic Disease Overview In: Adam MP, Ardinger HH, Pagon RA, Wallace SE, Bean LJH, Stephens K and Amemiya A, eds. *GeneReviews*((R)) Seattle (WA): University of Washington, Seattle University of Washington, Seattle GeneReviews is a registered trademark of the University of Washington, Seattle. All rights reserved.; 1993.
4. Pomianowski P, et al. The genetics and genomics of thoracic aortic disease. *Ann Cardiothorac Surg.* 2013;2:271–279. [PubMed: 23977594]
5. Brownstein AJ, et al. Genes Associated with Thoracic Aortic Aneurysm and Dissection: An Update and Clinical Implications. *Aorta (Stamford).* 2017;5:11–20. [PubMed: 28868310]
6. Brownstein AJ, et al. Genes Associated with Thoracic Aortic Aneurysm and Dissection: 2018 Update and Clinical Implications. *Aorta (Stamford).* 2018;6:13–20. [PubMed: 30079932]
7. Renard M, et al. Clinical Validity of Genes for Heritable Thoracic Aortic Aneurysm and Dissection. *J Am Coll Cardiol.* 2018;72:605–615. [PubMed: 30071989]
8. Hiratzka LF, et al. 2010 ACCF/AHA/AATS/ACR/ASA/SCA/SCAI/SIR/STS/SVM guidelines for the diagnosis and management of patients with thoracic aortic disease. *Circulation.* 2010;121:e266–369. [PubMed: 20233780]
9. Erbel R, et al. 2014 ESC Guidelines on the diagnosis and treatment of aortic diseases: Document covering acute and chronic aortic diseases of the thoracic and abdominal aorta of the adult. The Task Force for the Diagnosis and Treatment of Aortic Diseases of the European Society of Cardiology (ESC). *Eur Heart J.* 2014;35:2873–2926. [PubMed: 25173340]
10. Wallace SE, et al. MYLK pathogenic variants aortic disease presentation, pregnancy risk, and characterization of pathogenic missense variants. *Genet Med.* 2019;21:144–151. [PubMed: 29925964]
11. Bradley TJ, et al. The Expanding Clinical Spectrum of Extracardiovascular and Cardiovascular Manifestations of Heritable Thoracic Aortic Aneurysm and Dissection. *Can J Cardiol.* 2016;32:86–99. [PubMed: 26724513]
12. Regalado ES, et al. Aortic Disease Presentation and Outcome Associated With ACTA2 Mutations. *Circ Cardiovasc Genet.* 2015;8:457–464. [PubMed: 25759435]
13. Sherry ST, et al. dbSNP: the NCBI database of genetic variation. *Nucleic Acids Res.* 2001;29:308–311. [PubMed: 11125122]
14. Landrum MJ, et al. ClinVar: public archive of relationships among sequence variation and human phenotype. *Nucleic Acids Res.* 2014;42:D980–985. [PubMed: 24234437]
15. Lek M, et al. Analysis of protein-coding genetic variation in 60,706 humans. *Nature.* 2016;536:285–291. [PubMed: 27535533]
16. Loeyls BL, et al. The revised Ghent nosology for the Marfan syndrome. *J Med Genet.* 2010;47:476–485. [PubMed: 20591885]
17. Guo DC, et al. Heritable Thoracic Aortic Disease Genes in Sporadic Aortic Dissection. *J Am Coll Cardiol.* 2017;70:2728–2730. [PubMed: 29169482]
18. Ziganshin BA, et al. Routine Genetic Testing for Thoracic Aortic Aneurysm and Dissection in a Clinical Setting. *Ann Thorac Surg.* 2015;100:1604–1611. [PubMed: 26188975]
19. Weerakkody R, et al. Targeted genetic analysis in a large cohort of familial and sporadic cases of aneurysm or dissection of the thoracic aorta. *Genet Med.* 2018;20:1414–1422. [PubMed: 29543232]
20. Michelena HI, et al. Natural history of asymptomatic patients with normally functioning or minimally dysfunctional bicuspid aortic valve in the community. *Circulation.* 2008;117:2776–2784. [PubMed: 18506017]

21. Kwartler CS, et al. Variants of Unknown Significance in Genes Associated with Heritable Thoracic Aortic Disease Can Be Low Penetrant “Risk Variants”. *Am J Hum Genet.* 2018;103:138–143. [PubMed: 29961567]
22. Richards S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med.* 2015;17:405–424. [PubMed: 25741868]

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

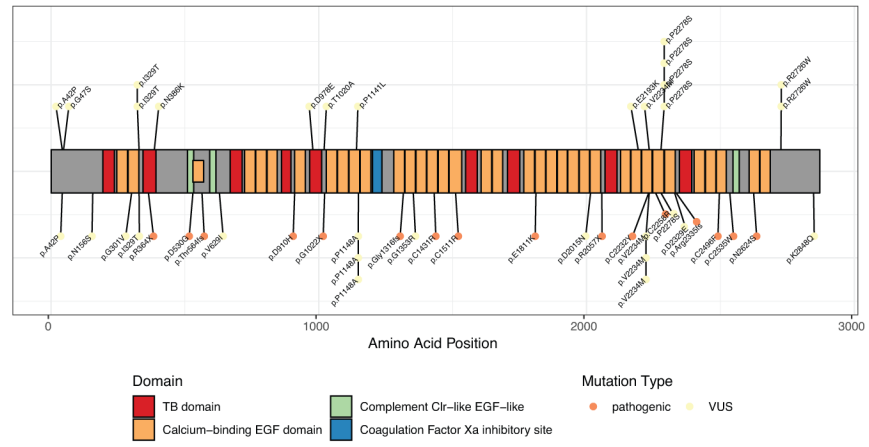


Figure 1. Distribution of pathogenic and variants of unknown significance in Fibrillin 1 (*FBNI*). Each point is a sample, with controls above the protein diagram and cases below.

Table 1.

Classification of 24 Pathogenic Variants

Chromosome:position	Reference Allele	Alternate Allele	Mutation type	Gene	HGVS protein notation	ClinVar 9/30/18	rsID dbSNP 151
2:189858169	G	A	Nonsynonymous	<i>COL3A1</i>	p.G378D	NA	
3:30732950	G	A	Stop Gain	<i>TGFBR2</i>	p.W521*	VUS for non-aortic phenotype	
5:121412592	CCAGA	C	Frameshift	<i>LOX</i>	p.Cys244fs	NA	rs779512296 [†]
10:53227579	G	A	Nonsynonymous	<i>PRKGI</i>	p.R177Q	Pathogenic	rs397515330
15:48707913	T	C	Nonsynonymous	<i>FBN1</i>	p.N2624S	VUS	
15:48713849	G	C	Nonsynonymous	<i>FBN1</i>	p.C2535W	Pathogenic	
15:48714232	C	A	Nonsynonymous	<i>FBN1</i>	p.C2496F	Likely pathogenic	
15:48719947	TGAAGCAGTACCCCTTCCC	T	Frameshift	<i>FBN1</i>	p.R2335fs	NA	
15:48722967	A	G	Nonsynonymous	<i>FBN1</i>	p.C2258R	Pathogenic	rs1057520617
15:48725107	C	T	Nonsynonymous	<i>FBN1</i>	p.C2232Y	Pathogenic	rs1060501054
15:48730109	G	A	Stop Gain	<i>FBN1</i>	p.R2057*	Pathogenic	rs763091520
15:48744873	C	T	Nonsynonymous	<i>FBN1</i>	p.E1811K	Conflicting interpretation of pathogenicity	rs761857514 [†]
15:48760660	A	G	Nonsynonymous	<i>FBN1</i>	p.C1511R	Likely pathogenic	rs397515811
15:48764793	A	G	Nonsynonymous	<i>FBN1</i>	p.C1431R	NA	
15:48773870	C	CT	Frameshift	<i>FBN1</i>	p.G1316fs	Likely pathogenic	
15:48782066	C	A	Stop Gain	<i>FBN1</i>	p.G1022*	NA	rs794728171
15:48786401	C	G	Nonsynonymous	<i>FBN1</i>	p.D910H	NA	
15:48802264	G	GT	Frameshift	<i>FBN1</i>	p.Thr564fs	Likely pathogenic	
15:48802366	T	C	Nonsynonymous	<i>FBN1</i>	p.D530G	VUS	
15:48808561	T	C	Essential Splice Site	<i>FBN1</i>	.	Pathogenic	rs397515756
15:48812913	G	A	Stop Gain	<i>FBN1</i>	p.R364*	Pathogenic	rs794728165
15:48888576	C	T	Essential Splice Site	<i>FBN1</i>	.	Likely pathogenic	
15:67457370	TGAA	T	In frame deletion	<i>SMAD3</i>	p.K116del	NA	
15:67462935	TA	T	Frameshift	<i>SMAD3</i>	p.Asn218fs	Pathogenic	rs587776881

[†] Also present in gnomAD version 2.1

Table 2.

Comparison Between Clinical Diagnosis and Pathogenic Variants Identified With Whole Exome Sequencing

	Clinical Diagnosis Matched		Clinical Diagnosis Changed		Diagnostic improvement and implications for clinical care	
	Number of Variants	Genes	Number of Variants	Genes	Number of Variants	Genes
Clinical genetic testing previously performed	5	<i>FBNI</i> [*] , <i>SMAD3</i> [*] , <i>PRKG1</i> [*]	0	0	0	-
No prior clinical genetic testing	12	<i>FBNI</i> [†]	1	<i>TGFBR2</i> [‡]	8	<i>FBNI</i> [§] , <i>SMAD3</i> [§] , <i>LOX</i> [§] , <i>COL3A1</i> [§]

* Clinical diagnosis and clinical genetic testing were consistent with the whole exome sequencing results

† Clinical diagnosis based on the Revised Ghent Nosology without clinical genetic testing was consistent with whole exome sequencing results.

‡ Clinical diagnosis without clinical genetic testing was inconsistent with whole exome sequencing results

§ Clinical diagnosis without clinical genetic testing would be improved by the whole exome result.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Table 3.

Demographic and Clinical Characteristics at the Time of Dissection

Variables	All Patients N= 240	Non-Pathogenic N=214	Pathogenic N=26	P
Age of onset, years	56 (45, 66)	57 (47, 67)	38 (26, 48)	<.001
Age of dissection, years	56 (45, 67)	57 (47, 67)	41 (29, 50)	<.001
Male	159 (66)	146 (68)	13 (50)	0.102
Race (% Caucasian)	212 (88)	190 (89)	22 (85)	0.76
Ethnicity (% non-Hispanic)	224 (93)	198 (93)	26 (100)	0.30
Thoracic aortic indications				
Root aneurysm	78 (33)	64 (30)	14 (54)	0.025
Ascending aneurysm	119 (50)	107 (50)	12 (46)	0.87
Arch aneurysm	59 (25)	55 (26)	4 (15)	0.34
Descending aneurysm	71 (30)	66 (31)	5 (19)	0.32
Max aneurysmal diameter, mm	48 (42, 57)	47 (42, 55)	57 (48, 71)	0.03
Type A aortic dissection	144 (60)	126 (59)	18 (69)	0.42
Type B aortic dissection	91 (38)	84 (39)	7 (27)	0.31
Rupture	5 (2.1)	4 (1.9)	1 (3.8)	0.441
Risk Factors				
HTN	126 (53)	122 (57)	4 (15)	<.001
Dyslipidemia	42 (18)	40 (19)	2 (7.7)	0.27
Smoking history (former/current)	102 (43)	97 (45)	5 (19)	0.02
Type 2 diabetes mellitus	6 (2.5)	6 (2.8)	0 (0)	1.00
Medications				
ACE-I	29 (12)	27 (13)	2 (7.7)	0.75
Calcium channel blocker	11 (4.6)	11 (5.1)	0 (0)	0.61
ARB	14 (5.8)	13 (6.1)	1 (3.8)	1.00
Beta-Blocker	68 (28)	62 (29)	6 (23)	0.69
Anti-HTN medications (% yes)	83 (35)	77 (36)	6 (23)	0.28
Number of HTN medications				0.40
0	157 (65)	137 (64)	20 (77)	
1	50 (21)	46 (21)	4 (15)	
2	27 (11)	26 (12)	1 (3.8)	
3	6 (2.5)	5 (2.3)	1 (3.8)	
Family history, first-degree relative				
Mother	41 (17)	31 (15)	10 (50)	0.008
Father	47 (20)	39 (18)	8 (31)	0.22
Sibling, at least one known	42 (18)	30 (14)	12 (46)	<.001
Child, at least one known	18 (7.5)	10 (5)	8 (31)	<.001
CLIA genetic testing (% yes)	20 (8.0)	15 (7.0)	5 (19.2)	0.05
Pathogenic variant	5 (2.0)	0 (0)	5 (19.2)	<.001
Likely pathogenic or VUS	8 (3.8)	8 (3.8)	0 (0)	0.604
No variant identified	7 (3.3)	7 (3.3)	0 (0)	1.0

Values are median (IQR) or n (%).

Correction for multiple statistical tests was not performed.

ACE-I=angiotensin converting enzyme inhibitor; ARB=Angiotensin II receptor blocker; CLIA: Clinical Laboratory Improvement Amendments; HTN=hypertension

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Table 4.

Risk factors for cases with a pathogenic variant

Variables	OR	95% Wald Confidence Limits		P-value
		Lower	Upper	
Age 50 vs > 50	5.5	1.6	19.7	0.008
Sex (female vs male)	1.1	0.3	3.8	0.84
Caucasian	0.7	0.1	3.1	0.60
Root aneurysm	1.7	0.6	5.2	0.34
Hypertension	5.6	1.4	22.3	0.015
Smoking history	2.6	0.7	9.9	0.16
Family history				
Mother	5.7	1.4	22.3	0.013
Father	0.3	0.1	1.6	0.17
Siblings	5.1	1.1	23.9	0.04
Children	6.0	1.4	26.7	0.017

Hypertension was defined as no hypertension versus had a diagnosis of hypertension. Smoking history was defined as no smoking history versus had a smoking history. Family history was defined as aortic disease noted within a first-degree relative.