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Targeted Sequencing of Human Aorta Tissue Reveals Undiagnosed Heritable Thoracic Aortic Disease

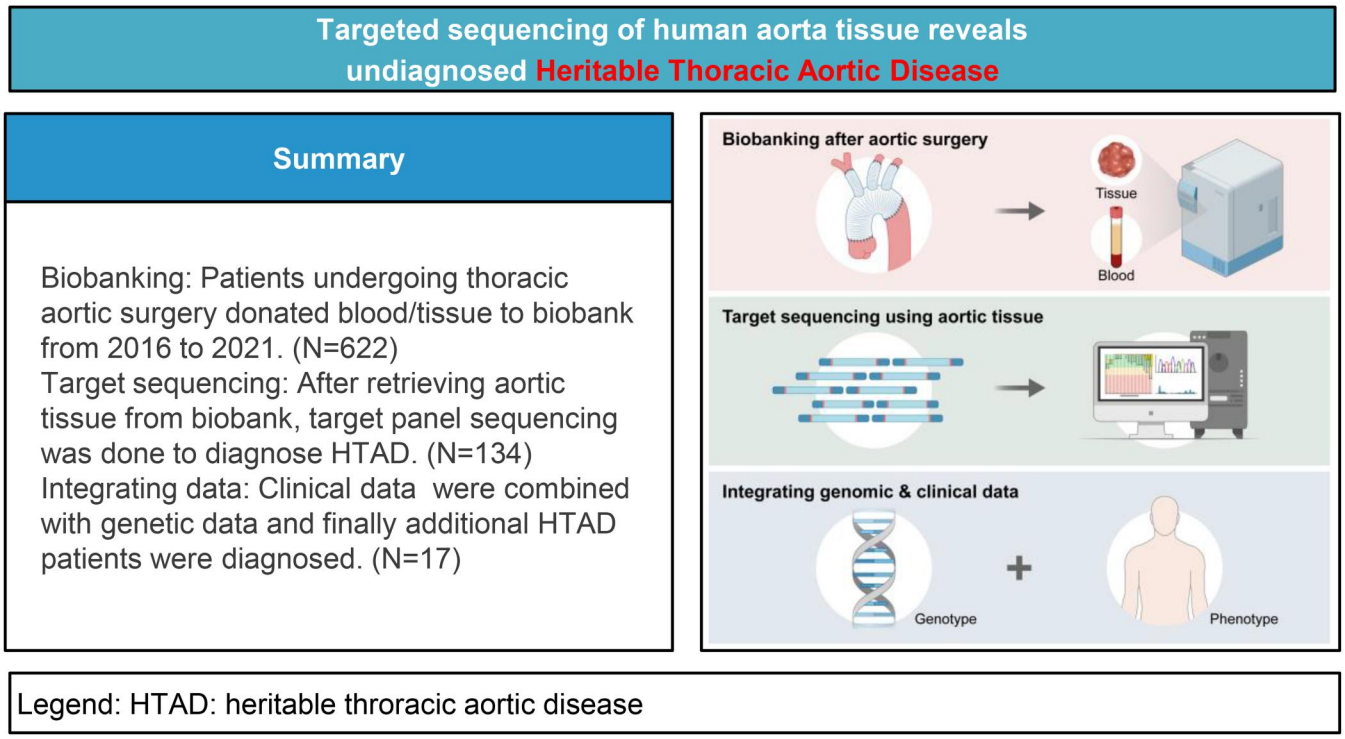
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Graphical abstract



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Abstract

Objectives: To diagnose undiagnosed heritable thoracic aortic disease (HTAD) using targeted next-generation sequencing technology on human aorta tissue obtained from a single-centre biobank.

Methods: From May 2016 to November 2021, 622 patients undergoing surgical repair for thoracic aortic aneurysm or acute aortic syndrome donated blood or tissue samples to the research biobank. A total of 134 aortic tissue samples were retrieved from patients suspected of HTAD. The inclusion criteria were (1) family history, (2) age ≤ 45, (3) annuloaortic ectasia, (4) bicuspid aortic valve, (5) patent ductus arteriosus, (6) polycystic kidney disease, or (7) patients previously classified as variant of uncertain significance by germline sequencing ($n = 17$). Exclusion criteria included patients previously classified as likely pathogenic or pathogenic by germline sequencing. The targeted panel included 96 genes.

Results: A total of 29 variants were identified including *FBN1*, *MYH11*, *COL11A1*, *SMAD3*, *SMAD6*, *ACTA2*, *COL3A1*, *FLNA*, *PKD2*, *THSD4*, *ACVRL1*, and *FBN2*. A total of 27 patients (20.1%) had variants with 2 patients having digenic variants. Clinical data were combined with genetic data, and finally, 17 patients (12.7%) were additionally diagnosed with HTAD.

Conclusions: Genomic data analysis of human aortic tissue allowed for additional diagnoses of undiagnosed HTAD, contributing to resolving discrepancies between clinical and genomic data. Considering the surgical treatment of HTAD, human aortic tissue can be a valuable resource for genomic analysis.

Keywords: heritable thoracic aortic disease; next-generation sequencing; Biobank; rare disease.

INTRODUCTION

Thoracic aortic aneurysm (TAA), characterized by progressive dilatation of the thoracic aorta, may culminate in acute aortic syndrome (AAS), including dissection or rupture.[1] Surgery remains the primary therapeutic principle in TAA, involving excision of the diseased aorta and replacement with a synthetic graft. This process yields substantial pathological tissue. While this tissue is often discarded, it offers unique opportunities for translational research, particularly for cardiovascular surgeons who are uniquely positioned to bridge the gap between the operating theatre and the laboratory.

Approximately 20% of TAA cases are heritable thoracic aortic disease (HTAD), caused by genetic mutations.[2] Since the early 2000s, next-generation sequencing (NGS) has revolutionized DNA sequencing,[3] improving diagnosis of rare genetic diseases and identifying over 50 causative genes for HTAD. Despite diagnostic advances, clinical identification of HTAD remains challenging. Barriers include financial concerns, discordance between phenotype and genotype due to de novo mutations or mosaicism,[4] and variable expressivity with incomplete penetrance.[5] These difficulties highlight the unmet clinical need that this study aims to address.

On the other hand, the concept of "biobank" has become a cornerstone of precision medicine.[6] In rare diseases such as HTAD, where genotype-phenotype discordance is common, databases from biobanks are essential to resolve discrepancies. The utilization of aortic tissue rather than peripheral blood offers distinct advantages, including the potential to detect tissue-specific somatic mosaicism. This study aims to diagnose undiagnosed HTAD by analysing aortic tissue donated to a biobank after surgery at a single centre.

METHODS

Biobanking of human aorta tissue

This study was approved by the Institutional Review Board of Gangnam Severance Hospital, Yonsei University College of Medicine (Yonsei Institutional Review Board no. 3-2021-0434). Participant-derived data and biological materials were collected and stored in accordance with the WMA Declaration of Taipei. Informed consent for the donation of human-derived materials was obtained from patients who underwent either elective surgery for TAA or emergency surgery due to AAS. Written consent was obtained from all participants or legal guardians. Aortic tissue or blood samples were stored in the Gangnam Severance Hospital Biobank. After surgery, the harvested aortic tissues were refrigerated at 4°C for 1-4 days, after which a portion was cut into 1 × 1 cm pieces and was stored at −80°C in a deep freezer.

Patient cohort and statistical analysis

Between May 2016 and November 2021, a total of 622 patients' aortic tissue or blood samples were stored in the Gangnam Severance Hospital Biobank. Among these, 573 patients had aortic tissue stored. Aortic tissue samples were retrieved from 140 patients who met one or more of the following inclusion criteria: (1) family history (defined as a history of aortic valve or aortic surgery, unexplained sudden death, or heart failure in first-degree relatives), (2) age ≤ 45 years, (3) annuloaortic ectasia (AAE, defined as a diameter of 4 cm or more), (4) bicuspid aortic valve (BAV), (5) patent ductus arteriosus, (6) polycystic kidney disease, or (7) patients previously classified as a variant of uncertain significance (VUS) in

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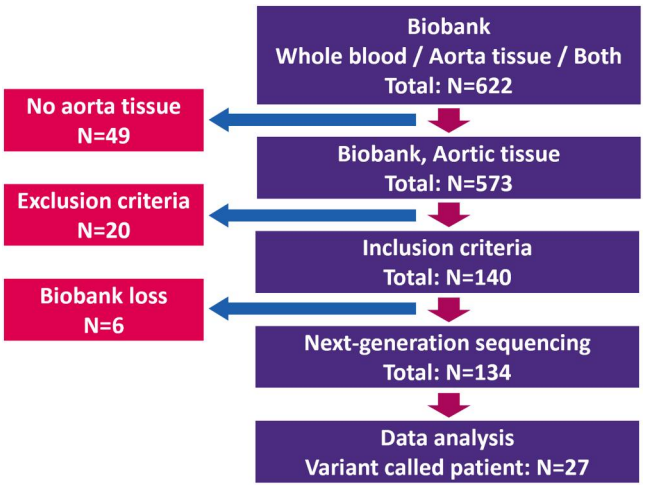


Figure 1. Study Flow Chart. This figure visually summarizes patient recruitment, the Biobanking process, application of inclusion/exclusion criteria, and the final cohort size for NGS and data analysis, enhancing the study's transparency and reproducibility. Abbreviation: NGS, next-generation sequencing. "Biobank Loss" means missing samples during sample retrieving

prior genetic testing ($n = 17$). Of the initial 140 patients meeting the inclusion criteria, 6 aortic tissue samples were lost due to biobank processing issues, resulting in a final selection of 134 patients. Twenty patients with previously confirmed likely pathogenic (LP) or pathogenic (P) variants from genetic testing were excluded (**Figure 1**).

Comparisons were performed using Pearson's chi-squared test, the Wilcoxon rank sum test, and Fisher's exact test. Logistic regression analysis was used to identify factors associated with the variant group compared with the non-variant group and calculate odds ratios (ORs) and 95% confidence intervals (CIs). All analyses were performed using R 4.5.1 (R Foundation for Statistical Computing, http://www.r-project.org).

DNA extraction and quality control

To enable simultaneous detection of germline variants and potential somatic mosaicism, genomic DNA was extracted from frozen aortic tissue specimens using the Invitrogen Easy-DNA^{TM} kit (Thermo Fisher Scientific, USA) according to the manufacturer's instructions. DNA concentration and integrity were assessed with the Agilent TapeStation 4200 (Agilent Technologies, USA), which provides a DNA integrity number (DIN) as a standardized measure of DNA fragmentation on a scale of 1-10.

Next-generation sequencing

Ninety-six genes were included in the targeted NGS panel for HTAD. Gene selection was informed by the 2022 ACC/AHA Guideline for the Diagnosis and Management of Aortic Disease, ClinGen gene-disease validity assessments, and the Genomics England TAA or dissection panel (https://panelapp.genomicsengland.co.uk/panels, accessed August 2021). In addition, relevant published literature was reviewed to ensure comprehensive coverage of genes implicated in HTAD (**Table S1**).[7,8] For NGS, a library was prepared using the customized Capture probes produced by Celemics, Inc. The libraries were prepared using nucleic acid input according to the manufacturer's instructions. The constructed library was used for sequencing with the Illumina NextSeq 550Dx platform (Illumina, San Diego, CA, United States) with the 2×150 base pair paired-end reads.

Bioinformatics pipeline for NGS gene panel analysis

Quality control of paired-end FASTQ reads was performed using FastQC. Subsequently, Trimmomatic (v0.39) was employed to trim adapter sequences and eliminate low-quality sequences. The processed reads were aligned to the reference genome (GRCh37/hg19) using BWA-MEM (v0.7.17). Picard tools (v2.27.1) were then used to mark duplicated reads and sort BAM files. Variant calling for single-nucleotide variants and small insertions/deletions (indels) was carried out with the Genome Analysis Toolkit (GATK) HaplotypeCaller (v4.2), with VarScan (v2.4.4) used as a complementary method. Multi-allelic sites were normalized and left-aligned using Bcftools (v1.10.2). Copy number variations (CNVs) were identified using ExomeDepth. Variant annotation was performed with ANNOVAR and the Ensembl Variant Effect Predictor.

For clinical interpretation, variants were classified as P, LP, or VUS according to the ACMG/AMP standards and guidelines, with refinements based on specific recommendations.[9–11] In addition, we applied a study-specific operational framework to further stratify VUS. Variants were categorized into 3 groups: (1) P/LP; (2) VUS—favour pathogenic (VUS-FP), defined as VUS occurring in genes with reported association to HTAD that were close to the LP threshold and could be reclassified upon confirmation of a disease-consistent phenotype, segregation, or functional evidence; and (3) VUS—with reclassification potential (VUS-RP), referring to VUS with more limited baseline evidence but with a plausible path to future reclassification (eg, predicted loss-of-function variants supported by rarity or computational data). VUS-FP and VUS-RP were collectively considered as candidate variants for reclassification and were retained for targeted follow-up analyses, whereas non-candidate VUS were excluded from further consideration.

RESULTS

Patient characteristics and risk factors for variant detection

Variants were identified in 27 patients (20.1% of the cohort), and no variants were identified in 107 patients (70.9%). The demographics of this cohort were

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characterized by a median age at surgery of 49 years, with an interquartile range of 38-62 years. The variant group had a higher proportion of females (44%) compared to the no-variant group (18%), a difference that was statistically significant ($P = .003$). AAE was also significantly more common in the variant group (81%) compared to the no-variant group (57%) ($P = .019$) (**Table 1** and **Table S2**). By multivariate analysis, female (OR 4.87, 95% CI 1.80-13.75; $P = .002$) and AAE (OR 3.46, 95% CI 1.26-11.32; $P = .024$) were significant risk factors for detecting variants in this study cohort (**Table 2**, **Figure 2**).

Not all members of the cohort are related by family or genetically related.

Quality control of human aorta tissues

Quality control tests were performed on all 134 aortic tissue samples. The average DNA concentration was 79.25 ng/μL, and the DIN showed a median of 7.3 (range 2.9-9.9). A median DIN of 7.3 suggests generally good DNA quality suitable for NGS (**Figure 3**).

Table 1. Patient Demographics and Clinical Characteristics

Characteristic	Overall $N = 134$[a]	No-variant group $N = 107$[a]	Variant group $N = 27$[a]	*P*-value[b]
Sex				.003
Female	31 (23%)	19 (18%)	12 (44%)	
Male	103 (77%)	88 (82%)	15 (56%)	
Age at surgery (years)	49 (38, 62)	50 (39, 62)	48 (36, 60)	.4
Family history	33 (25%)	23 (21%)	10 (37%)	.094
AAE	83 (62%)	61 (57%)	22 (81%)	.019
BAV	29 (22%)	26 (24%)	3 (11%)	.14
PDA	3 (2.2%)	0 (0%)	3 (11%)	NA
Arch surgery				>.9
1PAR	11 (8.2%)	9 (8.4%)	2 (7.4%)	
2PAR	31 (23%)	25 (23%)	6 (22%)	
TAR	59 (44%)	48 (45%)	11 (41%)	
Root surgery				.011
Bentall	29 (22%)	24 (22%)	5 (19%)	
BioBentall	11 (8.2%)	11 (10%)	0 (0%)	
VSARR	42 (31%)	26 (24%)	16 (59%)	
DTA intervention/surgery				.085
ET	13 (9.7%)	13 (12%)	0 (0%)	
FET	8 (6.0%)	7 (6.5%)	1 (3.7%)	
DTAr	2 (1.5%)	2 (1.9%)	0 (0%)	
TAAAr	1 (0.7%)	0 (0%)	1 (3.7%)	
Emergency op.	72 (54%)	56 (52%)	16 (59%)	.5
Dissection type				.6
Aneurysm	54 (40%)	45 (42%)	9 (33%)	
Type A	69 (51%)	53 (50%)	16 (59%)	
NonA nonB	4 (3.0%)	4 (3.7%)	0 (0%)	
Type B	7 (5.2%)	5 (4.7%)	2 (7.4%)	

Abbreviations: 1PAR, 1 partial arch replacement; 2PAR, 2 partial arch replacement; AAE, annuloaortic ectasia; BAV, bicuspid aortic valve; DTA, descending thoracic aorta; DTAr, descending thoracic aorta replacement; ET, elephant trunk; FET, frozen elephant trunk; NA, not applicable; PDA, patent ductus arteriosus; TAAAr, thoraco-abdominal aorta replacement; TAR, total arch replacement; VSARR, valve sparing aortic root reimplantation.

[a]*n* (%); Median (Q1, Q3).

[b]Pearson's Chi-squared test; Wilcoxon rank sum test; Fisher's exact test.

Table 2. Multivariate Logistic Regression Analysis of Risk Factors for the Variant Group

Characteristic	Univariate analysis			Multivariate analysis		
	OR	95% CI	*P*-value	OR	95% CI	*P*-value
Sex (female)	3.71	1.49-9.24	.005	4.87	1.78-13.33	.002
Family history	2.15	0.85-5.28	.1			
Age	0.98	0.95-1.02	.3	0.97	0.94-1.00	.088
AAE	3.32	1.25-10.5	.024	3.46	1.18-10.19	.024
BAV	0.39	0.09-1.23	.15			
Other anomaly[a]	3.22	0.60-15.5	.14			

Abbreviations: AAE, annuloaortic ectasia; BAV, bicuspid aortic valve; CI, confidence interval; OR, odds ratio.

[a]Other anomaly: patent ductus arteriosus, polycystic kidney disease, coarctation of aorta, ventricle septal defect and aberrant left subclavian artery were included.

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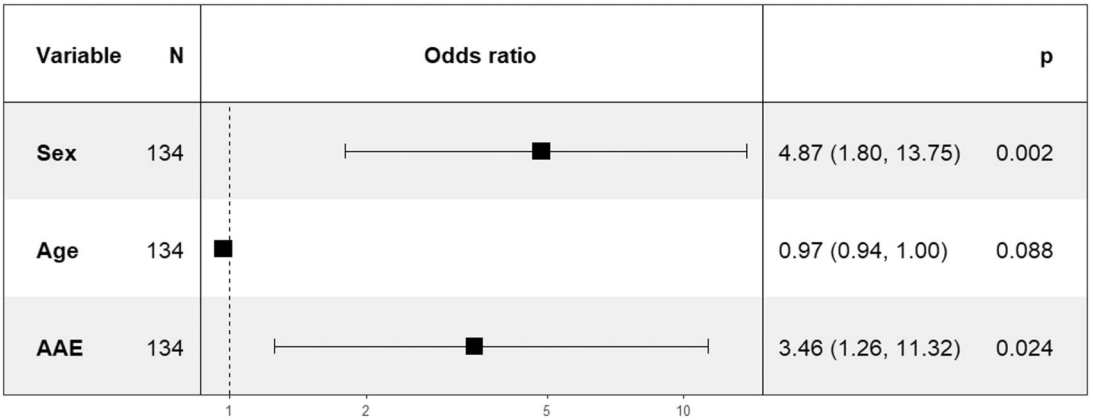


Figure 2. Forest Plot of Multivariate Analysis of Risk Factors for the Variant Group. Abbreviation: AAE, annuloaortic ectasia

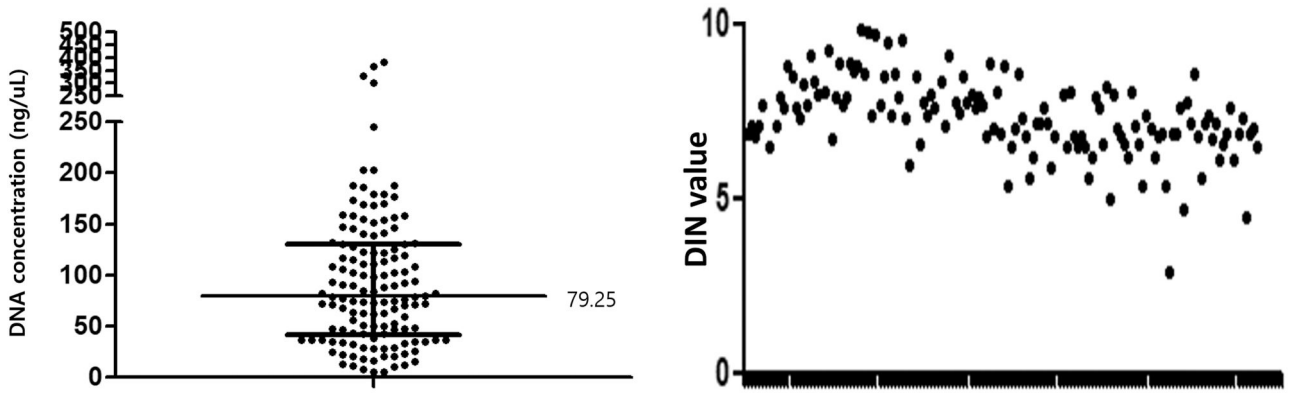


Figure 3. Quality Control of Human Aorta Tissues. This figure visualizes the distribution of DNA concentration (ng/μL) and DIN values for the 134 samples using a histogram or box plot, allowing for immediate assessment of the quality of input material used for genomic analysis. Abbreviation: DIN, DNA integrity number. DIN Scale, 1<DIN < 10; DIN = 1, highly degraded DNA; DIN = 10, highly intact DNA

Genetic variants identified in HTAD

A total of 29 variants were identified with variants in 12 specific genes: *FBN1*, *MYH11*, *COL11A1*, *SMAD3*, *SMAD6*, *ACTA2*, *COL3A1*, *FLNA*, *PKD2*, *THSD4*, *ACVRL1*, and *FBN2* (**Figure 4**). Among the 29 variant-called genes, 13 variants were classified as P/LP, 7 as VUS-FP, and 9 as VUS-RP. Variants in 2 genes were observed in 2 patients: one had both an *ACVRL1* variant and an *FBN1* variant, and the other had both an *FBN2* variant and a *SMAD3* variant (**Table 3**).

Integrating clinical and genetic data with VUS-FP variants

The 7 patients initially classified with VUS-FP variants underwent re-evaluation by a multidisciplinary team consisting of diagnostic genetic experts and aortic specialists. Of these 7 patients, 6 were ultimately reclassified as LP after integrating clinical and genetic data. For reclassification, (1) strong family history, (2) functional study, (3) population study, and (4) database updates were used.

Detailed reclassification cases

Patient 8 (*SMAD3* c.695G>T variant, p.(Trp232Leu)): Reclassified as LP, leading to a final diagnosis of LDS (**Table 3**). This was supported by a strong family history of sudden death in the mother and post-operative death of a sister due to aortic surgery, as well as in silico prediction of pathogenicity.

Patient 14 (*ACTA2* c.739G>A variant, p.(Gly247Arg)): Reclassified as LP. The patient's father had a family history of aortic valve surgery, and in silico prediction suggested pathogenicity.

Patient 21 (*MYH11* whole gene duplication): Reclassified as LP. The patient also had an ABCC6 exon 2-31 duplication, diagnosing a chromosome 16p13.11 duplication (**Figure S1**). High-level functional analysis and population studies exist[12,13] suggesting pathogenicity.

Patient 22 (*FBN1* c.1147G>A variant, p.(Glu383Lys)): Reclassified as LP. Although this variant was classified as VUS or a conflicting interpretation in 2023, updated evidence and reanalysis after 2 years led to its final reclassification as LP in 2025.

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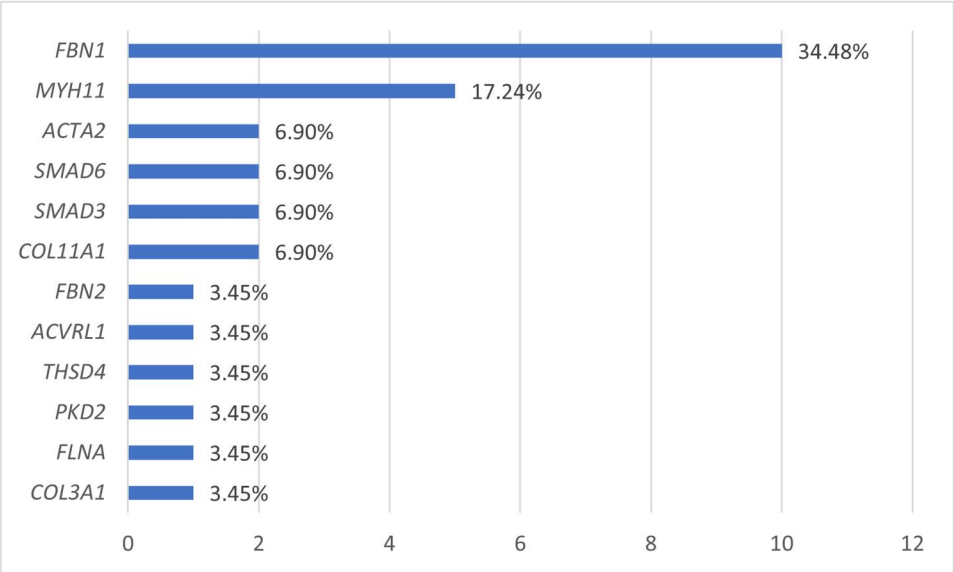


Figure 4. Variant-Called Genes of HTAD. Total 29 genes were called for HTAD-related variants. This figure presents a bar chart showing the frequency of variants identified in the 12 specific genes (*FBN1*, *MYH11*, *COL11A1*, *SMAD3*, *SMAD6*, *ACTA2*, *COL3A1*, *FLNA*, *PKD2*, *THSD4*, *ACVRL1*, and *FBN2*). *FBN1* (10 patients), *MYH11* (5 patients), *COL11A1* (2 patients), *SMAD3* (2 patients), and *SMAD6* (2 patients) were the most frequently identified, while other genes were found in one patient each. Abbreviation: HTAD, heritable thoracic aortic disease

Patient 23 (*THSD4* c.1862G>A nonsense variant, p.(Trp621Ter)): Reclassified as LP. It is predicted to result in loss of function through premature termination, consistent with the haploinsufficiency mechanism proposed for *THSD4* in heritable thoracic aortic disease. Although not previously reported, truncating variants in *THSD4* have been described in affected families, supporting loss-of-function as a relevant pathogenic mechanism.[14]

Patient 27 (*FBN2* c.6881-2A>G splice site variant and *SMAD3* c.1153A>G variant, p.(Arg385Gly)): The *SMAD3* variant was reclassified as LP. There was a strong family history including the sudden death of the grandfather, aortic surgery in the father, and a history of cerebrovascular disease and dilated cardiomyopathy in a younger brother, and in silico prediction suggested pathogenicity.

Interpreting digenic patients

In patient 26, who carried both ACVRL1 (c.936C>G, p.His312Gln) and FBN1 (c.2529del, p.Ile844SerfsTer3) variants, the phenotype was primarily driven by the FBN1 frameshift mutation, consistent with Marfan syndrome features (AAE) (**Table 3**). The ACVRL1 variant is associated with vascular malformations, not with AAE.

In patient 27, who carried both FBN2 (c.6881-2A>G) and SMAD3 (c.1153A>G, p.Arg385Gly) variants, we prioritized SMAD3 as the causative gene. While the FBN2 splice site variant is classified as LP, FBN2 mutations have a less clear association with aggressive aortic disease.

DISCUSSION

This study could not definitively diagnose de novo variants, which require trio testing with parental samples. Only clinical suspicion based on family history was possible, highlighting a methodological limitation. Similarly, no cases of somatic mosaicism were detected, likely due to its low prevalence of <1% and the limited cohort. For example, Arnaud et al[15] identified 5 cases of somatic mosaicism in a study of 1961 MFS patients, and Yang et al[16] also diagnosed 2 cases of somatic mosaicism for *FBN1* pathogenic variants among 376 HTAD patients.

Four patients in this study were diagnosed with HTAD despite being aged 60 years or older and having no family history. According to the guidelines,[8] HTAD is not suspected in TAA patients aged 60 or older without a family history, suggesting the need to lower the threshold for genetic testing in atypical presentations of TAA, potentially broadening the diagnostic scope and improving patient outcomes. In this study, according to multivariate analysis and receiver operating characteristic curve analysis of age (**Figure S2**), strict inclusion criteria by age may have led to an underestimation of HTAD prevalence in the total cohort.

One patient's *FBN1* variant was reclassified from VUS to likely pathogenic upon reanalysis. According to a report by Liu et al,[17] the diagnostic yield for rare diseases in a cohort of 250 patients who underwent whole exome sequencing increased from 24.8% in 2012 to 46.8% upon reanalysis in 2017. Regular review of variants is essential, as new evidence can resolve previously uncertain cases, influencing diagnosis and management. Therefore, regular reanalysis is needed for the 10 patients in this study who still have VUS.

In the case of *MYH11* whole gene duplication, high-level functional analysis and population studies exist.[12,13] A study by Kwartler et al[12] showed that overexpression of the Myh11 protein due to *MYH11* whole gene duplication in mouse aortic vascular smooth muscle cells causes aortic aneurysm formation. Therefore, *MYH11* whole gene duplication can be reclassified as pathogenic based on functional evidence. Although *FLNA* whole gene

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Table 3. Clinical and Genetic Data of Patients with VUS, LP, and Pathogenic Variants

No	Sex	Age	Diagnosis	Operation	F.Hx	AAE	BAV	PDA	PKD	Gene	Nucleotide change	Protein change	Variant	Effect	ACMG	OMIM
1	M	29	AAE	Bentall	+	+	–	–	–	*FBN1*	c.4927dup	p.(Thr1643AsnfsTer5)	indel	Frameshift	LP/P	MFS
2	M	35	TAAA	TAAA	–	+	–	–	–	*FBN1*	c.6295T>C	p.(Cys2099Arg)	SNP	Missense	LP/P	MFS
3	F	57	AAE	VSARR	+	+	–	–	–	*FBN1*	c.4927dup	p.(Thr5643AsnfsTer5)	indel	Frameshift	LP/P	MFS
4	F	29	ATAAD	VSARR+TAR	+	+	–	–	–	*FBN1*	c.6296G>T	p.(Cys2099Phe)	SNP	Missense	LP/P	MFS
5	F	34	AAE	VSARR	+	+	–	–	–	*FBN1*	c.4911C>G	p.(Tyr1637Ter)	SNP	Nonsense	LP/P	MFS
6	M	36	ATAAD	2PAR	+	–	–	–	–	*COL3A1*	c.2689G>A	p.(Gly897Ser)	SNP	Missense	LP/P	vEDS
7	M	45	CTAAD	Bentall	–	+	–	–	–	*FLNA*	whole gene duplication		CNV		VUS-RP	Cardiac valvular dysplasia
8	M	48	CTAAD	Bentall+2PAR	+	+	–	–	–	*SMAD3*	c.695G>T	p.(Trp232Leu)	SNP	Missense	VUS-FP	LDS3
9	M	48	BAV	2PAR	–	–	+	–	–	*COL11A1*	c.2758C>A	p.(Pro920Thr)	SNP	Missense	VUS-RP	Stickler syndrome
10	M	53	CTAAD	Bentall+TAR	–	+	–	–	–	*SMAD6*	exon 1 deletion		CNV		VUS-RP	Aortic valve disease 2
11	M	56	AAE	VSARR+TAR	–	+	+	+	–	*SMAD6*	exon 1 deletion		CNV		VUS-RP	Aortic valve disease 2
12	F	63	ATAAD	TAR	–	+	–	–	–	*MYH11*	c.3719T>G	p.(Leu1240Arg)	SNP	Missense	VUS-FP	Aortic aneurysm, familial thoracic 4
13	F	65	ATAAD	2PAR	–	+	–	–	–	*FBN1*	c.4588C>T	p.(Arg1530Cys)	SNP	Missense	LP/P	MFS
14	F	36	ATAAD	VSARR+TAR	+	+	–	–	–	*ACTA2*	c.739G>A	p.(Gly247Arg)	SNP	Missense	VUS-FP	Aortic aneurysm, familial thoracic 6
15	M	60	AAE	VSARR	–	+	–	–	–	*FBN1*	c.6695G>A	p.(Cys2232Tyr)	SNP	Missense	LP/P	MFS
16	F	64	ATAAD	VSARR+TAR	+	+	–	–	–	*FBN1*	c.5431G>A	p.(Glu1811Lys)	SNP	Missense	LP/P	MFS
17	F	64	ATAAD	1PAR	–	–	–	–	+	*PKD2*	exon 3-5 deletion		CNV		LP/P	PKD2
18	M	36	CTAAD	VSARR+1PAR	–	+	–	+	–	*MYH11*	c.4661_4681del	p.(Glu1554_Asp1560del)	indel		VUS-RP	Aortic aneurysm, familial thoracic 4
19	F	37	AAE	VSARR	–	+	–	–	–	*COL11A1*	c.3688C>G	p.(Gln1230Glu)	SNP	Missense	VUS-RP	Stickler syndrome
20	M	41	ATAAD	VSARR+TAR	–	+	–	+	–	*ACTA2*	c.338A>G	p.(Asn113Ser)	SNP	Missense	VUS-RP	Aortic aneurysm, familial thoracic 6
21	M	50	ATAAD	VSARR+2PAR	–	+	–	–	–	*MYH11*	whole gene duplication		CNV		VUS-FP	Aortic aneurysm, familial thoracic 4
22	M	51	ATAAD	VSARR+TAR	–	+	–	–	–	*FBN1*	c.1147G>A	p.(Glu383Lys)	SNP	Missense	VUS-FP	MFS
23	M	61	AAE	VSARR+2PAR	–	+	+	–	–	*THSD4*	c.1862G>A	p.(Trp621Ter)	SNP	Nonsense	VUS-FP	Aortic aneurysm, familial thoracic 12
24	F	67	AAE	VSARR+TAR	–	+	-	–	–	*MYH11*	c.4358T>G	p.(Phe1453Cys)	SNP	Missense	VUS-RP	Aortic aneurysm, familial thoracic 4
25	M	42	ATAAD	VSARR+TAR	–	+	–	–	–	*MYH11*	c.3391C>T	p.(Arg1131Trp)	SNP	Missense	VUS-RP	Aortic aneurysm, familial thoracic 4
26	F	46	AAE	VSARR	–	+	–	–	–	*ACVRL1*	c.936C>G	p.(His312Gln)	SNP	Missense	LP/P	Telangiectasia, hereditary haemorrhagic, type 2
										FBN1	c.2529del	p.(Ile844SerfsTer3)	indel	Frameshift	LP/P	MFS
27	F	37	ATAAD	VSARR+TAR	+	+	–	–	–	*FBN2*	c.6881-2A>G		SNP	Splice site	LP/P	Contractural arachnodactyly, congenital
										SMAD3	c.1153A>G	p.(Arg385Gly)	SNP	Missense	VUS-FP	LDS3

Abbreviations: 1PAR, 1 partial arch replacement; 2PAR, 2 partial arch replacement; AAE, annuloaortic ectasia; ATAAD, acute type A aortic dissection; BAV, bicuspid aortic valve; Bentall, Bentall operation; CNV, copy number variation; CTAAD, chronic type A aortic dissection; F.Hx, family history; indel, insertion-deletion; LDS3, Loeys-Dietz syndrome type 3; LP, likely pathogenic; MFS, Marfan syndrome; P, pathogenic; PDA, patent ductus arteriosus; PKD, polycystic kidney disease; SNP, single-nucleotide polymorphism; TAAA, thoracoabdominal aortic aneurysm (repair); TAR, total arch replacement; vEDS, vascular-type Ehlers-Danlos syndrome; VSARR, valve sparing aortic root reimplantation; VUS-FP, variant of uncertain significance-favour pathogenic; VUS-RP, variant of uncertain significance-reclassification potential.

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duplication was identified in this study, it was concluded as a VUS due to a lack of functional analysis or population studies. However, considering that *FLNA* is involved in the cytoskeletal system, similar to *MYH11*, *FLNA* whole gene duplication should be considered a potential cause of HTAD, requiring additional functional analysis.[18] In the case of *SMAD6* exon 1 deletion, it was diagnosed in 2 unrelated patients. Considering recent genetic studies linking *SMAD6* to BAV and TAA,[19] and the high conservation score of the exon 1 region,[20] exon 1 deletion should also be considered for reclassification if additional evidence accumulates. The detailed discussion of specific CNVs (*MYH11*, *FLNA*, and *SMAD6*) underscores the growing importance of structural variants beyond single-nucleotide variants in HTAD pathogenesis.

In this study, RNA sequencing was also attempted but failed due to low RNA integrity numbers (RINs), largely from RNA degradation during 1-4 days of refrigeration before freezing. Such methodological issues directly influenced outcomes, stressing the importance of prompt processing. For example, Mizrak et al[21] obtained RIN values ≥7 by immediately embedding aorta tissue in optimal cutting temperature compound and performed spatial transcriptomics analysis. This indicates that with optimized institutional protocols, transcriptomics and RNA sequencing could be feasible in future research retrieving aortic tissues from the biobank.

Limitations

This study has several limitations. First, it is a single-centre retrospective study with a relatively small sample size, which limits the generalizability of the findings and the statistical power to detect rare phenomena like mosaicism. Second, the refrigeration delay for tissue processing precluded successful RNA analysis. Third, we were unable to perform trio analysis for all patients to confirm de novo status.

CONCLUSION

Targeted panel sequencing of 134 aortic tissue samples from a single-institution biobank led to an additional 12.7% HTAD diagnosis rate. Of 6 VUS-FP cases, 5 were reclassified as LP, emphasizing the value of combining clinical and genetic data. The study indicates the feasibility of genomic research using human aortic tissue and highlights the need to suspect HTAD even in elderly or family history–negative patients. These findings contribute to improving HTAD diagnosis, resolving clinical uncertainty, and advancing precision medicine.

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AUTHOR CONTRIBUTIONS

H.L., Y.J.K., M.S.K., K.A.L., S.W.S: Conceptualization. H.L., M.S.K., Y.J.K.: Data curation. H.L., Y.J.K.: Formal analysis. K.A.L., S.W.S.: Funding acquisition. H.L., Y.J.K.: Investigation. H.L., Y.J.K., M.S.K., K.A.L., S.W.S.: Methodology. H.L., Y.J.K., M.S.K., K.A.L., S.W.S.: Project administration. H.L., Y.J.K., M.S.K., K.A.L., S.W.S.: Resources. Y.J.K., K.A.L.: Software. K.A.L., S.W.S.: Supervision. H.L., Y.J.K.: Validation. H.L., Y.J.K.: Visualization. H.L., Y.J.K.: Writing—original draft. K.A.L., S.W.S.: Writing—review & editing.

SUPPLEMENTARY MATERIAL

Supplementary material is available at *ICVTS* online.

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CONFLICTS OF INTEREST

None declared.

DATA AVAILABILITY

The data underlying this article will be shared on reasonable request to the corresponding author K.A.L. and S.W.S.

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