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ABSTRACT

Purpose

Thoracic aortic aneurysm/aortic dissection (TAAD) is a disorder with highly variable age of onset and phenotype. We sought to determine the prevalence of pathogenic variants in TAAD-associated genes in a mixed cohort of sporadic and familial TAAD patients and identify relevant genotype-phenotype relationships.

Methods

We used a targeted PCR and next generation sequencing-based panel for genetic analysis of 15 TAAD associated genes in 1025 unrelated TAAD cases.

Results

We identified 49 pathogenic or likely pathogenic (P/LP) variants in 47 cases (4.9% of those successfully sequenced). Almost half of the variants were in non-syndromic cases with no known family history of aortic disease. Twenty-five variants were within *FBN1* and two patients were found to harbour two P/LP variants. Presence of a related syndrome, younger age at presentation, family history of aortic disease and involvement of the ascending aorta increased the risk of carrying a P/LP variant.

Conclusions

Given the poor prognosis of TAAD that is undiagnosed prior to acute rupture or dissection, genetic analysis of both familial and sporadic cases of TAAD will lead to new diagnoses, more informed management and possibly reduced mortality through earlier, preclinical diagnosis in genetically determined cases and their family members.

Key Words: Thoracic Aortic Aneurysm/ Aortic Dissection (TAAD); TAAD Genetics; FBN1; Sporadic TAAD; High-Throughput DNA Sequencing

INTRODUCTION

Thoracic aortic aneurysm/aortic dissection (TAAD) manifests a devastating clinical course if undiagnosed and untreated [1]. Recent studies of post-mortem computerised tomographic autopsy indicate that 7% of out-of-hospital sudden deaths are due to Type A aortic dissection [2].

The major mortality and morbidity associated with TAAD lies with acute dissection or rupture, both of which are difficult to predict [1,3]. Early perioperative mortality of a ruptured aneurysm remains high (28-46%) despite recent advances in surgical technology compared with the safety of elective treatment of unruptured thoracic aneurysms where surgical results have improved significantly (6-7% mortality) [4]. Aneurysm size (5-5.5cm) is currently the major determinant of the timing of surgery[5]. In syndromic aortic disease (Marfan (MFS), Loeys-Dietz (LDS), Ehlers-Danlos Syndrome (EDS)), earlier surgical intervention is recommended as dissection can occur at diameters less than 5.0cm [6] although these associated syndromes may be difficult to diagnose [7,8]. Whilst genetic testing is recommended in familial and syndromic TAAD, it is not widely available in sporadic TAAD (STAAD) and is generally restricted to those with a strong suspicion of a genetic aetiology[5,9].

The study of large pedigrees with multiply affected members has led to the identification of causal genes in both syndromic and non-syndromic TAAD [6,10]. Causative mutations have been identified in non-syndromic FTAAD pedigrees within the smooth muscle contractile (SMC) genes *ACTA2*, *MYH11*, *PRKG1* and *MYLK* in non-syndromic patients [11–13]. Syndromic TAAD is associated with numerous connective tissue disorders and their corresponding genes; Marfan syndrome (*FBN1*), Loeys-Dietz syndrome (*TGFBR1*, *TGFBR2*, *SMAD3* & *TGFB2*), Ehlers-Danlos syndrome (*COL1A1*, *COL1A2*, *COL3A1*, *COL5A1* and *COL5A2*), Arterial tortuosity syndrome (*SLC2A10*) and Shprintzen-Goldberg syndrome (*SKI*) [10,14].

Many of the genes in syndromic TAAD are associated with dissections occurring in patients with an aortic size below the 5.5cm threshold recommended for reparative surgery [6]. The identification of causal variants in probands can lead to additional diagnoses by family screening in family members who have yet to develop clinical manifestations of the disorder [9]. In addition, endovascular repair is contraindicated in cases diagnosed with a connective tissue syndrome, when open surgery is recommended [15]. Genetic testing can therefore identify causal variants leading to a definitive preclinical diagnosis that can better determine the optimal timing and technique of prophylactic surgery.

Next generation sequencing (NGS) is increasingly used for mutation testing and clinical diagnosis [16]. Using a cost-effective PCR and NGS-based targeted sequencing approach [17,18], we sought to identify the prevalence of causative genetic variants in known TAAD genes and to elucidate genotype-phenotype relationship in a mixed cohort of 1,025 cases of familial and sporadic TAAD from the UK and USA.

METHODS

Patient Cohorts

Surgical intervention in 927 consecutive unrelated cases was undertaken over a 10 year period at the Aortic Institute at Yale-New Haven (Yale University, New Haven, USA): 785 surgical samples from these cases were used for DNA analysis within this study (Yale cohort) and 240 unrelated cases, treated and followed up at three UK centres from 2000-2013 (St Mary's hospital, London; Royal Brompton & Harefield hospitals, London; Liverpool Heart & Chest Hospital, Liverpool) (UK cohort) were recruited to the study. Twenty-five cases within the Yale cohort were also present in a previous whole-exome sequencing study [9]. The Yale cohort was approved by the Human Investigation Committee of Yale University (IRB protocol 12617) and the UK

cohort was approved by the West London Research Ethics Committee (REC reference 11/LO/0883). Both cohorts complied with the Declaration of Helsinki and written informed consent was obtained from all participants. MFS and LDS diagnoses in the UK cohort were identified by clinical case note review in which diagnoses were made by consultant clinical geneticists following a standard referral pathway. MFS diagnoses in the Yale cohort were made according to the Revised Ghent Nosology[19]. There were no LDS cases in the Yale cohort.

DNA Extraction

For the UK cohort, saliva samples were collected using the Oragene DNA kit (Genotek, Ontario, Canada) and QIAamp DNA Blood Midi kit (Qiagen, Venlo, Netherlands) was used to extract DNA from whole-blood samples. For the Yale cohort, DNA from fresh frozen aortic specimens collected at surgery were extracted using the DNeasy Blood & Tissue kit (Qiagen, Venlo, Netherlands). All DNA samples were subsequently normalized to 25-50ng/ul.

Targeted Exon Sequencing

Targeted exon sequencing was carried out on two Fluidigm assays named Aortopathy panel 1 (TAAD-X), which included 363 primer-pairs for *FBN1*, *TGFBR1*, *TGFBR2*, *MYH11*, *ACTA2*, *SMAD3* and *MYLK* and Aortopathy panel 2 (TAAD-Z) containing 493 primer pairs for *SKI*, *TGFB2*, *SLC2A10*, *COL1A1*, *COL1A2*, *COL3A1*, *COL5A1* and *COL5A2* (Table S1 & S2). Multiplex PCR was performed using the Access Array System (Fluidigm, South San Francisco, CA) and the MiSeq sequencing platform (Illumina, San Diego, CA) as previously described [17,18].

Read Mapping, Variant Calling & Annotation

FastQC was used to assess the sequencing read quality. Primer sequences were trimmed from FASTQ files using cutadapt (v 1.9.1) [20] prior to read mapping to

GRCh37/hg19 human reference sequence using BWA-MEM V0.7.12 [21]. Realignment of reads around indels and base quality score recalibration was performed using GATK v3.4 [22]. The GATK UnifiedGenotyper was used for calling variants [23]. Variants were annotated using Ensembl Variant Effect Predictor v84 [24].

Variant and Sample Filtering

Synonymous variants and intronic variants (excluding splice sites) were omitted from downstream analysis unless annotated as pathogenic or disease-causing in ClinVar [25] or HGMD [26] databases. Variants with a minor allele frequency greater than 0.1% in either ExAC release 0.3.1 [27] or dbSNP146 [28] datasets were excluded. We found a high frequency of false positives in variants within SKI exon 1 and those with an allele balance below 0.3. These variants were therefore excluded from the datasets. Samples with less than 80% of target bases covered by more than 49 reads were not considered for downstream analysis.

Pathogenicity Assignment

Variants passing filters were assigned to one of three categories: either 'pathogenic', 'likely pathogenic', 'variant of uncertain significance (VUS)' or 'likely benign' based on the presence of one or more pieces of evidence (Table S3 & S4). Variants were assigned to the 'pathogenic' or 'likely pathogenic' category if they met any of the following criteria: if the variant is already reported as disease causing in HGMD or ClinVar unless sufficient evidence could not be found for categorising it as 'pathogenic/likely pathogenic' (P/LP) (Table S5); if the variant results in the same amino acid substitution as a variant already reported as disease causing in HGMD or ClinVar; premature termination of translation; a substitution of a glycine residue within a GlyXY repeat in collagen triple helical domains; an insertion of amino acids disrupting the GlyXY repeat sequence; alteration of a key residue in a protein feature (e.g. active site, disulphide bond) in keeping with previously ascribed molecular

mechanisms for a given gene (see Table S6). Variants predicted to be damaging by PolyPhen and SIFT and with CADD scores above 10 were assigned to the VUS category, as were variants resulting in a substitution at an amino acid position at which a different substitution is assigned disease-causing status in ClinVar or HGMD [25,26,29–31]. Absence of PolyPhen or SIFT predictions for missense variants resulted in classification as VUS. Remaining variants were assigned 'likely benign' status. All variants assigned P/LP status were validated by Sanger sequencing.

Statistical Analysis

Significant differences in categorical variables between individuals in different genotype or phenotype groups were estimated by Fisher's exact test. The unpaired Wilcoxon rank-sum test was used to assess non-parametric phenotypic continuous measurements.

RESULTS

Patient Demographics and Clinical Characteristics

Of the total of 785 cases recruited to the Yale cohort and 240 to the UK cohort, sequence depth reached the assay threshold of 50 reads in at least 80% of target bases in 93% of the Yale cohort and 98% of the UK cohort. A total of 732 patients in the Yale cohort and 235 patients in the UK cohort were therefore taken forward for further analyses: their demographic and clinical characteristics are given in Table 1.

Variants Identified by NGS

We identified 17 pathogenic variants, 32 likely pathogenic variants and 68 VUS within the whole cohort. Two patients, Y_91_1 and Y_17_1, each carried two P/LP variants, giving a total of 49 P/LP variants in 47 patients, constituting 4.9% of the 967 samples sequenced to our target threshold (Tables 2 and S7). The majority of the identified

P/LP variants were in the *FBN1* gene (n = 25) (Figure 1, Table S7). A total of 68 VUS were found within 67 patients (Table S8).

We also tested our pipeline with a more lenient allele frequency threshold of 1%, which yielded three more P/LP variants. However, each of these additional variants were reclassified to VUS upon inspection of the literature (Table S5), so a maximum allele frequency of 0.1% was used for all variant filtering.

Genotype-phenotype correlation with P/LP variants

A lower age at diagnosis was found to significantly increase the likelihood of identifying a P/LP variant. The median age at diagnosis was 46.0 years for patients carrying a P/LP variant compared to 55.5 years for those carrying a VUS ($p=3.5e^{-3}$), and 61.0 for those in the likely benign and no variant group ($p=3.4e^{-8}$) (Figure 2a). Patients who were diagnosed under 50 years of age were far more likely to carry a P/LP variant with 11.6% of those diagnosed before 50 carrying a P/LP variant compared to 3.0% in the over 50 category ($p=1.3e^{-7}$) (Figure 2b). Removing Marfan cases from these datasets increases the median age at diagnosis for patients harbouring a P/LP variant to 51.5 (Figure 2c) and decreases the percentage of patients under 50 harbouring a P/LP variant to 7.8% (Figure 2d). The median age of TAAD diagnosis in clinically suspected MFS cases was 33.5 years in our cohort.

TAAD patients with a family history were four times more likely to carry a P/LP variant than those without a family history ($p=1.05e^{-4}$; Figure S1a). Gender did not significantly influence the likelihood of harbouring a P/LP variant within our cohorts (p=0.29; Figure S1b). A median maximum aortic size of 5.25cm was found in patients harboring P/LP variants compared to 5.1cm in those not harboring a P/LP variant (p = 0.45). Although aortic size is the main determinant for surgery, no association was found in our cohorts between maximum aortic size and variant categories

(unpublished data). Similarly, splitting the data by pathology type and location yielded nothing significant (unpublished data).

To estimate the influence of clinical characteristics in determining the likelihood of obtaining a genetic diagnosis, we calculated the relative risk of detecting a P/LP variant by clinical phenotype (Table 3). Likelihood of genetic diagnosis was associated with presence of a related syndrome, a younger age at diagnosis, family history of aortic disease and ascending aortic aneurysm compared to other locations. All but 7 of patients harbouring a P/LP variant possessed at least one of these phenotypes. Other clinical features yielded small, non-significant risk.

A median age at diagnosis of 39 and 29 was observed in patients who carried a P/LP variant within *FBN1* (n=25) and *TGFBR2* (n=3) respectively (Figure S2). Of the 24 cases of clinically diagnosed MFS, 10 cases were identified as harbouring a P/LP variant in *FBN1*; the remaining case (Y_35_28) in whom a presumptive genetic diagnosis was made had a likely pathogenic variant in *MYH11* (Table 2). Although no pathogenic *FBN1* variants were found in this patient by our panel assay, subsequent clinical exome analysis identified a probable copy number variant in *FBN1* in this patient (unpublished data), suggesting two possible genetic causes for this patient's TAAD.

DISCUSSION

To date, the majority of genetic abnormalities in TAAD have been identified in syndromic or familial cases [6]. The aim of our study was to determine the prevalence of likely disease-causing variants in a mixed, clinically relevant cohort of patients with sporadic and familial TAAD. We sequenced 967 of 1025 unselected TAAD patients from the UK and USA above our target coverage threshold and identified a total of 49 P/LP variants in 47 patients that are the likely cause of their disease. To our knowledge, this is the largest genetic analysis of familial and sporadic cases of TAAD

to date and defines a lower limit of 4.9% for the prevalence of TAAD in our cohorts with a pathogenic abnormality amongst most of the currently known TAAD genes. This frequency is similar to that of deleterious mutations (3.9%) reported previously in a series of 102 TAAD patients analysed by exome sequencing [9].

The majority (87.8%) of identified P/LP variants were found within known syndromic genes. Mutations in *FBN1* accounted for more than half of all identified P/LP variants, with the majority affecting functionally significant domains of the gene. P/LP variants in *FBN1* were discovered in 2.6% of the sequenced cohort and 5.8% of patients who had a probable or proven family history of TAAD. This is somewhat higher than was reported in a previous study of FTAAD, in which 2.7% of familial cases carried a pathogenic *FBN1* variant [32].

FBN1 is clearly an important contributor to Mendelian cases of TAAD and MFS. A recent genome-wide association study showed that common variants in *FBN1* are associated with STAAD, suggesting a common pathogenesis of thoracic aortic disease in MFS and STAAD [33]. We found that only 40% of those with a P/LP variant in *FBN1* had a known or suspected clinical diagnosis of MFS. Similarly, only 48.9% of those with a P/LP variant in any gene had a known or probable family history of TAAD. Although these results may be partly due to an incomplete clinical record (UK records did not definitively record the absence of a family history), they are also consistent with observations over the last 20 years that *FBN1* mutations are a cause of TAAD in patients who do not have clinical MFS [32,34]. In keeping with this, a recent study of probands with *FBN1* mutations found that, only 56-79% met formal clinical criteria for MFS by Ghent systemic scores [35].

Patients Y_91_1 and Y_17_1 were each found to harbour two P/LP variants (in *SLC2A10* and *MYH11*, and *FBN1* and *COL1A1* respectively). No common phenotypic features (young age-of-onset, family history) were seen in these two patients, although a much larger maximum aortic size was found in both patients

(7cm and 6.5cm respectively) compared to the median maximum aortic size identified in patients harbouring a P/LP variant (5.25cm).

The age at diagnosis within our patients that have a P/LP variant (46.0 years) is much lower than has been observed previously in FTAAD (56.8 years) and STAAD (64.3 years) patients [36]. This is likely due to the fact that 11 of the 47 patients harbouring a P/LP variant had clinically suspected MFS, 10 of whom had a P/LP variants in *FBN1*. The diagnosis of TAAD in MFS has been reported to have a lower age at diagnosis (24.8 years) than non-syndromic TAAD [36]; our cohort had a similarly lower age at diagnosis (33.5 years). If the patients in our cohort with suspected MFS are removed, the median age of TAAD diagnosis in our cohort rises from 46.0 to 51.5 years. Overall, the percentage of patients with a P/LP variant was 3-4x higher within the under 50 age group than in those over the age of 50.

Although aortic size is the main determinant for surgery, we found no association between maximum aortic size and variant categories. This may in part be explained by early evaluation and surgical intervention in cases with syndromic presentations or family history of TAAD.

Comparing all 47 patients with a P/LP variant with the rest of the cohort, we were able to identify statistically significant risks of carrying a P/LP variant associated with developing the disorder: a syndromic component (RR 11.94; 95% CI 7.06-20.20), a younger age at presentation (RR 3.83; 95% CI 2.20-6.67), a probable or known family history of aortic disease (RR 3.80; 95% CI 1.88-7.66) and an aneurysm or dissection occurring partially or wholly within the ascending aorta (RR 1.84; 95% CI 1.04-3.27). The first three factors are clearly suggestive of Mendelian disease and may be suitable criteria for prioritizing TAAD patients for genetic testing if genetic testing is not applied to all cases. The fourth factor, disease location in the ascending aorta, is reflective of the known stronger genetic aetiology of TAAD in the ascending aorta compared to the descending aorta [9].

Previous studies have suggested that genetic testing should be undertaken in patients who present at a young age without any additional risk factors [37], although it is unclear to what extent this is implemented in routine clinical practice. Non-syndromic TAAD can have a similarly severe clinical course to those with syndromic TAAD [38], highlighting the importance of identifying genetically predisposed, non-syndromic TAAD patients prior to the development of symptoms. Seven (14.9%) patients harbouring a P/LP variant did not possess any of the above phenotypes (syndromic features, young age-of-onset, family history, involvement of the ascending aorta) associated with greater risk of a genetic aetiology. Therefore, whilst these risk factors increase the likelihood of identifying P/LP variants in known TAAD genes, restricting genetic testing solely to cases with these risk factors would miss a significant, albeit low percentage (14.9%) of cases in whom a genetic diagnosis could be made.

Our study has a number of limitations that include possibly having underestimated the number of truly pathogenic variants. We employed intentionally stringent criteria for defining P/LP variants, but this may have led to a number of truly pathogenic variants being classified as VUS's. The difficulty in unequivocally designating variants as either P/LP or benign highlights the need for concerted efforts to systematically classify mutations in these disease genes, for example with prospective functional assays[39]. As more information becomes available we anticipate that many of the VUS identified in this study, particularly those with strong supporting *in silico* data, (Tables S8 and S9) may be unambiguously reclassified as causative variants. A further limitation to our study is that our sequencing assay is limited to the coding sequences and intron/exon boundaries of 15 TAAD genes that represent most of the common causes of TAAD - our study would not detect variants in as yet undiscovered genes or in non-coding sequence. In time, with greater understanding of genotype-phenotype correlation, whole genome sequencing could ultimately

detect a higher frequency of pathogenic variants than detected herein. Indeed, we were unable to identify a P/LP variant in 44 of 62 individuals with three or more of the risk factors we identified as increasing the likelihood of harbouring a P/LP variant, and only eight of these individuals were found to carry a VUS. These individuals with a high relative probability of carrying a P/LP variant but no genetic diagnosis after panel testing are likely to be worth prioritising for future whole genome/exome sequencing studies.

In summary, we found 4.9% of patients carried a P/LP variant as the underlying cause of their TAAD, predominantly within *FBN1* but with substantial contributions from *TGFBR* and *COL* genes. Consistent with previous reports, amongst these cases were patients with non-syndromic TAAD in whom we found P/LP variants in genes normally associated with connective tissue disease. A higher likelihood of harbouring a P/LP variant was found to be associated with a syndromic component to the disease, early age at presentation, positive family history and aneurysm location in the ascending aorta. However, restricting genetic testing only to TAAD patients with these features is likely to miss a small but significant number of cases in whom a definitive genetic diagnosis could be made.

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CONFLICT OF INTEREST

Tim Aitman reports grants from Wellcome, grants from National Institute for Health Research, grants from UK Medical Council, personal fees from Illumina, during the conduct of the study; personal fees from AstraZeneca, outside the submitted work.

Colin Bicknell reports personal fees from Medtronic, personal fees from Bolton Medical, non-financial support from Vascutek, non-financial support from Gore, outside the submitted work.

DATA ACCESS

All P/LP variants have been submitted to ClinVar database (SUB2992095). Detailed phenotype and genotype data is available on request. Source code for data cleaning and statistical analysis can be found at https://github.com/superDross/TAAD_analysis

FIGURE LEGENDS

Figure 1: Numbers of pathogenic or likely pathogenic (P/LP) variants identified by gene across both the Yale and UK cohorts. Percentages shown are the overall proportion of P/LP variants for each gene.

Figure 2: Influence of variant type and diagnosis of Marfan syndrome on age of diagnosis. In cases with more than one P/LP variant or VUS, only the most damaging was included in this analysis. Age distribution and impact of age on variant type in whole cohort (a, b), and in non-Marfan patients (c, d).

REFERENCES

1. Goldfinger JZ, Halperin JL, Marin ML, et al. Thoracic aortic aneurysm and dissection. *J Am Coll Cardiol*. 2014;**64**:1725-1739.
2. Tanaka Y, Sakata K, Sakurai Y, et al. Prevalence of Type A Acute Aortic Dissection in Patients With Out-Of-Hospital Cardiopulmonary Arrest. *Am J Cardiol*. 2016;**117**:1826-1830.
3. Booher AM, Eagle KA. Diagnosis and management issues in thoracic aortic aneurysm. *Am Heart J*. 2011;**162**:38-46.
4. Goodney PP, Travis L, Lucas FL, et al. Survival after open versus endovascular thoracic aortic aneurysm repair in an observational study of the medicare population. *Circulation*. 2011;**124**:2661-2669.
5. Erbel R, Aboyans V, Boileau C, et al. 2014 ESC guidelines on the diagnosis and treatment of aortic diseases. *Eur Heart J*. 2014;**35**:2873-2926.
6. Milewicz DM, Regalado ES. Use of genetics for personalized management of heritable thoracic aortic disease: How do we get there? *J Thorac Cardiovasc Surg*. 2015;**149**:S3-S5.
7. Faivre L, Collod-Beroud G, Adès L, et al. The new Ghent criteria for Marfan syndrome: What do they change? *Clin Genet*. 2012;**81**:433-442.
8. Williams JA, Loeys BL, Nwakanma LU, et al. Early Surgical Experience With Loeys-Dietz: A New Syndrome of Aggressive Thoracic Aortic Aneurysm Disease. *Ann Thorac Surg*. 2007;**83**:S757-S763.
9. Ziganshin BA, Bailey AE, Coons C, et al. Routine genetic testing for thoracic aortic aneurysm and dissection in a clinical setting. *Ann Thorac Surg*. 2015;**100**:1604-1612.
10. Lindsay ME, Dietz HC. Lessons on the pathogenesis of aneurysm from heritable conditions. *Nature*. 2011;**473**:308-316.
11. Wang L, Guo DC, Cao J, et al. Mutations in myosin light chain kinase cause familial aortic dissections. *Am J Hum Genet*. 2010;**87**:701-707.

12. Guo D-C, Pannu H, Tran-Fadulu V, et al. Mutations in smooth muscle alpha-actin (ACTA2) lead to thoracic aortic aneurysms and dissections. *Nat Genet*. 2007;**39**:1488-1493.
13. Guo DC, Regalado E, Casteel DE, et al. Recurrent gain-of-function mutation in PRKG1 causes thoracic aortic aneurysms and acute aortic dissections. *Am J Hum Genet*. 2013;**93**:398-404.
14. Doyle AJ, Doyle JJ, Bessling SL, et al. Mutations in the TGF-β repressor SKI cause Shprintzen-Goldberg syndrome with aortic aneurysm. *Nat Genet*. 2012;**44**:1249-1254.
15. Grabenwöger M, Alfonso F, Bachet J, et al. Thoracic endovascular aortic repair (TEVAR) for the treatment of aortic diseases: A position statement from the european association for cardio-thoracic surgery (EACTS) and the european society of cardiology (ESC). *Eur J Cardio-thoracic Surg*. 2012;**42**:17-24.
16. Baker MW, Atkins AE, Cordovado SK, et al. Improving newborn screening for cystic fibrosis using next-generation sequencing technology: a technical feasibility study. *Genet Med*. 2015;**18**:231-238.
17. Weerakkody RA, Vandrovcova J, Kanonidou C, et al. Targeted next-generation sequencing makes new molecular diagnoses and expands genotype-phenotype relationship in Ehlers-Danlos syndrome. *Genet Med*. 2016;**18**:1119-1127.
18. Vandrovcova J, Thomas ER a, Atanur SS, et al. The use of next-generation sequencing in clinical diagnosis of familial hypercholesterolemia. *Genet Med*. 2013;**15**:948-957.
19. Loeys BL, Dietz HC, Braverman AC, et al. The revised Ghent nosology for the Marfan syndrome. *J Med Genet*. 2010;**47**:476-485.
20. Martin M. Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet.journal*. 2011;**17**:10.
21. Li H. Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. March 2013. http://arxiv.org/abs/1303.3997. Accessed October 20, 2017.
22. Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler

transform. *Bioinformatics*. 2009;**25**:1754-1760.

23. McKenna A, Hanna M, Banks E, et al. The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Res*. 2010;**20**:1297-1303.
24. McLaren W, Gil L, Hunt SE, et al. The Ensembl Variant Effect Predictor. *Genome Biol*. 2016;**17**:122.
25. Landrum MJ, Lee JM, Benson M, et al. ClinVar: Public archive of interpretations of clinically relevant variants. *Nucleic Acids Res*. 2016;**44**:D862-D868.
26. Stenson PD, Mort M, Ball E V., et al. The Human Gene Mutation Database: Building a comprehensive mutation repository for clinical and molecular genetics, diagnostic testing and personalized genomic medicine. *Hum Genet*. 2014;**133**:1-9.
27. Karczewski KJ, Weisburd B, Thomas B, et al. The ExAC browser: Displaying reference data information from over 60 000 exomes. *Nucleic Acids Res*. 2017;**45**:D840-D845.
28. Sherry ST, Ward MH, Kholodov M, et al. dbSNP: the NCBI database of genetic variation. *Nucleic Acids Res*. 2001;**29**:308-311.
29. Adzhubei I, Jordan DM, Sunyaev SR. *Predicting Functional Effect of Human Missense Mutations Using PolyPhen-2.* Vol Chapter 7.; 2013.
30. Ng PC, Henikoff S. SIFT: Predicting amino acid changes that affect protein function. *Nucleic Acids Res*. 2003;**31**:3812-3814.
31. Kircher M, Witten DM, Jain P, et al. A general framework for estimating the relative pathogenicity of human genetic variants. *Nat Genet*. 2014;**46**:310-315.
32. Regalado ES, Guo DC, Santos-Cortez RLP, et al. Pathogenic FBN1 variants in familial thoracic aortic aneurysms and dissections. *Clin Genet*. 2016;**89**:719-723.
33. LeMaire SA, McDonald M-LN, Guo D-C, et al. Genome-wide association study identifies a susceptibility locus for thoracic aortic aneurysms and aortic dissections spanning FBN1 at 15q21.1. *Nat Genet*. 2011;**43**:996-1000.
34. Milewicz DM, Michael K, Fisher N, et al. Fibrillin-1 (FBN1) mutations in patients with

thoracic aortic aneurysms. *Circulation*. 1996;**94**:2708-2711.

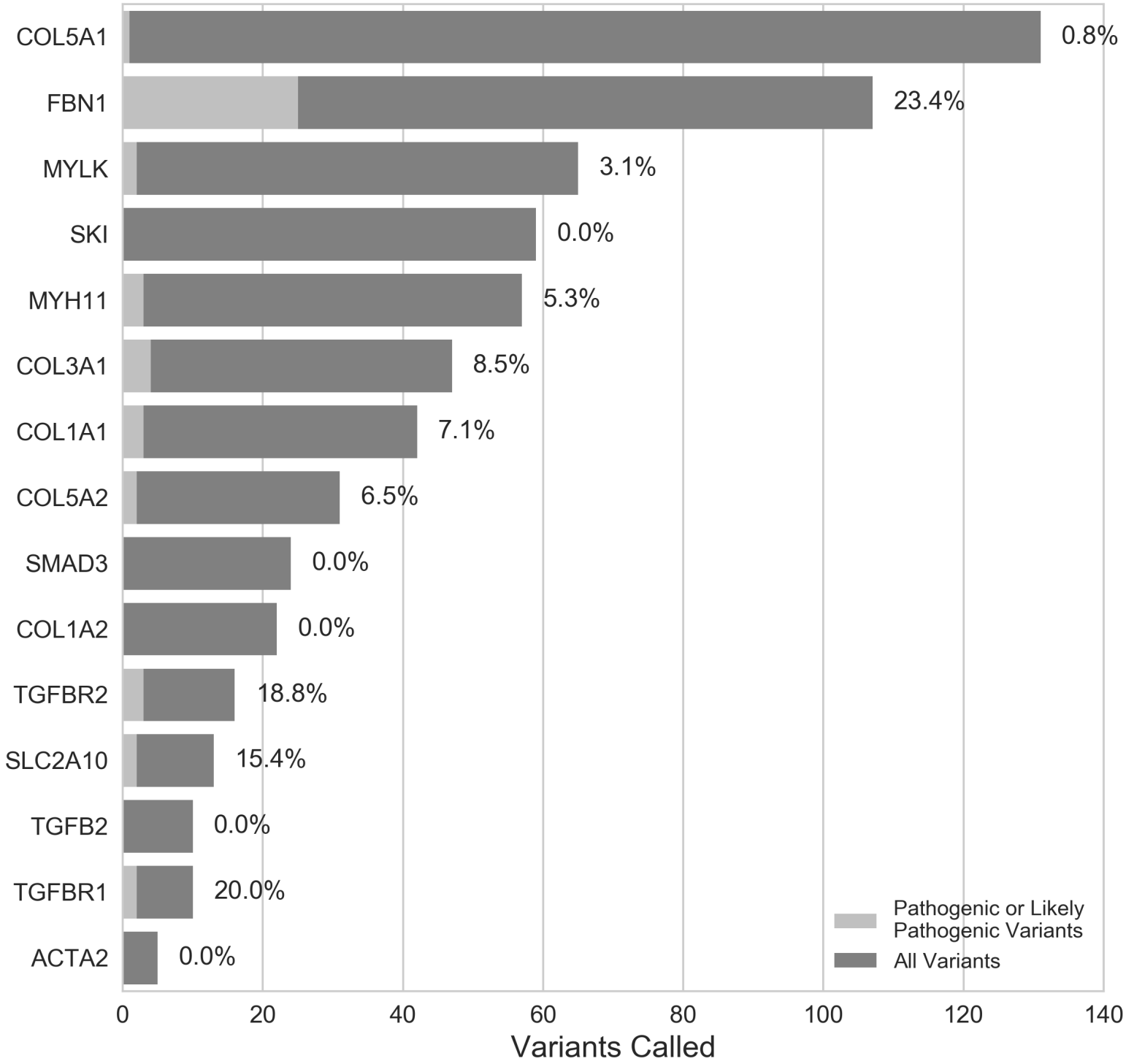
35. Faivre L, Collod-Beroud G, Child A, et al. Contribution of molecular analyses in diagnosing Marfan syndrome and type I fibrillinopathies: an international study of 1009 probands. *J Med Genet*. 2008;**45**:384-390.

36. Luyckx I, Loeys BL. The genetic architecture of non-syndromic thoracic aortic aneurysm. *Heart*. 2015;**101**:1678-1684.

37. Backer J De, Renard M, Campens L, et al. Genes in Thoracic Aortic Aneurysms and Dissections – Do they Matter? Translation and Integration of Research and Modern Genetic Techniques into Daily Clinical Practice. *Aorta*. 2013;**1**:135-145.

38. Keramati AR, Sadeghpour A, Farahani MM, Chandok G, Mani A. The non-syndromic familial thoracic aortic aneurysms and dissections maps to 15q21 locus. *BMC Med Genet*. 2010;**11**:143.

39. Majithia AR, Tsuda B, Agostini M, et al. Prospective functional classification of all possible missense variants in PPARG. *Nat Genet*. 2016;**48**:1570-1575.


COL5A1
FBN1
MYLK
SKI
MYH11
COL3A1
COL1A1
COL5A2
SMAD3
COL1A2
TGFBR2
SLC2A10
TGFB2
TGFBR1
ACTA2
0.8%
23.4%
3.1%
0.0%
5.3%
8.5%
7.1%
6.5%
0.0%
0.0%
18.8%
15.4%
0.0%
20.0%
0.0%
Pathogenic or Likely Pathogenic Variants
All Variants
0
20
40
60
80
100
120
140
Variants Called

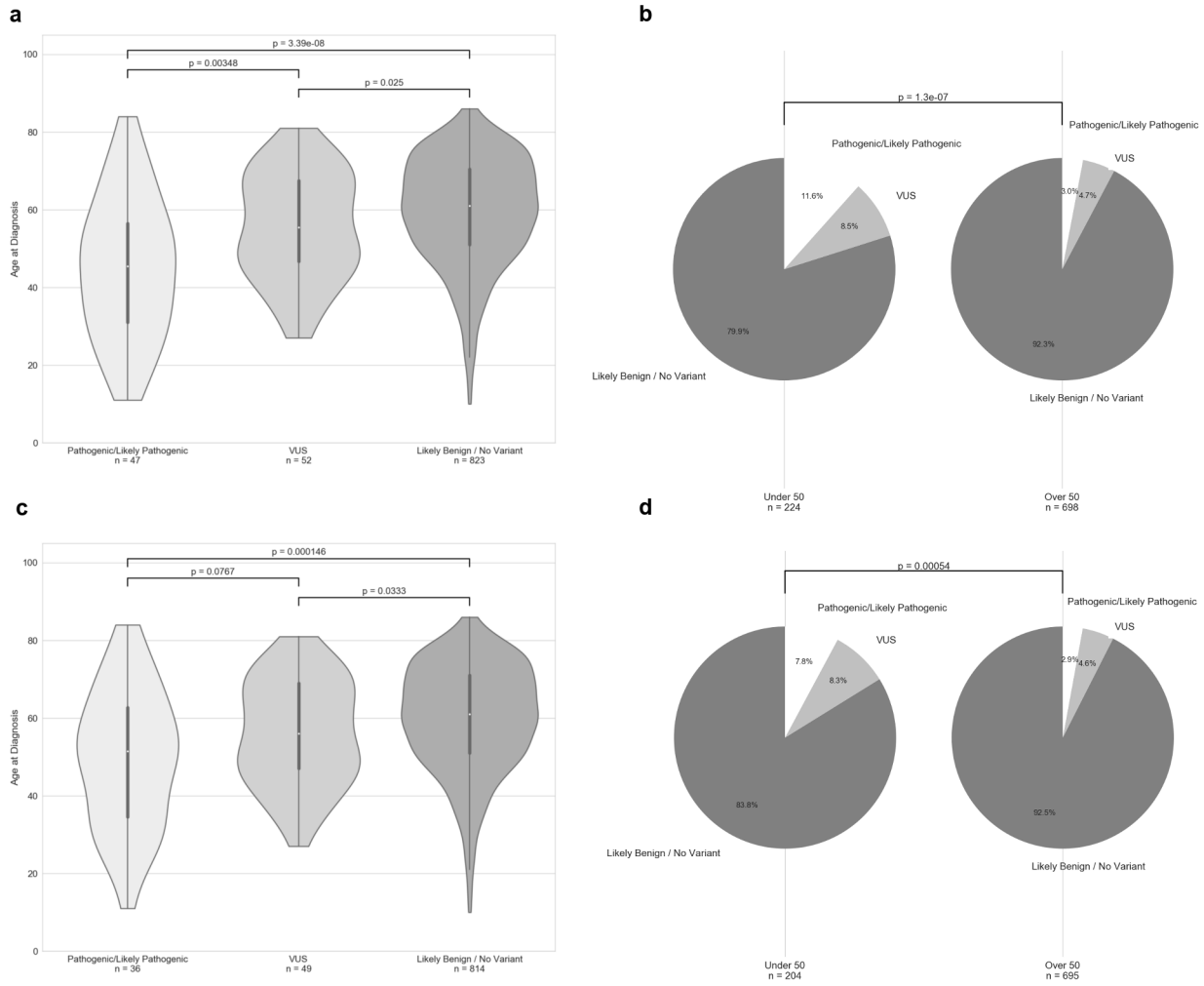

a
p = 3.39e-08
p = 0.00348
p = 0.025
Age at Diagnosis
Pathogenic/Likely Pathogenic
n = 47
VUS
n = 52
Likely Benign / No Variant
n = 823
b
p = 1.3e-07
Pathogenic/Likely Pathogenic
VUS
11.6%
8.5%
79.9%
Likely Benign / No Variant
Under 50
n = 224
3.0%
4.7%
92.3%
Over 50
n = 698
c
p = 0.000146
p = 0.0767
p = 0.0333
Age at Diagnosis
Pathogenic/Likely Pathogenic
n = 36
VUS
n = 49
Likely Benign / No Variant
n = 814
d
p = 0.00054
Pathogenic/Likely Pathogenic
VUS
7.8%
8.3%
83.8%
Likely Benign / No Variant
Under 50
n = 204
2.9%
4.6%
92.5%
Over 50
n = 695

Table 1 Patient demographics and clinical characteristics of the cohort.

	Yale Cohort	UK Cohort	Whole Cohort
Demographics			
Number (%)	732 (75.7)	235 (24.3)	967 (100)
Age at Diagnosis, Median	60	60	60
Min	10	11	10
Max	86	84	86
Male (%) [a]	503 (68.7)	154 (65.5)	657 (67.9)
Female (%)	223 (30.4)	80 (34.2)	303 (31.3)
Caucasian (%)	634 (86.7)	200 (85.2)	833 (86.1)
Probable/Proven Family History (%)	214 (29.2)	31 (13.2)	245 (25.3)
No Family History (%) [b]	444 (60.7)	NA*	NA*
Undergone Aortic Surgery (%)	732 (100)	199 (84.7)	931 (96.3)
Primary Aortic Pathology			
Aneurysm (%)	650 (88.8)	151 (64.3)	801 (82.8)
Dissection (%)	72 (9.8)	79 (33.6)	151 (15.6)
PAU/IMH (%)	10 (1.4)	4 (1.7)	14 (1.4)
Rupture (%)	3 (0.4)	2 (0.9)	5 (0.5)
Primary Anatomical Presentation			
Ascending/Arch (%)	683 (93.3)	152 (64.7)	835 (86.3)
Descending/Thoracoabdominal (%)	48 (6.6)	83 (35.3)	131 (13.5)
Aortic Size			
Maximum Aortic Diameter (cm), Median	5.1	5.5	5.1
Min	3.4	2.7	2.7
Max	11	13	13
Maximum Aortic Diameter < 5.5cm (%)	488 (66.7)	103 (43.8)	591 (61.1)
Known Syndrome [c]			
MFS (%)	4 (0.5)	20 (8.5)	24 (2.5)
LDS (%)	0 (0)	3 (1.3)	3 (0.3)
EDS (%)	0 (0)	0 (0)	0 (0)
Other (%) [d]	1 (0.1)	2 (0.9)	3 (0.3)

PAU, penetrating aortic ulcer. IMH, intramural haematoma. MFS, Marfan Syndrome. LDS, Loeys-Dietz syndrome. EDS, Ehlers-Danlos syndrome. NA, not available. * Absence of family history was only recorded for the Yale cohort. a. Gender information un available for 7 patients. b. Family history unavailable for 207 patients in UK cohort and 78 in Yale cohort. c. The majority of known Marfan patients in the Yale cohort were operated on as emergencies without research consent, explaining the low number of Marfan cases within the Yale cohort. d. One individual from the Yale cohort had several features suggestive of a connective tissue disorder but did not fit any classic syndrome presentations while two individuals from the UK cohort had scoliosis in addition to TAAD.

Table 2 Pathogenic and likely pathogenic variants identified by the NGS panels

Gene Affected	Variant	Functional Category	Classification	Previously Reported?	ID	Primary Diagnosis	Clinical Diagnosis	Family History
COL1A1	c.1042G>A:p.A348T	missense	Likely Pathogenic	yes	Y_109_21	Aneurysm	N/A	no
COL1A1	c.1042G>A:p.A348T	missense	Likely Pathogenic	yes	Y_17_1	Dissection	N/A	no
COL1A1	c.2932C>T:p.Pro978Ser	missense	Likely Pathogenic	yes	Y_12_61	Aneurysm	N/A	no
COL1A1	c.2932C>T:p.Pro978Ser	missense	Likely Pathogenic	yes	Y_50_38	Aneurysm	N/A	yes
COL3A1	c.1178G>A:p.Gly393Asp	missense	Likely Pathogenic	no	Y_5_23	Aneurysm	Other*	yes
COL3A1	c.1204G>A:p.Gly402Ser	missense	Likely Pathogenic	no	Y_112_51	Aneurysm	N/A	no
COL3A1	c.1744G>A:p.Gly582Ser	missense	Pathogenic	yes	UK_24_0727	Aneurysm	N/A	unknown
COL3A1	c.536delC:p.Pro179GlnfsTer43	frameshift	Pathogenic	no	Y_130_31	Aneurysm	N/A	yes
COL5A1	c.2504G>C:p.Gly835Ala	missense	Likely Pathogenic	no	Y_68_20	Aneurysm	N/A	no
COL5A2	c.3275G>A:p.Gly1092Asp	missense	Likely Pathogenic	no	UK_21_0261	Aneurysm	N/A	unknown
COL5A2	c.808G>A:p.Gly270Ser	missense	Likely Pathogenic	no	Y_1_1	Aneurysm	N/A	yes
FBN1	c.59A>G:p.Tyr20Cys	missense	Likely Pathogenic	yes	Y_128_61	Aneurysm	N/A	no
FBN1	c.626G>A:p.Cys209Tyr	missense	Likely Pathogenic	yes	Y_95_7	Dissection	N/A	unknown
FBN1	c.1090C>T:p.Arg364Ter	stop gained	Pathogenic	yes	UK_24_0907	Aneurysm	Marfan	yes
FBN1	c.1090C>T:p.Arg364Ter	stop gained	Pathogenic	yes	UK_24_0916	Aneurysm	Marfan	yes
FBN1	c.1422T>G:p.Cys474Trp	Missense	Likely Pathogenic	yes	UK_24_0712	Dissection	Marfan	yes
FBN1	c.1468+5G>A	splice region	Likely Pathogenic	yes	UK_24_0719	Aneurysm	N/A	unknown
FBN1	c.1468+5G>A	splice region	Likely Pathogenic	yes	UK_24_0720	Aneurysm	N/A	unknown
FBN1	c.2168-1G>T	splice acceptor	Likely Pathogenic	no	Y_82_41	Aneurysm	N/A	unknown
FBN1	c.2306G>A:p.Cys769Tyr	missense	Likely Pathogenic	yes	UK_24_0904	Aneurysm	Marfan	yes
FBN1	c.2554_2555dupAC:p.Cys853LeufsTer20	frameshift	Pathogenic	no	UK_21_0250	Dissection	Marfan	yes
FBN1	c.2581C>T:p.Arg861Ter	stop gained	Pathogenic	yes	Y_17_1	Dissection	N/A	no
FBN1	c.2645C>T:p.Ala882Val	missense	Likely Pathogenic	yes	UK_21_0003	Aneurysm	N/A	unknown
FBN1	c.2896G>T:p.Glu966Ter	stop gained	Pathogenic	yes	UK_21_0281	Dissection	N/A	unknown
FBN1	c.3012C>G:p.Tyr1004Ter	stop gained	Pathogenic	yes	UK_21_0083	Dissection	N/A	unknown
FBN1	c.3193delG:p.Glu1065LysfsTer23	frameshift	Pathogenic	yes	Y_21_18	Aneurysm	N/A	yes
FBN1	c.4406G>C:p.Arg1469Pro	missense	Likely Pathogenic	yes	Y_133_86	Aneurysm	Marfan	yes
FBN1	c.5235_5236dupTA:p.Thr1746IlefsTer148	frameshift	Pathogenic	no	UK_21_0242	Aneurysm	Marfan	yes
FBN1	c.5917+6T>C	splice region	Likely Pathogenic	yes	UK_24_0796	Aneurysm	Marfan	unknown
FBN1	c.5917+6T>C	splice region	Likely Pathogenic	yes	UK_24_0842	Aneurysm	Marfan	yes

FBN1	c.6402dupC:p.Asp2135ArgfsTer4	frameshift	Pathogenic	no	UK_21_0355	Aneurysm	Marfan	yes
FBN1	c.7039_7040delAT:p.Met2347ValfsTer19	frameshift	Pathogenic	yes	Y_26_51	Aneurysm	N/A	no
FBN1	c.7788C>A:p.Tyr2596Ter	stop gained	Likely Pathogenic	no	Y_94_61	Aneurysm	N/A	yes
FBN1	c.7956T>A:p.Cys2652Ter	stop gained	Pathogenic	no	Y_47_31	Aneurysm	N/A	yes
FBN1	c.8504dupC:p.Leu2836ThrfsTer3	frameshift	Likely Pathogenic	no	Y_56_99	Aneurysm	N/A	yes
FBN1	c.8504dupC:p.Leu2836ThrfsTer3	frameshift	Likely Pathogenic	no	Y_59_47	Aneurysm	N/A	yes
MYH11	c.1A>G:p.Met1?	start loss	Likely Pathogenic	no	Y_91_1	Aneurysm	N/A	unknown
MYH11	c.4861A>C:p.Lys1621Gln	missense	Likely Pathogenic	yes	Y_20_10	Aneurysm	N/A	no
MYH11	c.5273G>A:p.Arg1758Gln	missense	Likely Pathogenic	yes	Y_21_41	Aneurysm	N/A	no
MYH11	c.5273G>A:p.Arg1758Gln	missense	Likely Pathogenic	yes	Y_35_28	Aneurysm	Marfan	no
MYLK	c.2390+2T>C	splice donor	Likely Pathogenic	no	Y_19_18	Aneurysm	N/A	yes
MYLK	c.5275T>C:p.Ser1759Pro	missense	Likely Pathogenic	yes	Y_84_18	Dissection	N/A	yes
SLC2A10	c.394C>T:p.Arg132Trp	missense	Likely Pathogenic	yes	Y_51_21	Aneurysm	N/A	yes
SLC2A10	c.648C>G:p.Tyr216Ter	stop gained	Pathogenic	no	Y_91_1	Aneurysm	N/A	unknown
TGFBR1	c.974-2A>G	splice acceptor	Likely Pathogenic	no	Y_105_61	Dissection	N/A	no
TGFBR1	c.1255+1G>A	splice donor	Pathogenic	no	UK_21_1025	Dissection	N/A	unknown
TGFBR2	c.1489C>T:p.Arg497Ter	stop gained	Pathogenic	yes	Y_18_71	Aneurysm	N/A	yes
TGFBR2	c.1524+1G>T	splice donor	Pathogenic	no	UK_24_0795	Aneurysm	LDS	unknown
TGFBR2	c.1609C>T:p.Arg537Cys	missense	Likely Pathogenic	no	Y_112_30	Aneurysm	N/A	yes

LDS, Loeys-Dietz Syndrome.

*This individual had a suspected but unconfirmed connective tissue disorder

Table 3 Probability of harbouring a pathogenic or likely pathogenic variant according to phenotype

	Total	Number (Percentage) with a Pathogenic or Likely Pathogenic Variant Validated by Sanger	RR (95% CI)	P-Value
Syndromic	30	13 (43)	11.94 (7.06-20.20)	$9.89e^{-11}$
Young Age, < 50	237	26 (11)	3.83 (2.20-6.67)	$4.28e^{-6}$
Known or Probable Family History	257	23 (9)	3.80 (1.88-7.66)	$1.39e^{-4}$
Ascending Aorta	477	29 (6)	1.84 (1.04-3.27)	0.036
Male	694	28 (4)	0.68 (0.39-1.20)	0.20
Presence of Dissection	158	9 (6)	1.28 (0.63-2.59)	0.53
Short-Term Mortality*	575	24 (5)	1.18 (0.42-3.32)	1.00
Large Aortic Diameter (>5cm)	612	29 (5)	1.05 (0.60-1.84)	0.88

* Mortality data was only available from the Yale cohort