ORIGINAL RESEARCH

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

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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.

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

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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, although prevalence might be even higher according to recent series. TADs are often silent entities with a mortality of almost 80% when presenting as life-threatening emergencies. Consequently, early recognition and treatment are crucial elements for improving patient survival. Unlike syndromic TADs, nonsyndromic TADs (NS-TADs) lack overt clinical signs...
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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.5,9 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 Assessment13 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
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 \(-80 \, ^\circ\text{C}\) 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...
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, 69 (97%) blood sample collection for genetic testing, 43 (80% 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...
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 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 (5.6) | 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 (2.2) | 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) | 29.4 (3.5) |
| 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).
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 ($P=0.047$).

| 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 (8%) | 10 (50%) | 28 (46%) | 19 (11%) | 7 (29%) | 26 (31%) |
| 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 (60%) | 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.
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...
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.

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. 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 notwithstanding, however, the data suggest that cascade screening is feasible, is safe, and does identify relatives who require ongoing surveillance and secondary prevention.

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.
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. 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
A 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.

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Supplemental Material
Data S1–S4
Table S1–S7
Figure S1–S3

Screening in Nonsyndromic Thoracic Aortic Disease

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Disclosures
None.

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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.

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.

• 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-phenotype 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.

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: | 1 2 3 4 5           |                    |
| a. Medical history, physical examination                       |                     |                    |
| b. Blood sample                                               |                     |                    |
| c. Imaging (TTE, MRI)                                         |                     |                    |
| 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         | 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              | 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                |
| Category          | Item   | Description                                                                                                                                                                                                 | Reference |
|------------------|--------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------|
| 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 m² BSA above 2.0 m² should be added or 0.5 mm per BSA below 2.0 m² should be subtracted to the reference values for men; 0.5 mm per 0.1 m² BSA above 1.7 m² should be subtracted to the reference values for women.
added or 0.5 mm per BSA below 1.7 mm² 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%)                          |
| Not-Dilated (MRI)  | 1 (2%) (false positive)     | 34 (79%) (true negative)       | 35 (81%)                         |
|                    | 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       | G   | T   | 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      | GTT | G   | conservative inframe deletion | MYLK  | . | NM_053025.3:c.3196_3198del | Benign |
| 21:44483184      | A   | ATGA TCTG frameshift variant | CBS | . | NM_000071.3:c.832_833i | Benign |
| Chromosome:Position | Ref | Alt | Gene | HGVS | ACMG Classification | HGVS Notation |
|---------------------|-----|-----|------|------|---------------------|---------------|
| 9:137642654         | G   | A   | COL5A1 | c.1588G>A | Likely benign | NM_000093.4:c.1588G>A |
| 16:15811165         | G   | A   | MYH11  | c.5357C>T | Likely benign | NM_001040114.1:c.5357C>T |
| 3:123451773         | G   | C   | MYLK   | c.1486C>G | Benign | NM_053025.3:c.1486C>G |

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 | 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 | - |
|   |   |   |   |   |   |   |   |
|---|---|---|---|---|---|---|---|
| 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 | MYLK | MYLK |
| Sex | Age | Height | BMI | Symptoms and History | Family History | Aneurysm Size | Cause of Aneurysm | Family History | Diagnosis | Gene | Mutation | Significance |
|-----|-----|--------|-----|----------------------|----------------|--------------|----------------|----------------|------------|--------|----------|------------|
| 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 | 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 junctio) | Cheloid scars, Beighton score = 0/9 | Hypertension, previous AVR | Sister (mild ascending aorta dilation) | NM_007313.2:c.646 G>A | Uncertain significance | 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 | COL5A1 |
|   | Sex | Age | Height | Subtype | Family History | Aneurysm Measurements | Comorbidities | Genetics | Video Available |
|---|-----|-----|--------|---------|----------------|-----------------------|--------------|----------|----------------|
| F | 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 | 537C>T Likely benign |
| M | 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 | 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 |
| 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.257 G>A | MYLK |
|-----|----|-----|----|-------------------|-------------------|------------------------|-----------------------------------------------|---------------------------------------------------|-------------|------------------------|------|
|     |    |     |    |                   |                   |                        |                                               |                                                   |             | Uncertain significance |      |
|     |    |     |    |                   |                   |                        |                                               |                                                   |             |                        |      |
|     |    |     |    |                   |                   |                        |                                               |                                                   |             | NM_000138.4:c.6823 A>G | FBN1 |
|     |    |     |    |                   |                   |                        |                                               |                                                   |             | Uncertain significance |      |
|     |    |     |    |                   |                   |                        |                                               |                                                   |             | NM_001040114.1:c.5 357C>T | MYH11 |
|     |    |     |    |                   |                   |                        |                                               |                                                   |             | Likely benign           |      |
|   |   |   |   |   |   |   |   |   |
|---|---|---|---|---|---|---|---|---|
| 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.451A>G Uncertain significance |
| 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.3503 A>G Uncertain significance |
|   |   |   |   |   |   |   |   | NM_00071.2:c.832_833insCTGGGGTGCTGGGATCATCCAGGTGGGCTTTTGCTGGGCTTGAGCCCTGAA GCCGCGCCCTCTG FBN1; CBS |
|   |   |   |   |   |   |   |   |   |
|---|---|---|---|---|---|---|---|---|
| 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 | CAGATCA Benign |
| 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_017617.3:c.4168C>A Uncertain significance |
|   |   |   |   |   |   |   |   |   |   | NOTCH1 CBS |
|   |   |   |   |   |   |   |   |   |   | NM_000071.3:c.832_83 NM_000071.2:c.832_833insCTGGGGGTGG ATCAGGAGTGTTGG GCTTITGCTGGG CTTGAGCCCTGGA GCCGCGCCCTCTG CAGATCA 3 Benign |
|   |   |   |   |   |   |   |   |   |   | NM_000071.3:c.832_83 NM_000071.2:c.832_833insCTGGGGGTGG ATCAGGAGTGTTGG GCTTITGCTGGG CTTGAGCCCTGGA GCCGCGCCCTCTG CAGATCA 3 Benign |
|   |   |   |   |   |   |   |   |   |   | NM_000071.3:c.832_83 NM_000071.2:c.832_833insCTGGGGGTGG ATCAGGAGTGTTGG GCTTITGCTGGG CTTGAGCCCTGGA GCCGCGCCCTCTG CAGATCA 3 Benign |
| 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.