Journal of the American Heart Association

ORIGINAL RESEARCH

Evaluating the Feasibility of Screening Relatives of Patients Affected by Nonsyndromic Thoracic Aortic Diseases: The REST Study

Riccardo Giuseppe Abbasciano, MD; Giovanni Mariscalco, PhD; Julian Barwell, PhD; Gareth Owens, MSc (Oxon); Mustafa Zakkar, PhD; Lathishia Joel-David, BSc; Suraj Pathak, MD; Adewale Adebayo, PhD; Nora Shannon, MD; Rebecca Louise Haines, PhD; Hardeep Aujla, MRes; Bryony Eagle-Hemming, MSc; Tracy Kumar, BSc; Florence Lai, MPhil; Marcin Wozniak, PhD; Gavin Murphy, MD

BACKGROUND: Diseases of the thoracic aorta are characterized by a familial etiology in up to 30% of the cases. Nonsyndromic thoracic aorta diseases (NS-TADs) lack overt clinical signs and systemic features, which hinder early detection and prompt surgical intervention. We hypothesize that tailored genetic testing and imaging of first-degree and second-degree relatives of patients affected by NS-TADs may enable early diagnosis and allow appropriate surveillance or intervention.

METHODS AND RESULTS: We conducted a feasibility study involving probands affected by familial or sporadic NS-TADs who had undergone surgery, which also offered screening to their relatives. Each participant underwent a combined imaging (echocardiogram and magnetic resonance imaging) and genetic (whole exome sequencing) evaluation, together with physical examination and psychological assessment. The study population included 16 probands (8 sporadic, 8 familial) and 54 relatives (41 first-degree and 13 second-degree relatives) with median age 48 years (range: 18–85 years). No syndromic physical features were observed. Imaging revealed mild-to-moderate aortic dilation in 24% of relatives. A genetic variant of uncertain significance was identified in 3 families. Imaging, further phenotyping, or a form of secondary prevention was indicated in 68% of the relatives in the familial group and 54% in the sporadic group. No participants fulfilled criteria for aortic surgery. No differences between baseline and 3-month follow-up scores for depression, anxiety, and self-reported quality of life were observed.

CONCLUSIONS: In NS-TADs, imaging tests, genetic counseling, and family screening yielded positive results in up to 1 out of 4 screened relatives, including those in the sporadic NS-TAD group.

REGISTRATION: URL: https://www.clinicaltrials.gov; Unique identifier: NCT03861741.

Key Words: familial thoracic aortic aneurysm and dissection ■ genetic screening ■ whole exome sequencing

See Editorial by Cecchi et al.

Diseases of the thoracic aorta (TADs) account for 1% to 2% of all deaths in the Western countries and occur in approximately 1% of the general population,[1,2] although prevalence might be even higher according to recent series.[3] TADs are often silent entities with a mortality of almost 80% when presenting as life-threatening emergencies.[1,4,5] Consequently, early recognition and treatment are crucial elements for improving patient survival.[4,5] Unlike syndromic TADs, nonsyndromic TADs (NS-TADs) lack overt clinical signs

Correspondence to: Riccardo Giuseppe Abbasciano, Department of Cardiovascular Sciences, University of Leicester, Clinical Sciences Wing, Glenfield General Hospital, Leicester LE3 9QP, UK. E-mail: rga8@leicester.ac.uk

Supplemental Material for this article is available at https://www.ahajournals.org/doi/suppl/10.1161/JAHA.121.023741

For Sources of Funding and Disclosures, see page 11.

JAHA is available at: www.ahajournals.org/journal/jaha

CLINICAL PERSPECTIVE

What Is New?

- A screening initiative, combining genetics and imaging, could potentially optimize surveillance and management of nonsyndromic thoracic aortic disease patients, with no major psychological impact.

What Are the Clinical Implications?

- Nonsyndromic thoracic aortic diseases (NS-TAD) have a high mortality when presenting as emergencies; nonetheless, clear guidance on how to conduct surveillance is currently lacking.
- In this study, testing 70 relatives of patients affected by NS-TADs confirmed that there is often a familial etiology.
- This supports screening initiatives in families of patients with NS-TADs.

Nonstandard Abbreviations and Acronyms

FDR	first degree relative
NS-TAD	nonsyndromic thoracic aortic disease
SDR	second degree relative
TAD	thoracic aortic disease
VUS	variant of uncertain significance

and systemic features, hindering early detection and prompt surgical intervention.[6,7] Although both the European and American guidelines recommended the screening of first-degree relatives of a subject affected by TAD, tailored imaging and genetic screening programs have not been standardized to date.[4,5,8] As a result, there is uncertainty around the screening of relatives with regard to screening modality, prognosis, and genetic counseling.[6,9] Therefore, the present study aimed to investigate the feasibility of a tailored imaging and genetic testing approach in relatives of probands affected by both sporadic and familial NS-TAD.

METHODS

Study Design and Participants

The present study is a single-center, prospective, and noninterventional feasibility study, and it is registered at Clinicaltrials.gov (NCT03508505). Its detailed protocol with definition criteria is reported in Data S1 through S4, and it was approved by the East Midlands—Derby Research Ethics Committee (18/EM/0287). The data that support the findings of this study are available from the corresponding author upon reasonable request. Briefly, the study population consisted of probands affected by NS-TAD with at least 2 first-degree (FDR) or second-degree (SDR) relatives (in order to maximize recruitment in each of the families) aged ≥16 years willing to participate in the study screening program. Probands with a previous diagnosis of syndromic TAD or those affected by aortic lesions associated with other aortic etiologies, including trauma and infections, were excluded. The target recruitment included at least 8 probands with familial and 8 with sporadic NS-TADs. Participants were identified through the surgical database of the Glenfield Hospital (Leicester, United Kingdom) between January 2016 and December 2018 and subsequently were approached initially by mail and then by telephone consultation. Up to 8 FDRs and SDRs for each identified proband were enrolled. All participants were screened by a complete clinical evaluation (clinical history and examination), genetic tests, and imaging (transthoracic echocardiography [TTE] and magnetic resonance imaging [MRI]) for the presence of NS-TADs.

The present study was approved by the Health Research Authority (HRA; East Midlands—Derby n. 18.EM.0287—IRAS 247434), and complies with the Consolidated Standards of Reporting Trials (CONSORT) Statement (Figure 1).[10]

Clinical Assessment, Familial and Genetic Counseling

At the first study visit, after informed consent, all recruited participants underwent a detailed clinical evaluation, including cardiological, ophthalmological, and orthopedic assessments (when needed) to identify any syndromic features. Questionnaires assessing participants' perception and comprehension of each part of the study, health-related self-assessed Quality of Life questionnaire,[11] a Patient Health Questionnaire,[12] and a Generalized Anxiety Disorder Assessment[13] form were completed and assessed at baseline and at 3 months. Genetic counseling was offered to all participants before the recruitment, to discuss possible outcomes including variants of uncertain significance (VUSs) and incidental findings (eg, a section of the genetic code missing that includes another important gene as well) with a wider impact for the patient or family as well as implications for health insurance. Any genetic variants of uncertain significance were discussed by a multidisciplinary team, including 2 clinical geneticists, a cardiac surgeon, and a bioinformatician. Following this discussion, participants with VUSs that warranted further phenotyping were seen in an outpatient clinic along with their relatives, where results were communicated and contextualized by a clinical geneticist.

Imaging Tests

TTE was performed by a trained sonographer. Aortic diameter was measured from the parasternal long-axis view

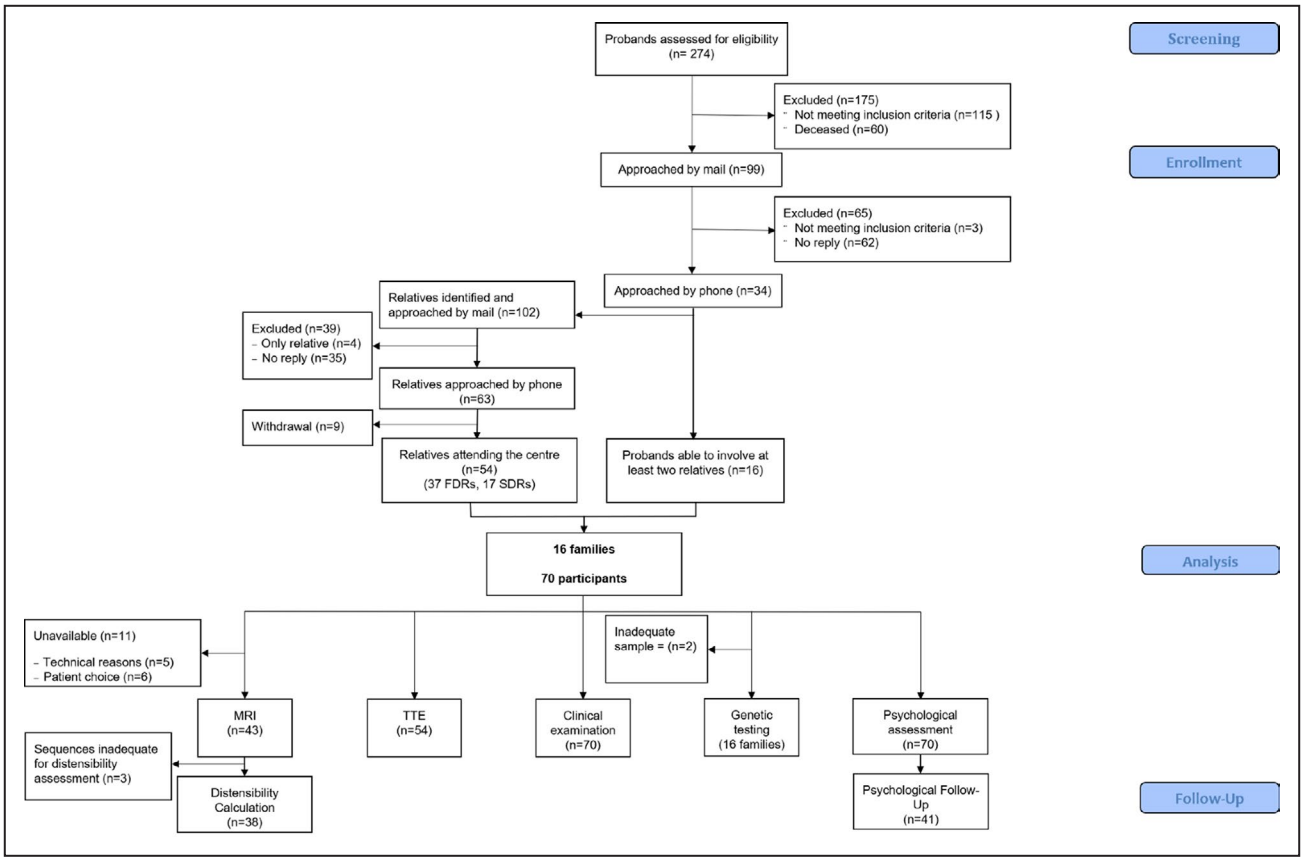


Figure 1. Consolidated Standards of Reporting Trials diagram for study recruitment and follow-up.
In 2 relatives a sufficient amount of blood could not be collected because of poor peripheral vasculature; the proband tested negative for variants in these cases. FDR indicates first-degree relative; MRI, magnetic resonance imaging; SDR, second-degree relative; and TTE, transthoracic echocardiogram.

at the level of aortic annulus, sinuses of Valsalva, widest level of the ascending aorta, aortic arch, and descending aorta.[14,15] Aortic index and *Z* score were calculated according to the published standards.[15–17] MRI of the thoracic aorta on a 3T research scanner was performed in all relatives able to attend the local hospital facility. All images used retrospective ECG gating unless arrhythmias were present in which case prospective gating was used. To decrease the breath-hold duration for the patient, parallel imaging was used in all acquisitions.[15] In patients with poor breath-holding, spatial resolution was decreased and free breathing allowed (increasing the averages to 3 for cine imaging). The internal diameters of the ascending and descending aorta were measured at the level of the pulmonary bifurcation,[15] and aortic distensibility analysis was performed as per previous recommendations.[18,19]

The adopted aortic values of references to define aortic dilatation are reported in the Tables S1 through S3.

Genetic Testing

A peripheral venous blood sample was obtained and stored at −80 °C for batch preparations of DNA suitable for genetic analysis. Samples from participants were processed internally, via a fully automated pipeline (QIAGEN QIAsymphony, Hilden, Germany), and externally subjected to whole-exome sequencing, on DNBseq platform (BGI Hong Kong Tech Solution NGS Lab, Hong Kong), where a high-throughput sequencing was performed for each captured library independently, to ensure that each sample would meet the desired average fold-coverage (x100). The bioinformatic workflow consisted of alignment, variant calling, and quality check through bwa, GATK4, and Haplotype Caller, respectively. Variants were annotated with *snpEff* (only high and moderate impact), *dbNSFP*, and *ClinVar*.[20–22] Variants were evaluated in line with the American College of Medical Genetics and Genomics guidelines for variant interpretation,[23] the Association for Clinical Genomic Science Best Practice Guidelines for variant classification in rare disease,[24] and the FBN1 Specific Variant Interpretation Guidelines from 2018.[25] Cascade sequencing was performed only when a VUS was detected in a proband.

Statistical Analysis

Continuous data are reported as mean±SD or median (range), and categorical data as number and/or

Table 1. Study Population Characteristics With Results From Physical Examination of Participants

Variables*	Familial		Sporadic		Participants
	Probands N=8	Relatives N=28	Probands N=8	Relatives N=26	Total N=70
Age, y	68.5 (51–74)	39.0 (18–85)	67.5 (41–84)	46.5 (20–77)	48.5 (18–85)
Sex	5 women; 3 men	22 women; 6 men	1 woman; 7 men	13 women; 13 men	41 women; 29 men
Height, cm	168.0 (160–177)	168.5 (156–184)	171.5 (155–183)	173.0 (157–204)	170 (155–204)
Weight, kg	70.3 (57–177)	63.8 (51–105)	83.6 (65–119)	80.0 (53–150)	71.5 (51.0–150.0)
Body mass index, kg/m^2	25.3 (22.3–33.2)	22.8 (19.9–33.0)	29.9 (21.7–38.4)	25.9 (21.0–44.3)	24.8 (19.9–44.3)
Body surface area, m^2	1.8 (1.6–2.2)	1.7 (1.5–2.2)	1.9 (1.7–2.3)	2.0 (1.5–2.7)	1.8 (1.5–2.7)
Systolic blood pressure, mm Hg	160.5 (124–202)	132.5 (96–177)	145 (109–172)	123.5 (96–171)	130.0 (96–202)
Diastolic blood pressure, mm Hg	98.5 (80–119)	87.0 (64–105)	93 (72–100)	83.0 (58–116)	86.0 (58–119)
Antihypertensive medications	8 (100%)	2 (7%)	8 (100%)	6 (23%)	24 (34%)
Beta blockers	5 (63%)	0 (0%)	4 (50%)	1 (4%)	10 (14%)
Angiotensin-converting enzyme inhibitor/ angiotensin receptor blocker	5 (63%)	2 (7%)	5 (63%)	3 (12%)	15 (21%)
Myocardial infarction	1 (13%)	0 (0%)	1 (13%)	1 (4%)	3 (4%)
Diabetes	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Cerebrovascular accident or transient ischemic attack	1 (13%)	0 (0%)	2 (25%)	0 (0%)	3 (4%)
Smoking	0 smokers 4 ex-smokers	2 smokers 4 ex-smokers	0 smokers 6 (ex-smokers)	2 smokers 4 ex-smokers	4 smokers 18 ex-smokers
Chronic obstructive pulmonary disease	0 (0%)	1 (4%)	2 (25%)	4 (15%)	7 (10%)
Impaired mobility	0 (0%)	0 (0%)	1 (13%)	0 (0%)	1 (1%)
Renal disease	0 (0%)	0 (0%)	1 (13%)	1 (4%)	2 (3%)
Peripheral vascular disease	1 (13%)	0 (0%)	0 (0%)	0 (0%)	1 (1%)

*Data expressed as median (range) and percentage or count.

percentage. Wilcoxon rank sum test, Kruskal-Wallis test, and unpaired *t* test were adopted for comparisons, as appropriate. Correlations between patient characteristics and aortic dilatation rate were assessed by Spearman's method. All statistical tests were 2 sided and a $P<0.05$ is described as statistically significant. Statistical analyses were performed using the *ggplot2*, *dplyr*, and *desctools* packages of R software (version 4.0; R Foundation for Statistical Computing, Vienna, Austria).[26–29]

RESULTS

Participants and Characteristics

A total of 276 probands operated on for TAD were identified (Figure 1), and 99 were eligible for the study criteria and approached by mail (Figure 1).

Thirty-four probands indicated a willingness to participate in the screening project (34% uptake), identifying 102 relatives who were approached by mail. However, 18 families were excluded because there were fewer than 2 eligible relatives available to be enrolled. Therefore, the final patient population included 16 probands (8 sporadic, 8 familial) and 54 relatives (41 FDRs and 13 SDRs). Of these, 70 underwent clinical examination, 68 (97%) blood sample collection for genetic testing, 54 (100% of the relatives) underwent echocardiography, 43 (80% of the relatives) underwent MRI screening, and 41 (59%) completed the psychological assessment (Figure 1).

The screened population had a median age of 49 years (range: 18 to 85 years), and 59% were women. Baseline characteristics are detailed in Table 1. As part of the physical examination, every participant underwent a series of tests to calculate the Beighton score for joint hypermobility with a mean of (0.98 ± 1.72). The prevalence of possible syndromic features detected during the clinical assessment are reported in Figure S1.

Imaging

The data obtained from imaging evaluations are summarized in Tables 2 and 3. Among all 54 relatives subjected to TTE, 10 (19%) were diagnosed with an aortic

Table 2. Imaging Features From First- and Second-Degree Relatives Involved in the Study

Imaging test	Measure*	All	Familial	Sporadic
Echocardiogram	End systolic diameter, mm	31.4 (5.4)	31.8 (4.5)	31.1 (6.3)
	End diastolic diameter, mm	46.2 (5.2)	45.4 (4.8)	47.1 (5.6)
	Septum thickness, mm	9.6 (2.0)	9.2 (2.3)	10.1 (1.7)
	Left ventricular ejection fraction, %	60.5 (5.8)	59.8 (3.3)	61.4 (7.6)
	E/A ratio	1.2 (0.4)	1.2 (0.4)	1.2 (0.3)
	Annulus, mm	22.7 (3.1)	21 (2.7)	24.3 (2.8)
	SOV, mm	30.8 (4.8)	29.4 (5)	32.4 (4.1)
	Ascending aorta, mm	30.8 (5.0)	29.5 (4.6)	32.2 (5.2)
	Distal arch, mm	24.2 (3.9)	23.9 (3.3)	24.5 (4.6)
	Abdominal aorta, mm	17.1 (2.7)	16.7 (2)	17.5 (3.2)
MRI (3-chambers view)	Annulus, mm	22.0 (2.7)	20.9 (2.1)	23.4 (2.8)
	SOV, mm	32.1 (4.8)	30.5 (5.4)	33.7 (3.6)
	Ascending aorta, mm	28.1 (5.1)	26.8 (5.3)	29.3 (4.5)
MRI (left ventricular outflow tract view)	Annulus, mm	23.8 (3.3)	22.4 (2.2)	25.5 (3.5)
	SOV, mm	32.8 (4.8)	31.7 (4.8)	34.2 (4.5)
	Ascending aorta, mm	28.9 (5.4)	27.6 (5.3)	30.3 (5.1)
MRI (distensibility)	Ascending aorta Distensibility (10^{-3} mm Hg^{-1})	5.1 (3.23)	5 (3.2)	5.2 (3.4)
	Descending aorta Distensibility (10^{-3} mm Hg^{-1})	5.3 (2.62)	5 (2.3)	5.6 (2.9)

MRI indicates magnetic resonance imaging; and SOV, sinuses of Valsalva.
*Data are reported as mean (SD).

dilatation. Five (18%) out of 28 relatives were in the familial group, and 5 (19%) out of 26 in the sporadic ones. In the familial group, the aortic dilatation was detected in 3 (17%) FDRs and 2 (20%) SDRs, respectively. In the sporadic group, aortic dilatation was observed in 4 (21%) FDRs and 1 (14%) SDRs, respectively.

Among the 43 (79%) relatives who underwent MRI, 8 (19%) were diagnosed with an aortic dilatation, including 4 (19%) out of 21 in the familial group, and 4 (18%) out of 22 in the sporadic group. In the familial NS-TAD group, the aortic dilatation was confirmed in 5 (18%) FDRs and 2 (25%) SDRs, respectively. In the sporadic NS-TAD group, the aortic dilatation was observed in 3 (20%) FDRs and 1 (14%) SDRs. MRI scanning provided additional phenotypic information in 6 screened relatives. MRI sequences allowed distensibility calculations in 38 (88%) scans. Aortic distensibility was abnormal for the ascending segment in 5 out of 38 scans (1/17 (13%)) from the familial group and 4/21 (19%) in the sporadic cohort; in the descending segment, distensibility was abnormal in 3 out of 38 scans (none of 17 participants from the familial cohort and 3/21 (14%) participants in the sporadic cohort). Aortic tortuosity was described in 1 case.

Agreement between MRI and TTE diagnoses was explored in an error matrix (Table S4). Taking the MRI positive results as confirmed cases of aortic dilatation, in our population TTE had 75% sensitivity and 97% specificity.

Overall, imaging tests identified 13 new cases with dilated aortas from all the 54 (24%) tested FDRs and SDRs. Family trees related to the sporadic and familial cohorts are presented in Figures S2 and S3. Figure 2 visually summarizes the imaging findings across all families involved in the study. In the 8 families of probands affected by familial NS-TAD, 6 (21%) relatives had aortic dilatation, with 4 out of 18 (22%) FDRs and 2 out of 10 (20%) SDRs affected. In the 8 families of probands affected by sporadic NS-TAD, 7 out of 26 (27%) had aortic dilatation with 6 out of 19 (32%) among FDRs and 1 out of 7 (14%) among SDRs. At least 1 relative in each (familial or sporadic) family was identified as affected by an aortic dilation. However, no participants fulfilled criteria for aortic surgery at the current time.

Genetic Testing

Sixty-eight participants (16/16 probands, 52/54 relatives, 34/36 familial, 34/34 sporadic, 35/37 FDR, and 17/17 SDR) underwent blood sample collection for the purpose of genetic testing. Analysis of the data occurred in probands initially. A Sankey chart demonstrating the analysis of the genetic test results is depicted in Figure 3. From the regions within the gene panel 431 variants were identified. Among these, 224 (52%) were nonsynonymous consequences (therefore the redundancy of the genetic code and the flexibility

Table 3. Results from the Imaging and Genetic Tests

Variables	Familial			Sporadic		
	FDR N (%)	SDR N (%)	Total N (%)	FDR N (%)	SDR N (%)	Total N (%)
Consented	18	10	28	19	7	26
History of smoking	1 (6%)	1 (10%)	2 (7%)	2 (11%)	0 (0%)	2 (8%)
Hypertension (at clinical assessment)	8 (44%)	5 (50%)	13 (46%)	6 (32%)	2 (29%)	8 (31%)
Antihypertensive medications	1 (6%)	1 (10%)	2 (7%)	4 (21%)	2 (29%)	6 (23%)
Underwent transthoracic echocardiogram	18 (100%)	10 (100%)	28 (100%)	19 (100%)	7 (100%)	26 (100%)
Aortic dilatation on echo	3 (17%)	2 (20%)	5 (18%)	4 (21%)	1 (14%)	5 (19%)
Underwent MRI	13 (72%)	8 (80%)	21 (75%)	15 (79%)	7 (100%)	22 (85%)
Aortic dilatation on MRI	2 (15%)	2 (25%)	4 (19%)	3 (20%)	1 (14%)	4 (18%)
Abnormal distensibility (MRI)	1 (8%)	0 (0%)	1 (5%)	3 (20%)	1 (14%)	4 (18%)
Genetic analysis finding	4 (22%)	2 (20%)	6 (21%)	2 (11%)	0 (0%)	2 (8%)
Genes affected	*NOTCH1*, *FBN1*			*FBN1*		
Disease variant	VUS	VUS	VUS	VUS	VUS	VUS
New positive genotype or phenotype	7 (54%)	4 (40%)	11 (39%)	8 (42%)	1 (14%)	9 (35%)
Imaging surveillance indicated (based on MRI)	2 (11%)	2 (20%)	4 (14%)	3 (20%)	1 (14%)	4 (15%)
Genetic medicine review indicated	4 (22%)	2 (20%)	6 (21%)	2 (11%)	0 (0%)	2 (8%)
Indication for surgery	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Secondary prevention indicated	8 (44%)	5 (50%)	13 (54%)	7 (37%)	2 (29%)	9 (35%)
Any surgery, prevention, imaging, or genetic surveillance	12 (67%)	7 (70%)	19 (68%)	12 (63%)	2 (29%)	14 (54%)

FDR indicates first-degree relative; MRI, magnetic resonance imaging; SDR, second-degree relative; TTE, transthoracic echocardiography; and VUS, variant of uncertain significance.

of protein formation would not compensate the mutation), and 207 (48%) were synonymous.

Fifty-nine (26%) of these variants were predicted to have high or moderate effects using snpEff variant predictor. Variants with this high/moderate impact rating occurred in 22 out of the 32 genes in the National Health Service Genomic Medicine Service aortopathy panel.[30] Three of the 59 had no rsIDs in dbSNP151 database. Twenty-eight of the 59 variants were considered to be rare (5% frequency based on gnomAD v2.1 exome and UK10K data in dbNSFP4.0 database, with 9 having no frequency data). After exclusion of benign and likely benign variants using ClinVar (pathogenic/likely pathogenic/uncertain significance versus benign/likely benign), a total of 14 variants were identified for interpretation according to the American College of Medical Genetics and Genomics guidelines (Table S5). Of these, 9 fulfilled the criteria for classification as a VUS and the rest were classified as benign or likely benign.

Figure 4 visually summarizes the findings from the genetic tests conducted in the enrolled families.

Among the 8 families affected by familial NS-TAD, 2 (25%) out of 8 probands demonstrated a finding of a VUS confirmed by variant interpretation according to the American College of Medical Genetics and Genomics criteria, which required additional phenotyping.[23,24] to look for specific features which might alter the classification. Four (100%) out of 4 FDRs and 2 (40%) out of 5 SDRs in these families shared the same variant identified in the proband. The genes involved were *NOTCH1* and *FBN1*. Among the 8 families of probands affected by sporadic NS-TAD, in 1 (13%) a VUS that required additional phenotyping was identified, with 2 (100%) out of 2 FDRs sharing the same variant as the proband. The gene involved was *FBN1*.

Clinical phenotyping did not provide support for these variants.

Assessments of Comprehension, Acceptability, Quality of Life, Anxiety, and Depression

There was no difference between baseline and 3-month follow-up scores for depression, anxiety, and self-reported quality of life. Only the perception of general health from the Quality of Life questionnaire was significantly lower at follow-up (P=0.009) (Table S6). Levels of comprehension and perception were comparable between probands and relatives, with the exception of the answer to "Becoming aware of the purpose of this study caused me uneasiness," which was reported as true more often in the relative cohort (P=0.047).

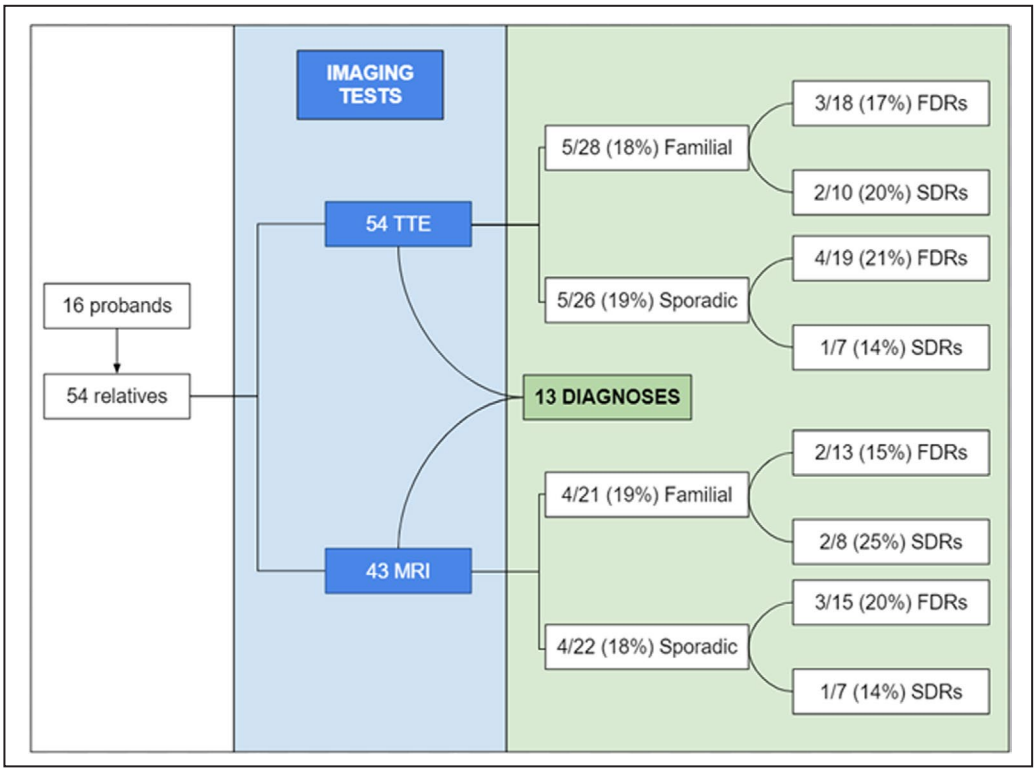


Figure 2. Summary of the findings related to the imaging study procedures.
Fifty-four participants underwent transthoracic echocardiogram as part of the study procedures, and 43 had both echocardiogram and MRI. Thirteen imaging diagnoses of mild-to-moderate aortic dilatation were reached. FDR indicates first-degree relative; MRI, magnetic resonance imaging; SDR, second-degree relative; and TTE, transthoracic echocardiogram.

Combined Clinical Assessment, Imaging, and Genetic Testing

The results of cascade tests in relatives (along with details related to the probands' diseases and imaging diagnoses) are reported in Table 3 and Table S7. In the familial NS-TAD group, 8 (44%) out of 18 FDRs and 5 (50%) out of 10 SDRs had clinical risk factors that required secondary prevention. In detail, 4 (22%) FDRs and 2 (20%) SDRs had either positive imaging tests, requiring ongoing surveillance, or a VUS requiring repeat phenotyping by a clinical geneticist. Overall, 12 (67%) FDR and 7 (70%) SDR required at least 1 subsequent management intervention (surveillance, repeat phenotyping, surgery, and/or secondary prevention).

In the sporadic NS-TAD group, 7 (37%) FDRs and 2 (29%) SDRs had clinical risk factors that required secondary prevention, whereas 6 (32%) FDRs and 1 (14%) SDR had positive imaging tests, requiring ongoing surveillance. Two (11%) FDRs had a VUS requiring repeat phenotyping and future variant review. No FDRs or SDRs had both abnormal imaging and a VUS. Overall, 12 (63%) FDRs and 2 (29%) SDRs required at least 1 subsequent management intervention (Figure 5).

DISCUSSION

The present study demonstrated the feasibility of cascade screening for relatives of probands affected by nonsyndromic thoracic aortic diseases. Although the uptake was only 34%, the detection of clinical aortopathy rate was significant, with 24% of screened relatives demonstrating a potential phenotype disease on imaging. The cascade testing identified 61% of relatives requiring further management, including surveillance, clinical genetics, surgery, or secondary prevention.

The major strength of the study was the inclusion of comprehensive clinical, imaging, and genetic testing, in an unselected cohort of probands with NS-TAD and their relatives. To our knowledge this is the first study to have included both sporadic and familial NS-TAD forms. Participants in the familial group were shorter with lower body mass index and had higher blood pressure readings despite similar levels of treatment for hypertension. Genetic testing did not detect any clinically actionable results (besides the necessity to rephenotype 3 participants to confirm the lack of clinical signs of syndromic conditions) but this is likely to be because of the sample size in the study. The frequency of positive imaging tests was comparable

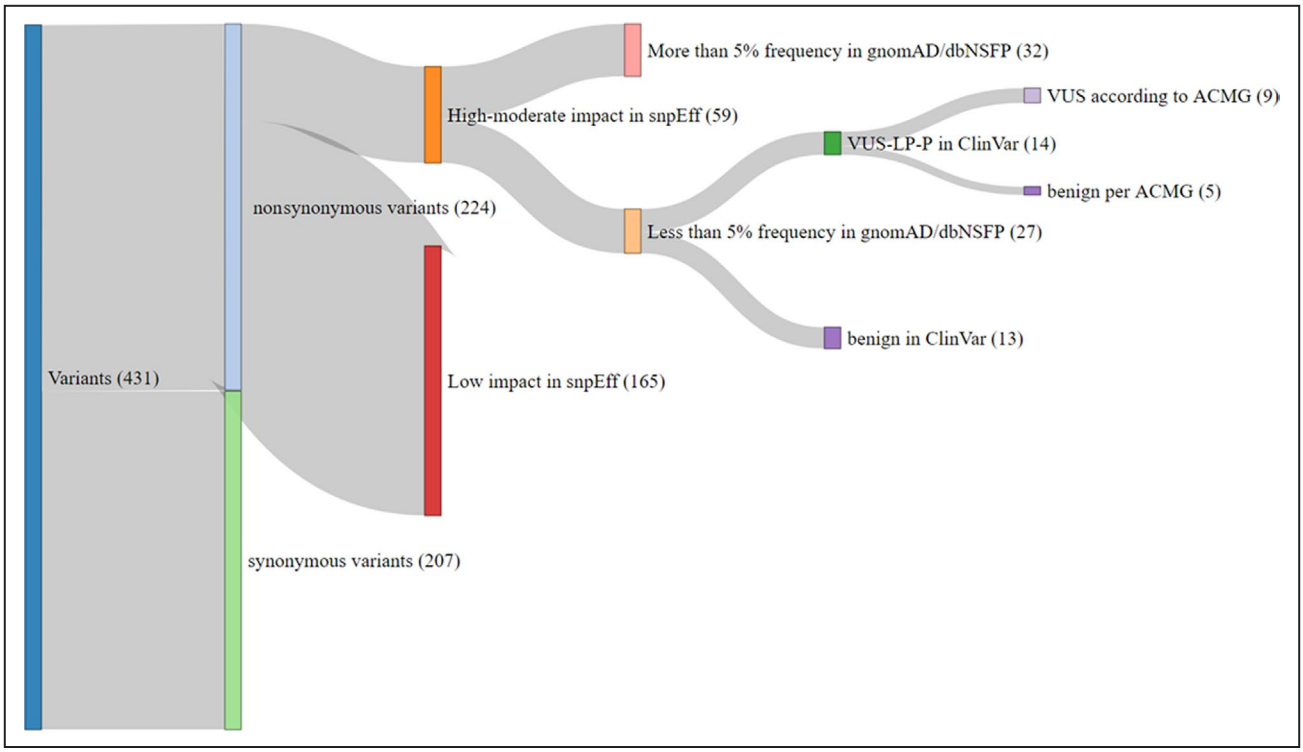


Figure 3. Flow chart describing the variant filtering and evaluation process.
The complete list was reduced by filtering for (in order) type of variant (synonymous vs nonsynonymous), rarity in gnomAD (less vs more than 5% of the general population), predicted impact of the mutation (high/moderate vs low impact), classification in ClinVar (pathogenic/likely pathogenic/uncertain significance vs benign/likely benign) and evaluated finally according to the American College of Medical Genetics and Genomics criteria. ACMG indicates American College of Medical Genetics and Genomics; VUS, variant of uncertain significance; and VUS-LP-P, variant of uncertain significance likely pathogenic-pathogenic.

in both familial and sporadic forms, highlighting the potential benefits of routine cascade screening in the often-overlooked sporadic group.

The study also had low levels of attrition for all of the assessments, allowing comparison of different testing modalities. TTE provided a specificity of 97% and a sensitivity of 75% for aortic dilatation as defined by the MRI gold standard. Abdominal aorta could be visualized by TTE in 85% of the participants, with no abdominal aortic dilatation diagnosed in our cohort. The lower specificity was offset by higher uptake in the TTE group (100% versus 79%, respectively) and the overall numbers of new disease phenotypes identified were the same for both modalities. In addition, false negatives and positives were attributable to diameters close to the limits of normal ranges indicating that diminished diagnostic accuracy may not be clinically important, particularly where repeat scans can be undertaken relatively cheaply compared with MRI. MRI provides useful additional data on distensibility and tortuosity that may have additional prognostic value; however, this requires further validation. Finally, the study demonstrated no effect of cascade screening on participant anxiety and depression levels. A small difference in 1 domain of the Quality of Life questionnaire that did not favor screening will need to be confirmed in an adequately powered study. This finding, were it confirmed on a larger population, might warrant the need for an increased care in communicating the screening rationale (and possibly the results) to specific categories of subjects. This is particularly meaningful given the relatively young age of participants and also has implications for assessments of cost-effectiveness in any future study.[31]

The major limitation of the study was the small sample size, and there is no certainty that these results would be representative of the findings of a larger study.[8] The present study was restricted to a single center without an established inherited cardiovascular disease service, and therefore uptake rates and detection rates may be higher than in some other centers. Moreover, the approach to cascade screening adopted in the study and the necessity for additional phenotyping of some participants do not reflect the standard management adopted in a clinical context and are mainly due to the research nature of the procedures described. These limitations not withstanding, however, the data suggest that cascade screening is feasible, is safe, and does identify relatives who require ongoing surveillance and secondary prevention.

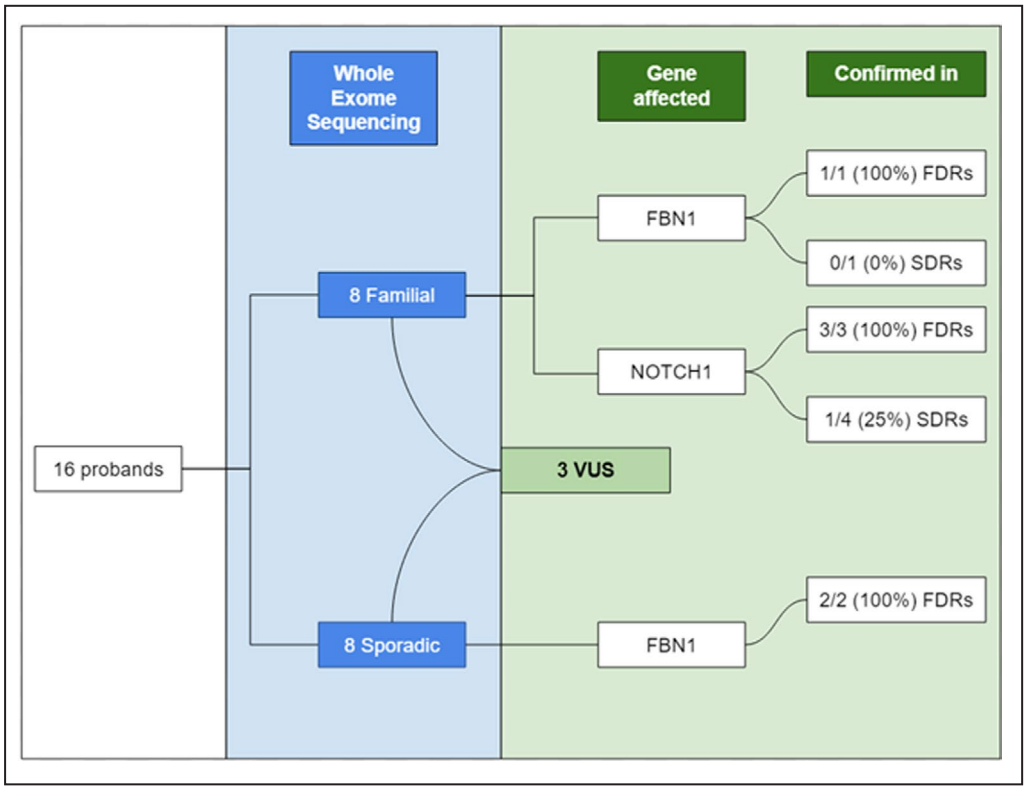


Figure 4. Summary of the findings related to the genetic test study procedures.
After ACMG evaluation and multidisciplinary team discussion, 3 participants were rephenotyped by a clinical geneticist where deep phenotyping might alter variant classification. FDR indicates first-degree relative; SDR, second degree relative; and VUS, variant of uncertain significance.

The study provided useful insights into the potential barriers to the wider introduction of such a program. First, uptake was low, accounting for 34%, possibly attributable to the limited understanding of the familial basis of TAD in people with the disease, but also more broadly, in primary and tertiary care. This suggests that education and overcoming institutional and individual barriers to cascade screening will be important components of any wider initiative. Decision support tools are increasingly used to help people make decisions around genetic testing in particular. Potential participants were also approached by mail, sometimes several years after the index admission of the proband. Uptake may be higher in the acute setting, as recommended by a recent Delphi exercise.[9] Second, in those probands who expressed an interest in the study, the uptake of cascade screening in their FDR and SDR was high, accounting for 54% of participants. This may reflect the desire of people at risk to know more about their likelihoods of developing the disease. As a matter of fact, cascade screening was identified as top research priority for aortic dissection survivors and their families in a recent survey (Aortic Dissection Awareness UK, personal communication). Third, the study identified participants with disease phenotypes and no detected genetic abnormality. This points toward a potential unmet need for further research into the interaction between genetic and environmental factors in the natural history of the condition. Finally, and accepting the limited power of the study sample size, the data constitute a potential argument in favor of imaging tests in FDRs and SDRs of both sporadic and familial groups. In contemporary clinical practice in the United Kingdom and United States, genetic and imaging testing are typically restricted to FDRs of familial cases in the first instance.[4,5,8]

Other larger studies in sporadic disease have reached similar conclusions.[32] Our work suggests that a clinical geneticist review should be sought where imaging results point toward a family history of the disease, to undertake phenotyping and aid variant interpretation. A final comment is that given the age of the participants and their comorbidities, only 1 out of the 3 probands who needed rephenotyping would have undergone testing according to the latest revision of the criteria of the National Genomic Test Directory.[30]

CONCLUSIONS

In conclusion, NS-TADs are conditions with an often-unrecognized genetic etiology. Cascade testing could return positive results in up to 1 out of 4 relatives, even in families with a first case of aortic dissection. A

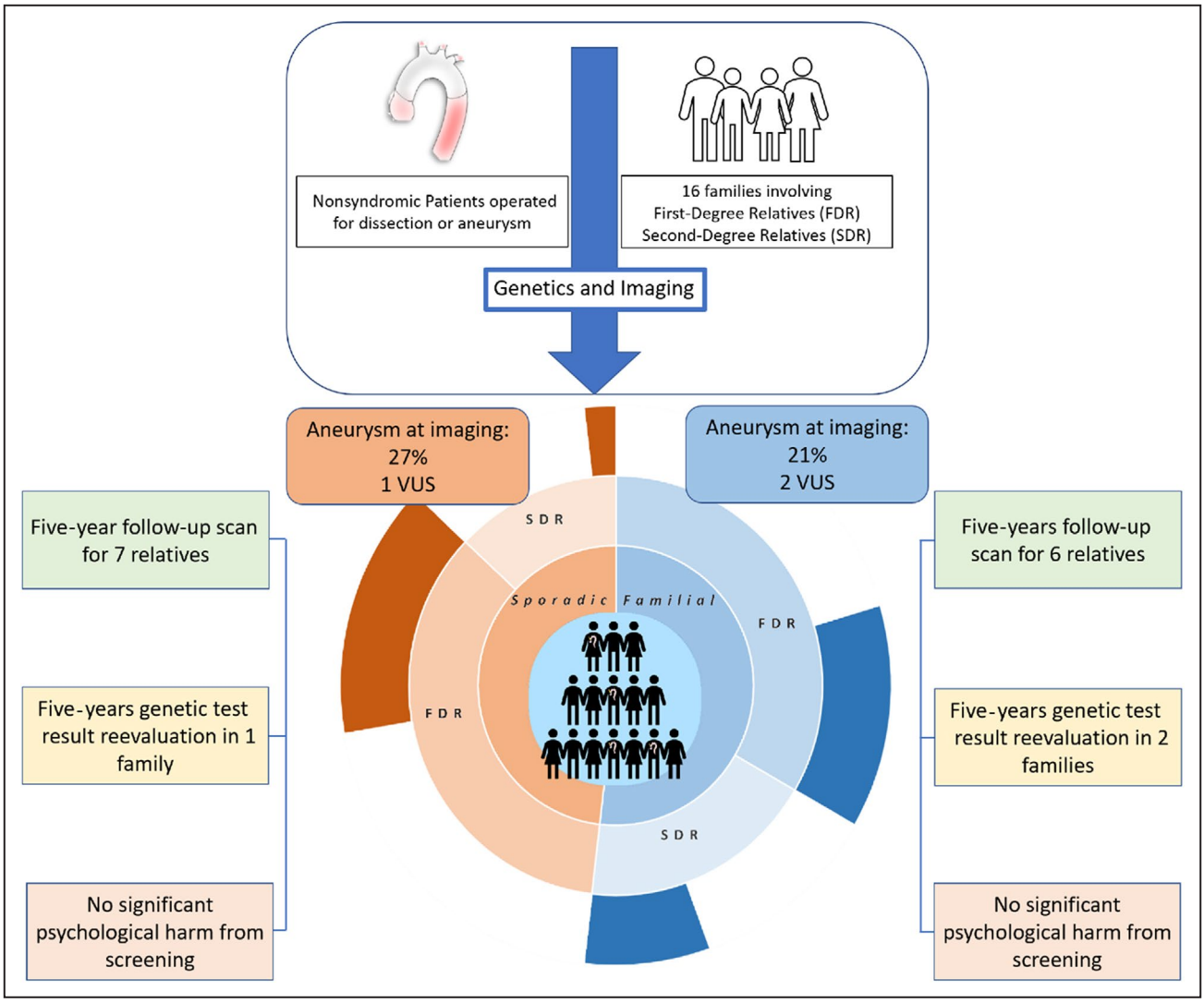


Figure 5. Summary of the overall study findings.
Sixteen families of patients with nonsyndromic thoracic aortic disease (NS-TAD) were involved in a feasibility study to evaluate a combined approach to screening for aortopathy. Results showed an aortic dilatation in 24% and a genotype that required rephenotyping in 15% of the relatives respectively. Thirteen participants required imaging follow-up, and 3 families a further clinical genetics reevaluation. FDR indicates first-degree relative; MRI, magnetic resonance imaging; SDR, second degree relative; TTE, transthoracic echocardiogram; and VUS, variant of uncertain significance.

tailored, focused screening program could potentially be helpful in optimizing surveillance, medical management, and prophylactic surgical intervention when required, by combining a careful review of a potential familial component with an imaging assessment that should be extended to SDRs.

ARTICLE INFORMATION

Received August 25, 2021; accepted December 22, 2021.

Affiliations

Leicester NIHR Biomedical Research Centre & Department of Cardiovascular Sciences, University of Leicester, UK (R.G.A., M.Z., S.P., A.A., H.A., B.E., F.L., M.W., G.M.); Department of Cardiac Surgery, University Hospitals of Leicester NHS Trust, Leicester, UK (G.M., L.J.); Department of Genetics and Genome Biology, University of Leicester, UK (J.B.); Aortic Dissection Awareness UK & Ireland, York, UK (G.O.); Department of Clinical Genetics, Nottingham University Hospitals, Nottingham, UK (N.S., R.L.H.); and Leicester Clinical Trials Unit, University of Leicester, Leicester, UK (T.K.).

Acknowledgments

The authors are obliged to Aortic Dissection Awareness UK & Ireland for the constant support and precious insights into the design and conduct of the study.

The authors are also extremely grateful to Anne-Marie Marsh, Manjit Sian, and Dianne Dennis (from National Institute for Health Research Leicester Biomedical Research Centre), and to Joanne Wormleighton, Tracey Alexander, Kelly Parker, and Anvesha Singh (from the University Hospitals of Leicester National Health Service Trust) for the help in managing and performing the imaging tests. The authors are also thankful to Leicester Cardiovascular Research Centre Precision Medicine Facility, in particular to Sarah Greatrex and Manish Asiani, for the assistance provided during the DNA extraction. The authors are also grateful for the invaluable administrative support from Ethaar El-Emir, Sue Page, and Kim Mason (University of

Leicester Cardiovascular Sciences Department). Lastly, the authors express their thanks to Cat Taylor, Yasmin Godhania, and the entire team at the Research Governance Office for the help in setting up and maintaining this research endeavor.

Sources of Funding

The present work was supported via the Van Geest Development Fund (years 2017–2018) from the University of Leicester (United Kingdom). Research echocardiograms were provided by the National Institute for Health Research Leicester Biomedical Research Centre, along with the support for appointing a Clinical Research Fellow (R.A.) following the project. This work was also supported by the British Heart Foundation [CH/12/1/29419 and AA18/3/34220 to GJM].

Disclosures

None.

Supplemental Material

Data S1–S4
Table S1–S7
Figure S1–S3

REFERENCES

1. Verstraeten A, Luyckx I, Loeys B. Aetiology and management of hereditary aortopathy. *Nat Rev Cardiol*. 2017;14:197–208. doi: 10.1038/nrcardio.2016.211
2. Sampson UK, Norman PE, Fowkes FG, Aboyans V, Song Y, Harrell FE Jr, Forouzanfar MH, Naghavi M, Denenberg JO, McDermott MM, Criqui MH. Global and regional burden of aortic dissection and aneurysms: mortality trends in 21 world regions. *Glob Heart*. 1990;2014:171–180.
3. Tanaka Y, Sakata K, Sakurai Y, Yoshimuta T, Morishita Y, Nara S, Takahashi I, Hirokami M, Yamagishi M. Prevalence of type A acute aortic dissection in patients with out-of-hospital cardiopulmonary arrest. *Am J Cardiol*. 2016;117:1826–1830. doi: 10.1016/j.amjcard.2016.03.015
4. Erbel RAV, Boileau C, Bossone E, Bartolomeo RD, Eggebrecht H, et al. 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. doi: 10.1093/eurheartj/ehu281
5. Hiratzka LFBG, Beckman JA, Bersin RM. 2010 ACCF/AHA/AATS/ACR/ASA/SCA/SCAI/SIR/STS/SVM guidelines for the diagnosis and management of patients with Thoracic Aortic Disease: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines, American Association for Thoracic Surgery, American College of Radiology, American Stroke Association, Society of Cardiovascular Anesthesiologists, Society for Cardiovascular Angiography and Interventions, Society of Interventional Radiology, Society of Thoracic Surgeons, and Society for Vascular Medicine. *Circulation*. 2010;121:e266–e369.doi: 10.1161/CIR.0b013e3181d4739e
6. Fletcher AJ, Syed MBJ, Aitman TJ, Newby DE, Walker NL. Inherited thoracic aortic disease: new insights and translational targets. *Circulation*. 2002;141:1570–1587. doi: 10.1161/CIRCULATIONAHA.119.043756
7. Faggion Vinholo T, Zafar MA, Ziganshin BA, Elefteriades JA. Nonsyndromic thoracic aortic aneurysms and dissections—is screening possible? *Semin Thorac Cardiovasc Surg*. 2019;31:628–634. doi: 10.1053/j.semtcvs.2019.05.035
8. Mariscalco G, Debiec R, Elefteriades JA, Samani NJ, Murphy GJ. Systematic review of studies that have evaluated screening tests in relatives of patients affected by nonsyndromic thoracic aortic disease. *J Am Heart Assoc*. 2018;7:e009302. doi: 10.1161/JAHA.118.009302
9. Abbasciano RG, Barwell J, Sayers R, Bown M, Milewicz D, Cooper G, Mariscalco G, Wheeldon N, Fowler C, Owens G, et al. Report of a Delphi exercise to inform the design of a research programme on screening for thoracic aortic disease. *Trials*. 2020;21:656. doi: 10.1186/s13063-020-04562-1
10. Eldridge SM, Chan CL, Campbell MJ, Bond CM, Hopewell S, Thabane L, Lancaster GA, et al. CONSORT 2010 statement: extension to randomised pilot and feasibility trials. *BMJ*. 2016;355:i5239. doi: 10.1136/bmj.i5239
11. Jenkinson C, Coulter A, Wright L. Short form 36 (SF36) health survey questionnaire: normative data for adults of working age. *BMJ*. 1993;306:1437–1440. doi: 10.1136/bmj.306.6890.1437
12. Kroenke K, Spitzer RL, Williams JBW. The PHQ-9: validity of a brief depression severity measure. *J Gen Intern Med*. 2001;16:606–613. doi: 10.1046/j.1525-1497.2001.016009606.x
13. Spitzer RL, Kroenke K, Williams JBW, Löwe B. A brief measure for assessing generalized anxiety disorder: the GAD-7. *Arch Intern Med*. 2006;166:1092–1097. doi: 10.1001/archinte.166.10.1092
14. Lang RM, Bierig M, Devereux RB, Flachskampf FA, Foster E, Pellikka PA, Picard MH, Roman MJ, Seward J, Shanewise JS, et al. Recommendations for chamber quantification: a report from the American Society of Echocardiography's Guidelines and Standards Committee and the Chamber Quantification Writing Group, developed in conjunction with the European Association of Echocardiography, a branch of the European Society of Cardiology. *J Am Soc Echocardiogr*. 2005;18:1440–1463. doi: 10.1016/j.echo.2005.10.005
15. Goldstein SA, Evangelista A, Abbara S, Arai A, Asch FM, Badano LP, Bolen MA, Connolly HM, Cuéllar-Calàbria H, Czerny M, et al. Multimodality imaging of diseases of the thoracic aorta in adults: from the American Society of Echocardiography and the European Association of Cardiovascular Imaging: endorsed by the Society of Cardiovascular Computed Tomography and Society for Cardiovascular Magnetic Resonance. *J Am Soc Echocardiogr*. 2015;28:119–182. doi: 10.1016/j.echo.2014.11.015
16. Devereux RB, de Simone G, Arnett DK, Best LG, Boerwinkle E, Howard BV, Kitzman D, Lee ET, Mosley TH, Weder A, et al. Normal limits in relation to age, body size and gender of two-dimensional echocardiographic aortic root dimensions in persons ≥15 years of age. *Am J Cardiol*. 2012;110:1189–1194. doi: 10.1016/j.amjcard.2012.05.063
17. Evangelista A, Flachskampf FA, Erbel R, Antonini-Canterin F, Vlachopoulos C, Rocchi G, Sicari R, Nihoyannopoulos P, Zamorano J, Pepi M, et al. Echocardiography in aortic diseases: EAE recommendations for clinical practice. *Eur J Echocardiogr*. 2010;11:645–658. doi: 10.1093/ejechocard/jeq056
18. Turkbey EB, Jain A, Johnson C, Redheuil A, Arai AE, Gomes AS, Carr J, Hundley WG, Teixido-Tura G, Eng J, et al. Determinants and normal values of ascending aortic diameter by age, gender, and race/ethnicity in the Multi-Ethnic Study of Atherosclerosis (MESA). *J Magn Reson Imaging*. 2014;39:360–368. doi: 10.1002/jmri.24183
19. Fung KBL, Aung N, Hann E, Paiva J, Lukaschuk E, et al. Reference values for aortic distensibility derived from UK Biobank cardiovascular magnetic resonance (CMR) imaging cohort. *Eur Heart J - Cardiovasc Imaging*. 2019;20(Supplement_2):jez114.
20. Cingolani P, Platts A, Wang LL, Coon M, Nguyen T, Wang L, Land SJ, Lu X, Ruden DM, et al. A program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff: SNPs in the genome of Drosophila melanogaster strain w1118; iso-2; iso-3. *Fly*. 2012;6:80–92. doi: 10.4161/fly.19695
21. Liu X, Wu C, Li C, Boerwinkle E. dbNSFP v3. 0: a one-stop database of functional predictions and annotations for human nonsynonymous and splice-site SNVs. *Hum Mutat*. 2016;37:235–241. doi: 10.1002/humu.22932
22. Landrum MJ, Lee JM, Benson M, Brown GR, Chao C, Chitipiralla S, Gu B, Hart J, Hoffman D, Jang W, et al. ClinVar: improving access to variant interpretations and supporting evidence. *Nucleic Acids Res*. 2018;46:D1062–D1067. doi: 10.1093/nar/gkx1153
23. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, Grody WW, Hegde M, Lyon E, Spector E, 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. *J Mol Diagn*. 2015;17:405–423. doi: 10.1038/gim.2015.30
24. Ellard SBE, Callaway A, Berry I, Forrester N, Turnbull C, et al. *ACGS Best Practice Guidelines for Variant Classification in Rare Disease 2020*. Association for Clinical Genomic Science. ACGS Guidelines; 2020.
25. Muiño-Mosquera L, Steijns F, Audenaert T, Meerschaut I, De Paepe A, Steyaert W, Symoens S, Coucke P, Callewaert B, Renard M, et al. Tailoring the American College of Medical Genetics and Genomics and the Association for Molecular Pathology Guidelines for the Interpretation of Sequenced Variants in the FBN1 Gene for Marfan Syndrome: proposal for a disease-and gene-specific guideline. *Circ Genom Precis Med*. 2018;11:e002039. doi: 10.1161/CIRCGEN.117.002039
26. Wickham HFR. ggplot2: elegant graphics for data analysis. Available at: https://ggplot2.tidyverse.org2016. Accessed September 20, 2021.

27. Wickham HFR, Henry L & Muller KJ. dplyr: a fast, consistent tool for working with data frame like objects, both in memory and out of memory. Available at: https://cran.r-project.org/web/packages/dplyr/index.html. Accessed September 20, 2021.
28. Signorell AAK, Alfons A, Anderegg N, Aragon T & DescTools AA. Tools for descriptive statistics. Available at: https://cran.r-pproject.org/web/packages/DescTools/DescTools.pdf. Accessed September 20, 2021.
29. Team RC. *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing. Available at: http://www.R-project.org/2013. Accessed September 20, 2021.
30. England N. National Genomic Test Directory. https://www.england.nhs.uk/publication/national-genomic-test-directories/2019
31. Pliskin JS, Shepard DS, Weinstein MC. Utility functions for life years and health status. *Oper Res*. 1980;28:206–224. doi: 10.1287/opre.28.1.206
32. Weerakkody R, Ross D, Parry DA, Ziganshin B, Vandrovcova J, Gampawar P, Abdullah A, Biggs J, Dumfarth J, Ibrahim Y, 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. doi: 10.1038/gim.2018.27

SUPPLEMENTAL MATERIAL

Data S1. Protocol Synopsis.

Study Title	A Feasibility Study on Screening of Relatives of Patients Affected by Non-Syndromic Thoracic Aortic Diseases
Internal ref. no.	0676
Study Design	Prospective, single-centre, non-interventional, feasibility study with a family-based approach
Study Participants	Relatives of 16 probands (index patients) affected by familial and sporadic non-syndromic aortopathies
Planned Sample Size	Up to 128 participants
Follow-up duration	3 months
Planned Study Period	18 months
Primary Objective	To evaluate the components of a screening program in non-syndromic thoracic aortic disease (NS-TAD).
Secondary Objectives	1. To establish referral and response rates among the 16 probands (index patients) and their first- and second-degree relatives. These will be used to model the sample sizes and outcomes that may be used in a definitive study. 2. To evaluate number/rate of relatives who will not undergo screening at the authors' institution, because of their residence in different geographic regions or their wish to be excluded from the study. This will include the withdrawal rate and the completeness of follow-up and data collection in this population of patients. These data will be used to model the sample sizes and outcomes that will be used in the definitive study. 3. To obtain the descriptive statistics for the primary and secondary outcomes of interest. 4. To evaluate the perceptions of probands and family members in term of appropriateness of the screening and consent process (baseline). 5. To evaluate the psychological impact related to screening process for the probands and their relatives (baseline and 3 months). 6. To evaluate the additional healthcare resource use attributable to the screening protocol.
Primary Endpoints	1. Frequency of first and second degree relatives with newly identified genetic loci associated with NS-TADs. 2. Frequency of newly diagnosed TAD through imaging modalities in first- and second-degree relatives of probands affected by NS-TADs.
Secondary Endpoints	1. Genetic variants associated with NS-TADs. 2. Rate of genetic carriers in each affected family. 3. Penetrance and mode of inheritance. 4. Male: female preponderance. 5. MRI features of affected and unaffected thoracic aortas (compliance and distensibility). 6. Rates of concomitant cardiovascular diseases (e.g. patent ductus

	arteriosus, cerebrovascular aneurysm) and external physical features (e.g. pectus excavates, livedo reticularis). 7. Response rates (recruitment) among the probands and their relatives. 8. Semi-quantitative evaluation of the participant experience awareness and acceptability of the screening and consent process, obtained by questionnaires administered to the patients and relatives. 9. Semi-quantitative evaluation of the impact of the screening process on anxiety and depression in probands and their relatives (baseline and 3 months). 10. Resource uses in terms of hospital visits, and unitary costs of genetic and imaging screening process.
Inclusion Criteria	The following 2 groups of patients will be included for screening purpose: 1) NS-TAD probands operated on (n=16). 2) FDR and SDR, aged 16 and above: a) At least two relatives willing to participate in the screening programme. b) Relatives able to understand English. Participant may not enter study if ANY of the following apply: 1) Probands with syndromic aortopathies, including MFS, LDS, EDS, SGS, AOS, arterial tortuosity syndrome, and *cutis laxa* syndrome. 2) Probands with aortic lesions associated with trauma and infections. 3) Probands/relatives unable to give informed consent.
Statistical Considerations	• As this is a feasibility study, the analyses of the data collected will be mainly descriptive, and any statistical comparisons made will be exploratory. Continuous data will be summarized as mean (standard deviation) or median (interquartile range), if data will be skewed, and categorical data will be summarised as number and/or percentage. • We will use mixed logistic regression model to explore the genotype-phonotype relationship in the family-based data and identify candidate genetic variants associated with NS-TADs. Exploratory comparison of the outcomes (e.g. aortic diameter, distensibility and pulse wave velocity) between mutation carriers and non-carriers may be conducted. Where appropriate, we will take into account of the family structure in the statistical analyses (family pedigree). • We will calculate the summary scores for SF36 (or EQ-5D) and HADs (or PHQ-9), as well as the score of each domain assessed by these instruments. The change in the scores (3-month follow-up minus the baseline) will be computed and as a measure to evaluate the psychological impact of the screening.
Governance	The trial funder is UoL. The trial sponsor is the University of Leicester. The trial will be conducted in accordance with the International Conference for Harmonisation of Good Clinical Practice (ICH GCP)

	guidelines (CPMP/ICH/135/95, July 1996), the UK Policy Framework for Health and Social Care Research, the European Union Directive 2001/20/EC on clinical trials and the European Directive on Clinical Trials (2001/20/EC, 04 April 2001 and subsequent amendments) European Directive on Good Clinical Practice (2005/28/EC).

Data S2. Baseline Questionnaire Form administered to participants.

When you signed the consent form to participate in the study, how well did you understand the following aspects of the study? *If you didn't understand the item at all, please circle 1. If you understood it very well, please circle 5. If you understood it somewhat, please circle a number between 1 and 5*

Rate from 1 to 5 how much you agree with the following sentences	I didn't understand this at all →				I understood this very well
1. What the researchers are trying to find out in the study	1	2	3	4	5
2. How long you will be in the study	1	2	3	4	5
3. The procedures you will undergo	1	2	3	4	5
4. The possible disadvantages of participating in the study	1	2	3	4	5
5. How participation in this study may benefit future patients	1	2	3	4	5
6. Whom you should contact if you have questions or concerns about the study	1	2	3	4	5
7. The fact that participation in the study is voluntary	1	2	3	4	5
8. The fact that you can withdraw from the study any time	1	2	3	4	5

9. Overall, how well did you understand the study when you signed the consent form?	1	2	3	4	5

Data S3. Follow Up Questionnaire Form administered to participants.

How much do you agree with the following sentences related to the different aspects of the study? *If you don't agree with the item at all, please circle 1. If you completely, please circle 5. If you agree with it somewhat, please circle a number between 1 and 5*

Rate from 1 to 5 how much you agree with the following sentences	I don't agree at all →				I completely agree
1. Becoming aware of the purpose of this study caused me uneasiness.	1	2	3	4	5
2. I experienced significant distress when undergoing these exams: a. Medical history, physical examination	1	2	3	4	5
b. Blood sample	1	2	3	4	5
c. Imaging (TTE, MRI)	1	2	3	4	5
3. I was anxious when waiting for the results.	1	2	3	4	5
4. I was concerned about the result being a false positive / false negative.	1	2	3	4	5
5. I am worried about the potential need for follow up exams in the future.	1	2	3	4	5
6. I believe answering to this questionnaire is inappropriate, distressful or not useful.	1	2	3	4	5
7. I think my quality of life has been negatively affected when I was recruited in this study.	1	2	3	4	5
8. I think the results I had or the information I am now aware of thanks to this study still negatively affect my quality of life.	1	2	3	4	5
9. I am concerned about leftover blood being kept for use in future research.	1	2	3	4	5
10. I think a program such as this study should not be extended to a wider population.	1	2	3	4	5

Data S4. CONSORT extension for Pilot and Feasibility Trials Checklist.

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a pilot or feasibility randomised trial in the title	1
	1b	Structured summary of pilot trial design, methods, results, and conclusions (for specific guidance see CONSORT abstract extension for pilot trials)	2
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale for future definitive trial, and reasons for randomised pilot trial	5-8
	2b	Specific objectives or research questions for pilot trial	5-8
Methods			
Trial design	3a	Description of pilot trial design (such as parallel, factorial) including allocation ratio	5-8
	3b	Important changes to methods after pilot trial commencement (such as eligibility criteria), with reasons	n.a.
Participants	4a	Eligibility criteria for participants	5-8
	4b	Settings and locations where the data were collected	5-8
	4c	How participants were identified and consented	8
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and	8-12

		when they were actually administered	
Outcomes	6a	Completely defined prespecified assessments or measurements to address each pilot trial objective specified in 2b, including how and when they were assessed	8-12
	6b	Any changes to pilot trial assessments or measurements after the pilot trial commenced, with reasons	n.a.
	6c	If applicable, prespecified criteria used to judge whether, or how, to proceed with future definitive trial	n.a.
Sample size	7a	Rationale for numbers in the pilot trial	n.a.
	7b	When applicable, explanation of any interim analyses and stopping guidelines	n.a.
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	n.a.
	8b	Type of randomisation(s); details of any restriction (such as blocking and block size)	n.a.
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	n.a.
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	n.a.
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how	n.a.

	11b	If relevant, description of the similarity of interventions	n.a.
Statistical methods	12	Methods used to address each pilot trial objective whether qualitative or quantitative	8
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were approached and/or assessed for eligibility, randomly assigned, received intended treatment, and were assessed for each objective	Figure 1
	13b	For each group, losses and exclusions after randomisation, together with reasons	Figure 1
Recruitment	14a	Dates defining the periods of recruitment and follow-up	6
	14b	Why the pilot trial ended or was stopped	6
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	Table 1
Numbers analysed	16	For each objective, number of participants (denominator) included in each analysis. If relevant, these numbers should be by randomised group	8 - 12
Outcomes and estimation	17	For each objective, results including expressions of uncertainty (such as 95% confidence interval) for any estimates. If relevant, these results should be by randomised group	8 - 12
Ancillary analyses	18	Results of any other analyses performed that could be used to inform the future definitive trial	8 - 12
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	8 - 12
	19a	If relevant, other important unintended consequences	8 - 12
Discussion			

Limitations	20	Pilot trial limitations, addressing sources of potential bias and remaining uncertainty about feasibility	12 - 15
Generalisability	21	Generalisability (applicability) of pilot trial methods and findings to future definitive trial and other studies	12 - 15
Interpretation	22	Interpretation consistent with pilot trial objectives and findings, balancing potential benefits and harms, and considering other relevant evidence	12 - 15
	22a	Implications for progression from pilot to future definitive trial, including any proposed amendments	12 - 15
Other information			
Registration	23	Registration number for pilot trial and name of trial registry	1
Protocol	24	Where the pilot trial protocol can be accessed, if available	5
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	16
	26	Ethical approval or approval by research review committee, confirmed with reference number	1

Citation: Eldridge SM, Chan CL, Campbell MJ, Bond CM, Hopewell S, Thabane L, et al. CONSORT 2010 statement: extension to randomised pilot and feasibility trials. BMJ. 2016;355.

Table S1. Transthoracic echocardiography reference values for thoracic aortic diameters according to the European Association for Echocardiography recommendations

Section	**Range**	**Indexed Range**
Aortic annulus	*20 – 31 mm*	*13 ± 1 mm/m2*
Sinuses of Valsalva	*29 – 45 mm*	*19 ± 1 mm/m2*
Sinotubular junction	*22 – 36 mm*	*15 ± 1 mm/m2*
Tubular ascending aorta	*22 – 36 mm*	*15 ± 2 mm/m2*
Aortic Arch	*22 – 36 mm*	-
Descending Aorta	*20 – 30 mm*	-

Adapted from *Evangelista A, Flachskampf FA, Erbel R, Antonini-Canterin F, Vlachopoulos C, Rocchi G, et al. Echocardiography in aortic diseases: EAE recommendations for clinical practice. 2010;11(8):645-58.*

Table S2. Normal aortic root and ascending aorta diameters by age adapted from Goldstein et al. and Turkbey et al.

AORTIC ROOT	***Age***	***Mean normal***	***Upper limit of normal***
Men	**15 – 29**	3.3	3.7
	30 – 39	3.4	3.8
	40 – 49	3.5	3.9
	50 – 59	3.6	4.0
	60 – 69	3.7	4.1
	≥ 70	3.8	4.2
Women	**15 – 29**	2.9	3.3
	30 – 39	3.0	3.4
	40 – 49	3.2	3.6
	50 – 59	3.2	3.6
	60 – 69	3.3	3.7
	≥ 70	3.4	3.9
ASCENDING AORTA	**Age**	**Median (average BSA indexed) (mm/m2)**	**95th percentile**
Men	**45 – 54**	1.59	1.95
	55 – 64	1.68	2.11
	65 – 74	1.78	2.18
	75 – 84	1.86	2.26
Women	**45 – 54**	1.67	2.07
	55 – 64	1.76	2.21
	65 – 74	1.81	2.21
	75 – 84	1.97	2.82

For the aortic root, for men with BSA of 2.0 m2 and for women with BSA of 1.7 m2, reference diameters are reported in cm. Upper limit considers a 95% Confidence Interval. 0.5 mm per 0.1 m^2 BSA above 2.0 m^2 should be added or 0.5 mm per BSA below 2.0 mm^2 should be subtracted to the reference values for men; 0.5 mm per 0.1 m^2 BSA above 1.7 m^2 should be

added or 0.5 mm per BSA below 1.7 mm^2 should be subtracted to the reference values for women.

Adapted from *Goldstein SA, Evangelista A, Abbara S, Arai A, Asch FM, Badano LP, et al. Multimodality imaging of diseases of the thoracic aorta in adults: from the American Society of Echocardiography and the European Association of Cardiovascular Imaging: endorsed by the Society of Cardiovascular Computed Tomography and Society for Cardiovascular Magnetic Resonance. 2015;28(2):119-82* and from *Turkbey EB, Jain A, Johnson C, Redheuil A, Arai AE, Gomes AS, Carr J, Hundley WG, Teixido-Tura G, Eng J, Lima JA. Determinants and normal values of ascending aortic diameter by age, sex, and race/ethnicity in the Multi-Ethnic Study of Atherosclerosis (MESA). Journal of Magnetic Resonance Imaging. 2014 Feb;39(2):360-8.*

BSA – Body Surface Area

Table S3. Normal reference values for distensibility in thoracic and descending aorta obtained from UK Biobank cohort and reported by Fung et al. (14)

	Age	*AA distensibility*	*DA distensibility*
Men	**45 – 54**	2.9 ± 1.1	3.8 ± 1.1
	55 – 64	2.0 ± 1.1	2.9 ± 1.1
	65 – 74	1.2 ± 0.6	2.2 ± 0.9
Women	**45 – 54**	3.1 ± 1.3	3.8 ± 1.1
	55 – 64	1.7 ± 1.1	2.6 ± 1.0
	65 – 74	1.2 ± 0.6	2.0 ± 0.8

AA – ascending aorta; DA – descending aorta. Data for distensibility are reported in 10^{-3} $mmHg^{-1}$.

Adapted from *Fung K, Biasiolli L, Aung N, Hann E, Paiva J, Lukaschuk E, et al. 282 Reference values for aortic distensibility derived from UK Biobank cardiovascular magnetic resonance (CMR) imaging cohort. 2019;20(Supplement_2):jez114.*

Table S4. Error matrix for comparison between MRI and echocardiographic diagnoses.

MRI (n = 43)	**Diagnosed as Dilated (TTE)**	**Diagnosed as Non-dilated (TTE)**		**TTE**
Dilated (MRI)	6 (14%) (true positive)	2 (5%) (false negative)	8 (19%)	Sensitivity 75%
Not-Dilated (MRI)	1 (2%) (false positive)	34 (79%) (true negative)	35 (81%)	Specificity 97%
	7 (16%)	36 (84%)	43	

MRI, magnetic resonance imaging; *TTE*, transthoracic echocardiography.

Table S5. List of the variants after filtering according to ClinVar and subsequent bioinformatic interpretation according to the ACMG guidelines, in the 16 samples collected from the probands, for the 32 genes in the NHS Genomic Medicine Service panel.

Genomic position*	Ref	Alt	Effect	HUGO Gene Symbol	gnomAD AF	HGVS notation	ACMG class
9:**139391512**	**G**	**A**	**missense variant**	***NOTCH1***	***0.00000929***	***NM_017617.3:c.6679C>T***	*Uncertain significance*
15:**48779358**	**T**	**C**	**missense variant**	***FBN1***	***0.0000366***	***NM_000138.4:c.3503A>G***	*Uncertain significance*
15:**48722916**	**T**	**C**	**missense variant**	***FBN1***	**.**	***NM_000138.4:c.6823A>G***	*Uncertain significance*
7:73474254	GTGGCTCCTGGTGTCGGTGTGGCTCCTGGAGTTGGCT[b]	G	disruptive inframe deletion	*ELN*	.	*NM_001278939.1:c.1551_1586del*	*Uncertain significance*
16:15809118	G	A	missense variant	*MYH11*	*0.000156*	*NM_001040114.1:c.5537C>T*	*Uncertain significance*
3:123471294	C	T	missense variant	*MYLK*	*0.0000366*	*NM_053025.3:c.257G>A*	*Uncertain significance*
9:139400180	G	T	missense variant	*NOTCH1*	*0.000531*	*NM_017617.3:c.4168C>A*	*Uncertain significance*
9:133738189	G	A	missense variant	*ABL1*	*0.000366*	*NM_007313.2:c.646G>A*	*Uncertain significance*
X:153587375	T	C	missense variant	*FLNA*	*0.000953*	*NM_001110556.1:c.4451A>G*	*Uncertain significance*
3:123419116	GTTC	G	conservative inframe deletion	*MYLK*	.	*NM_053025.3:c.3196_3198del*	*Benign*
21:44483184	A	ATGATCTG	frameshift variant &	*CBS*	.	*NM_000071.3:c.832_83 NM_000071.2:c.832_833i*	*Benign*

		CAGAGGGCGCGGCTTCAGGGCTCAAGCCCAGCAAAAGCCCCACCTGGATGATCCACCCCAG	stop gained			*nsCTGGGGTGGATCATCCAGGTGGGGCTTTTGCTGGGCTTGAGCCCTGAAGCCGCGCCCTCTGCAGATCA 3*	
9:137642654	G	A	missense variant	*COL5A1*	*0.0342*	*NM_000093.4:c.1588G>A*	*Likely benign*
16:15811165	G	A	missense variant	*MYH11*	*0.000817*	*NM_001040114.1:c.5357C>T*	*Likely benign*
3:123451773	G	C	missense variant	*MYLK*	*0.000298*	*NM_053025.3:c.1486C>G*	*Benign*

Variants for which an additional clinical evaluation was needed are bold and underlined in the table. *Genome build is hg19 (GRCh37); *ACMG,* American College of Medical Genetics; *HGVS*, Human Genome Variation Society notations obtained from dbSNP; *HUGO*, Human Genome Organization Nomenclature.

Table S7. Combined results from the imaging and genetic screening in all family members with aortic dilatation.

SEX	AGE	HEIGHT	WEIGHT	PRESENTATION	FAMILY HISTORY	SIZE	PHYSICAL EXAMINATION	RISK FACTORS	DIAGNOSIS IMAGING IN RELATIVES	GENOMIC VARIANT AND ACMG CLASSIFICATION	GENE
F	64	164	62	Ascending aorta and hemi-arch aneurysm (redo after aortic valve)	Familial form; Grandmother (maternal side of the family) had an aneurysm	4.3 cm (proximal arch)	No Signs, Beighton score = 0/9	Hypertension, Ex-Smoker	Mother (mild ascending aorta dilation) Sister (mild ascending aorta dilation) Niece (borderline, MRI diagnosis)	*NM_001278939.1:c.1551_1586del* *Uncertain significance*	*ELN*
M	38	173	65	Severe aortic stenosis and ascending aorta aneurysm	No Family History	4.3 cm (ascending aorta)	No Signs, Beighton score = 0/9	Hypertension, Bicuspid Valve	No diagnosis	none identified	-

				(after the sino-tubular junction)							
M	67	169	72	Root and ascending aorta aneurysm	Familial form; Sister affected by aneurysm	6.7 cm (ascending aorta)	No Signs, Beighton score = 0/9	Hypertension, Ex-Smoker, Impaired EF, Pulmonary Hypertension	No diagnosis	none identified	-
M	72	177	80	Severe aortic stenosis and ascending aorta dilatation	Familial form	6.0 cm (ascending aorta)	Pes planus, Beighton score = 0/9	Ex-Smoker	Brother (dilated aortic root)	none identified	-
F	48	177	104	Type A Dissection	Familial form	[acute aortic syndrome]	No Signs, Beighton score = 3/9	Hypertension, Ex-Smoker	No diagnosis	*NM_053025.3:c.3196_3198del* *benign* *NM_053025.3:c.1486C>G* *benign*	*MYLK* *MYLK*

M	68	165	83	Severe aortic stenosis, CAD and ascending aorta dilatation	No Family History	5.0 cm (aortic root)	Osteoarthritis, Beighton score = 0/9	Hypertension, Ex-Smoker	No diagnosis	*NM_000093.4:c.1588 G>A* *Likely benign*	*COL5A1*
F	62	167	63	Root and ascending aorta aneurysm (previous AVR)	Familial form; Several sudden deaths at young age in the family, father and grandmother affected	4.0 cm (loss of sinotubular junction)	Cheloid scars, Beighton score = 0/9	Hypertension, previous AVR	Sister (mild ascending aorta dilation)	*NM_007313.2:c.646 G>A* *Uncertain significance* *NM_017617.3:c.6679 C>T* *Uncertain significance*	*ABL1* *NOTCH1*
M	57	176	119	Aortic Regurgitation, Root and ascending aorta	No Family History	5.0 cm (ascending aorta)	No Signs, Beighton score = 1/9	Hypertension, Smoker, COPD	Brother (mild ascending aorta dilation)	*NM_000093.4:c.1588 G>A* *Likely benign* *NM_001040114.1:c.5*	*COL5A1*

				aneurysm						*537C>T* *Likely benign*	*MYH11*
F	68	160	57	Ascending aorta and hemi-arch aneurysm	Familial form	6.6 cm (ascending aorta), 4.0 cm (aortic root)	Myopia, Osteoarthritis, Scoliosis, Plain Flat Foot, Beighton score = 0/9	Hypertension, Moderate renal impairment	No diagnosis	none identified	-
M	69	171	77	Type A Dissection	Familial form; Sister affected by aneurysm	[acute aortic syndrome]	No Signs, Beighton score = 0/9	Hypertension, Extracardiac Arteriopathy	Niece (mild ascending aorta dilation) Nephew (mild root and ascending aorta dilation)	none identified	-
M	63	170	85	Aortic Regurgitation, Root and ascending aorta	No Family History	5.3 cm (sinus), 4.2 (ascending aorta)	Myopia, Beighton score = 0/9	Hypertension, Poor Mobility, Impaired EF	Son (mild aortic root dilation) Daughter (borderline aortic root	none identified	-

				aneurysm					dilation) Niece (borderline aortic root, mild ascending aorta dilation)		
M	79	170	79	Type A Dissection	No Family History	[acute aortic syndrome]	Myopia, Osteoarthritis, Beighton score = 0/9	Moderate renal impairment, extracardiac arteriopathy	No diagnosis	*NM_053025.3:c.257G>A* *Uncertain significance* *NM_000138.4:c.6823A>G* *Uncertain significance* *NM_001040114.1:c.5357C>T* *Likely benign*	*MYLK* *FBN1* *MYH11*

F	83	155	75	Aortic Regurgitation and ascending aorta aneurysm	No Family History	5.5 cm (ascending aorta)	Myopia, Beighton score = 0/9	Hypertension, Ex-Smoker, Severe renal impairment, Extracardiac arteriopathy	Son (mild aortic root and ascending aorta dilation) Daughter (mild ascending aorta dilation) Son (borderline aortic root dilation)	*NM_001110556.1:c.4451A>G* *Uncertain significance*	*FLNA*
F	69	164	69	Type A Dissection	Familial form; Mother died from aneurysm	[acute aortic syndrome]	No Signs, Beighton score = 0/9	Hypertension, Moderate renal impairment	No diagnosis	*NM_000138.4:c.3503A>G* *Uncertain significance* *NM_000071.2:c.832_833insCTGGGGTGGATCATCCAGGTGGGGCTTTTGCTGGGCTTGAGCCCTGAAGCCGCGCCCTCTG*	*FBN1;* *CBS*

										CAGATCA *Benign*	
M	51	178	111	Aortic Regurgitation, Root and ascending aorta aneurysm	No Family History	6.0 cm (ascending)	Cheloid scars, Beighton score = 0/9	Hypertension	No diagnosis	*NM_017617.3:c.4168C>A* *Uncertain significance* *NM_000071.3:c.832_83* *NM_000071.2:c.832_833insCTGGGGTGGATCATCCAGGTGGGGCTTTTGCTGGGCTTGAGCCCTGAAGCCGCGCCCTCTGCAGATCA 3* *Benign*	*NOTCH1* *CBS*
M	68	183	90	Aortic Regurgitation, Root and ascending	No Family History	5.3 cm (root)	Myopia, Osteoarthritis, Beighton score = 0/9	Hypertension, Ex-Smoker, Extracardiac arteriopathy	Brother (mild aortic root dilation at MRI)	*NM_000071.3:c.832_83* *NM_000071.2:c.832_833insCTGGGGTGGATCATCCAGGTGG*	*CBS*

				aorta aneurysm						*GGCTTTTGCTGGG CTTGAGCCCTGAA GCCGCGCCCTCTG CAGATCA 3* *Benign*	

Table S6. Summary table comparing psychological evaluations at baseline versus follow up

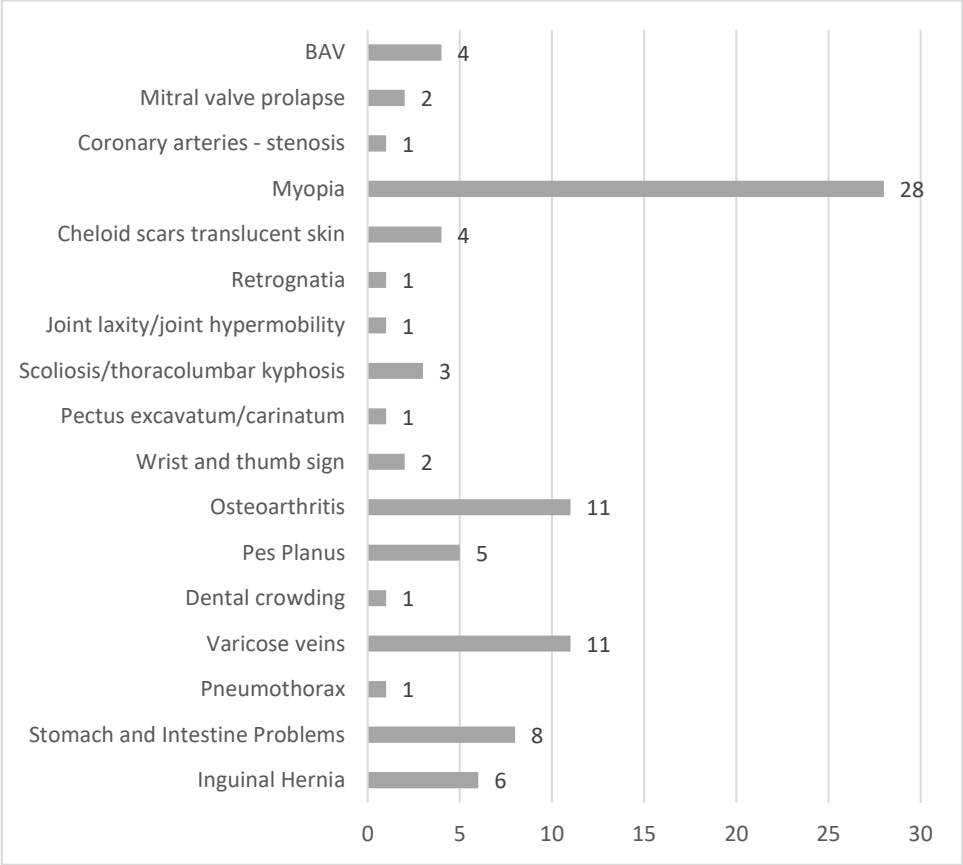
.

Question	Score* (baseline)	Score* (follow-up)	*P* value
Overall PHQ – 9 Score	2.9 ± 3.6	2.5 ± 3.3	*P* = .89
Overall GAD – 7 Score	2.2 ± 3.1	1.6 ± 2.7	*P* = .079
Physical functioning	89.1 ± 20.9	89 ± 17.8	*P* = .34
Role limitations due to physical health	85.7 ± 30.6	89.6 ± 25	*P* = .45
Role limitations due to emotional problems	88.1 ± 26.6	88.6 ± 28.5	*P* = .67
Energy/ fatigue	64.2 ± 17.6	62.7 ± 20.5	*P* = .54
Emotional well being	80.5 ± 16.4	80.5 ± 15.4	*P* = .12
Social functioning	89.8 ± 18.7	93 ± 14.8	*P* = .68
Bodily pain	82.7 ± 19.7	87.1 ± 13.3	*P* = .32
General health	73.5 ± 17.6	69.1 ± 19.2	*P* = .009

FDR, first degree relative; GAD-7, Generalised anxiety disorder questionnaire-7; PHQ-9, Patient health questionnaire-9; SDR, second degree relative; SF-36, Short Form 36 Questionnaire.

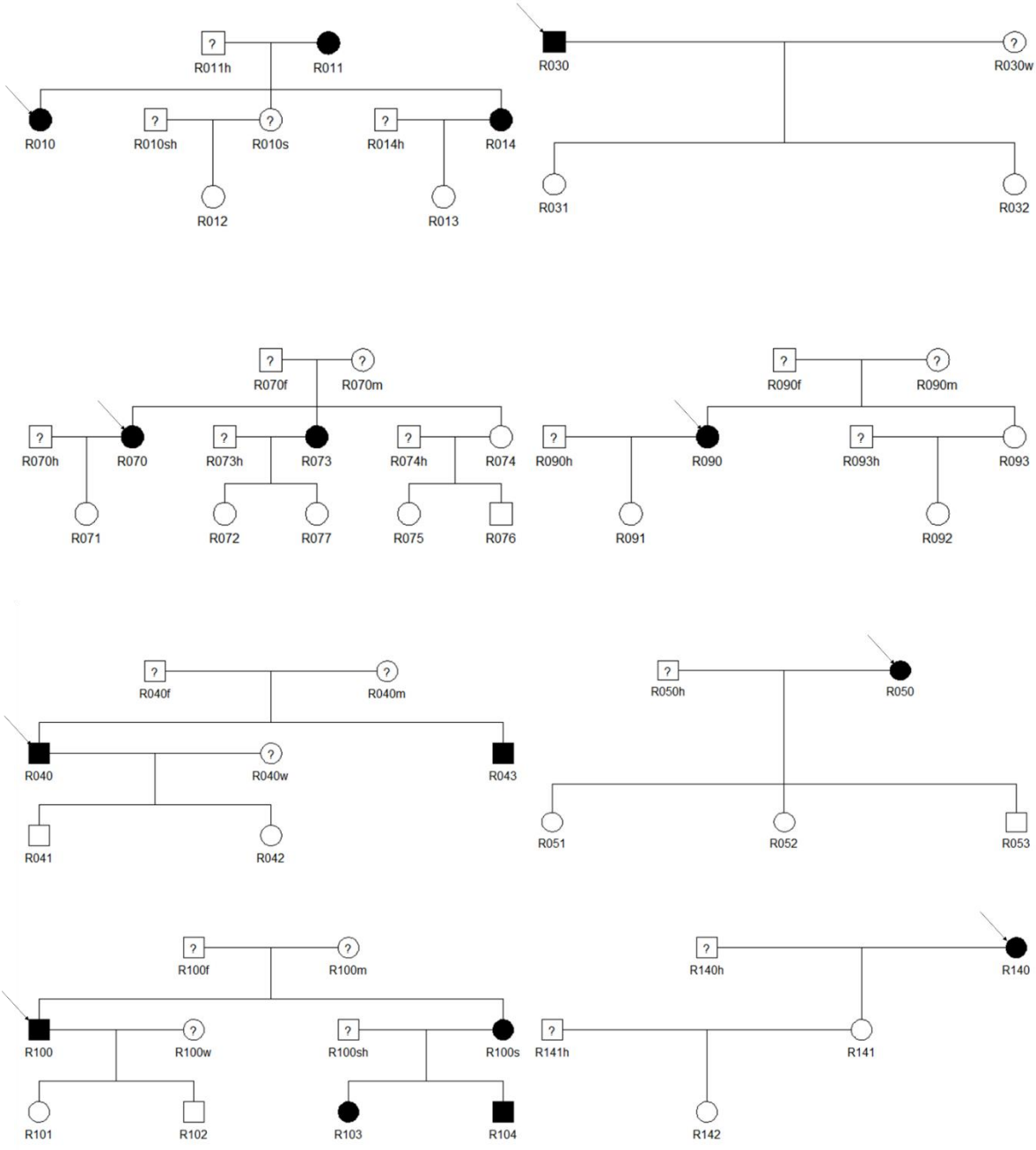
*Data are reported as mean (standard deviation)

Figure S1. Prevalence of syndromic features in the study population.



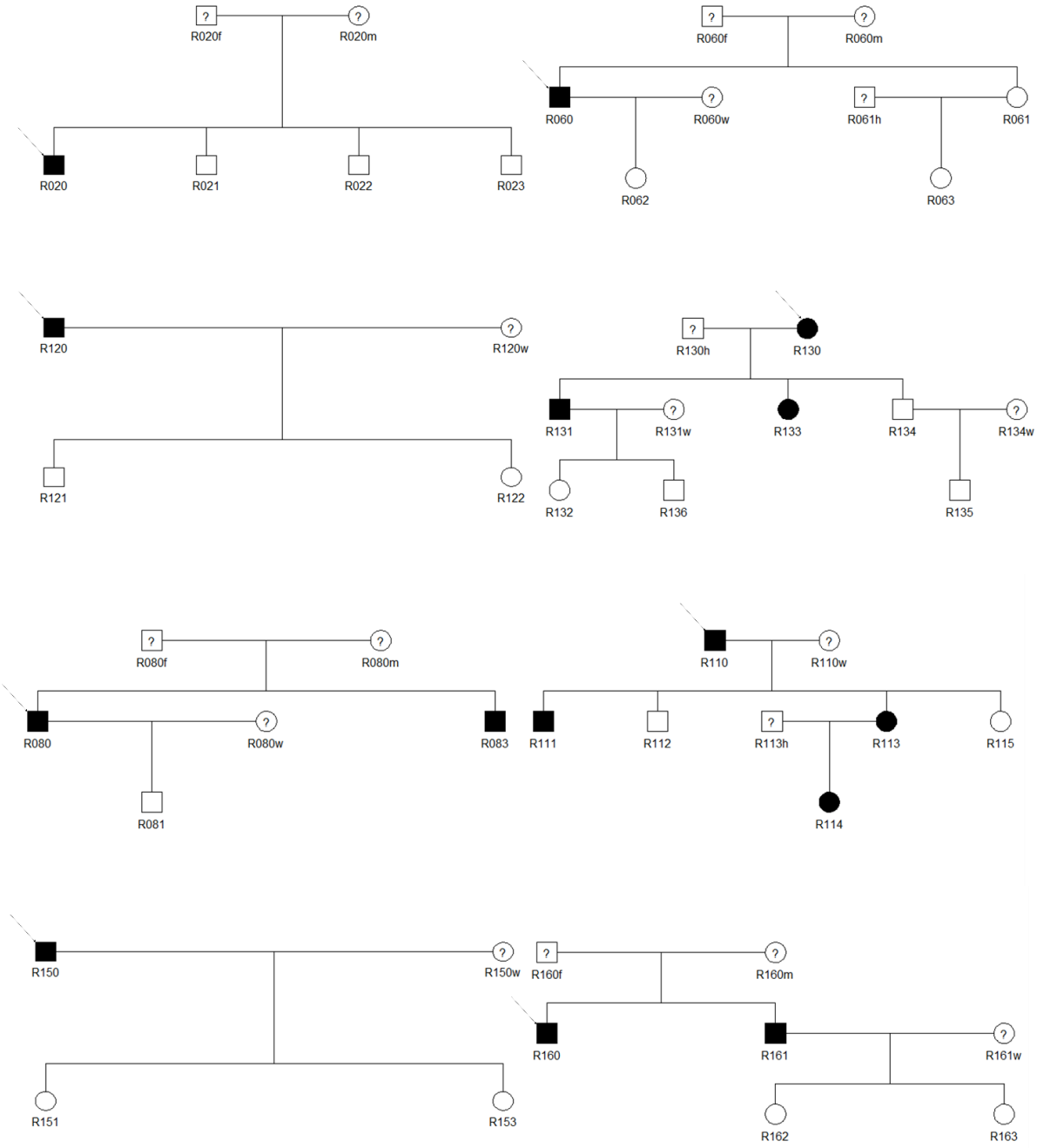
BAV – bicuspid aortic valve.

Figure S2. Family trees and imaging diagnoses in the familial cohort.



Shaded symbols indicate a positive phenotype, either for disease manifestation (probands, marked by an arrow), or recognised by imaging.

Figure S3. Family trees and imaging diagnoses in the sporadic cohort.



Shaded symbols indicate a positive phenotype, either for disease manifestation (probands, marked by an arrow), or recognised by imaging.