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ORIGINAL RESEARCH

Contributions of Germline and Somatic Mosaic Genetics to Thoracic Aortic Aneurysms in Nonsyndromic Individuals

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BACKGROUND: Thoracic aortic aneurysm (TAA) is associated with significant morbidity and mortality. Although individuals with family histories of TAA often undergo clinical molecular genetic testing, adults with nonsyndromic TAA are not typically evaluated for genetic causes. We sought to understand the genetic contribution of both germline and somatic mosaic variants in a cohort of adult individuals with nonsyndromic TAA at a single center.

METHODS AND RESULTS: One hundred eighty-one consecutive patients <60 years who presented with nonsyndromic TAA at the Massachusetts General Hospital underwent deep (>500×) targeted sequencing across 114 candidate genes associated with TAA and its related functional pathways. Samples from 354 age- and sex-matched individuals without TAA were also sequenced, with a 2:1 matching. We found significant enrichments for germline (odds ratio [OR], 2.44, P=4.6×10^{-6} [95% CI, 1.67–3.58]) and also somatic mosaic variants (OR, 4.71, P=0.026 [95% CI, 1.20–18.43]) between individuals with and without TAA. Likely genetic causes were present in 24% with nonsyndromic TAA, of which 21% arose from germline variants and 3% from somatic mosaic alleles. The 3 most frequently mutated genes in our cohort were *FLNA* (encoding Filamin A), *NOTCH3* (encoding Notch receptor 3), and *FBN1* (encoding Fibrillin-1). There was increased frequency of both missense and loss of function variants in TAA individuals.

CONCLUSIONS: Likely contributory dominant acting genetic variants were found in almost one quarter of nonsyndromic adults with TAA. Our findings suggest a more extensive genetic architecture to TAA than expected and that genetic testing may improve the care and clinical management of adults with nonsyndromic TAA.

Key Words: filamin a ■ genetics ■ NOTCH3 ■ thoracic aortic aneurysm

Aortic aneurysm is a serious condition associated with significant morbidity and mortality, accounting for nearly 10 000 deaths in the United States per year.[1] Based on clinical behavior, aneurysms are commonly subdivided based on location as abdominal aortic aneurysms or thoracic aortic aneurysms (TAA). Whereas abdominal aortic aneurysms generally develop in the setting of atherosclerosis and are driven by vascular risk factors such as hypertension and smoking history,[2] TAAs are more likely to have a genetic component, with up to 20% of individuals with TAA having a first-degree relative with a dilated aorta.[3] TAA is often defined as "syndromic" when associated with conditions with extravascular manifestations such as Marfan syndrome, Loeys-Dietz syndrome, or vascular Ehlers–Danlos syndrome and others, whereas "nonsyndromic" describes individuals with TAA where these clinical features are absent.[4,5]

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CLINICAL PERSPECTIVE

What Is New?

- Although adults with nonsyndromic thoracic aortic aneurysm (TAA) typically are not evaluated for genetic causes, genetic variants that likely contribute to TAA were found in 24% of this cohort of nonsyndromic adults with TAA, suggesting a more extensive genetic basis of TAA than previously suspected.
- The 3 genes most frequently mutated in this cohort were *FLNA* (encoding Filamin A), *NOTCH3* (encoding Notch receptor 3), and *FBN1* (encoding Fibrillin 1).

What Are the Clinical Implications?

- Deep genetic sequencing in certain adults with nonsyndromic TAA may reveal pathological variants, thereby further informing care of the patient or screening of family members for this potentially lethal disease.

Nonstandard Abbreviations and Acronyms

HGMD	Human Gene Mutation Database
LOF	loss-of-function
NsynD	predicted damaging missense
TAA	thoracic aortic aneurysm

Currently, there are 11 well-established TAA genes curated by the ClinGen Aortopathy Working Group, including *FBN1*, *FBN2*, *ACTA2*, *TGFBR1*, and *TGFBR2*.[2,6,7] As part of clinical care, genetic testing is recommended for syndromic individuals, those with a strong family history of TAA, or early age of disease onset.[8,9] However, patients with sporadic TAA rarely undergo genetic testing during routine clinical care.[10] Previous studies in research-based collections have established a higher frequency of variants of unknown significance in patients with sporadic TAA compared with individuals without TAA; however, fewer have systematically explored the role of germline variants in nonsyndromic patients who present later in life.[6] For this study, nonsyndromic was defined as those with no history of significant features commonly associated with Marfan's diagnosis/Loeys-Dietz disease at time of presentation, that is, hyperflexibility, tall stature etc. Due to the heritable nature of aortic diameter, distensibility, and strain, we sought to understand the genetic contribution in an adult cohort with nonsyndromic TAA and predominantly without a known family history of aortic disease.[11] Furthermore, the possibility that some disease-causing TAA variants might be somatic, that is, present in some but not all cells of the body, has not been examined.

We report the use of deep targeted sequencing of 114 established and candidate genes for aortic aneurysm to reveal a significant contribution of germline variants in a cohort of consecutive nonsyndromic adult patients with TAA <60 years of age who presented to a large single center (Massachusetts General Hospital) across 15 years. We also observe a modest but significant contribution of mosaic variants to disease. Genetic contribution was ascertained using the quantified variant excess against a cohort of 2:1 matched individuals with no known cardiac disease.

METHODS

Study Population

This study was approved by the Institutional Review Boards of the Massachusetts General Hospital and Boston Children's Hospital. The cohort consisted of patients from the institutional database at the Thoracic Aortic Center at the Massachusetts General Hospital. Patients who clinically presented with a TAA and who consented to genetic testing were systematically enrolled in the registry. Full medical records, medical imaging, and outcomes are included in the registry along with biobanking of blood for genetic analysis. The cohort of patients with TAA were sequentially enrolled between 2003 and 2015 and were included in this study if they (1) were between 21 and 60 years of age, (2) had no history of syndromic disease at time of presentation, and (3) were able to provide a blood sample for genetic analysis. The data that support the findings of this study are available from the corresponding author upon reasonable request.

Patients with TAA with syndromic features were excluded from the study. No family members were included in our study. Clinical demographics and cardiovascular history and family history were extracted from the registry and the medical records. A control group was created from blood samples from individuals without TAA who were age, sex, and ethnicity matched, derived from the National Institute of Mental Health Repository and Genomics Resource (Table S1). All individuals within the control cohort had no known history of cardiac disease and were recruited to achieve a 2-to-1 matching to the cohort with TAA.

Gene-Based Panel Sequencing

Custom gene-based sequencing panels were designed to enable ultra-deep coverage of exons and flanking intronic regions across 114 candidate and known TAA genes including 89 genes from the TGF-β (transforming growth factor beta), MAPK (mitogen-activated

protein kinase), RAS, and NOTCH signaling pathways (Table S2). Genomic DNA was isolated from peripheral blood mononuclear cells using standard methods for all sequencing library preparations. All samples were sequenced to >500× average read coverage using Illumina HiSeq with 150 bp reads (see Data S1 for further details).

Data Processing

Raw sequencing data were analyzed by quality assessment using FastqC and extraction of 8nt unique molecular identifiers with unique molecular identifier tools.[12,13] The data were then aligned to the human reference genome (hg19) with Burrows–Wheeler Aligner-mem using the default settings,[14] Cutadapt and GATK v3.7.[15,16]

Genetic variants, including single nucleotide variants and short insertions and deletions (indel), were detected using the CLC Genomics Workbench v12. For germline alleles, the Fixed Ploidy Variant Detection v2.02 module within the CLC Genomics Workbench, and Somatic events were detected using the Low Frequency Detection v2.02 module (see Data S1 for further details). Individual-level variant call formats were merged and filtered to remove clustered artifacts using BCFtools (SnpGap: 20, IndelGap: 20) and GATK variant filtration (clusterWindowSize: 30).[16,17]

Identification of Candidate Variants

All genetic variants occurring at sites covered by both molecular inversion probe-based and HaloPlex panels were annotated using our custom pipeline based on Annovar (see Data S1 for details on included databases).[18] Variants were then categorized by predicted impact: Loss-of-function (LOF) alleles were classified as frameshift-inducing, stop-gain, and canonical splice site variants with an estimated positive predictive value of 99.5%, accuracy of 98.2% and sensitivity of 98.9% based on benchmarking of known pathogenic and benign variants based on ClinVar.[19] Noncanonical splice-site variants were predicted with use of the combination of Spidex and SpliceAI.[20,21] Similarly, predicted damaging missense alleles (NsynD) were defined similar to previous analyses as more than 3 damaging predictions and fewer than 10 benign predictions (see Data S1 for details on included databases).[19] Sensitivity in detecting NsynD variants was 82% with an estimated positive predictive value of 98.4% and an accuracy of 82%.

Candidate damaging germline variants were identified by stringent filtering requiring: population frequency <0.1%, allele counts (AC) in Genome Aggregation Database (gnomAD) exomes <15, AC genomes <10, AC in cohort with TAA <=2, and CLC-based genotype quality score>100. Candidate damaging somatic variants required the following: population frequency<0.05%, AC in gnomAD exomes <5, AC in gnomAD genomes <5, cohort with TAA=1, and CLC-based genotype quality score >150. Variants meeting these criteria were filters to select those with predicted damaging impacts based on our custom classification approach, including classifications of NsynD and LOF. Although this classification approach was benchmarked against American College of Medical Genetics variant interpretation guidelines, all resulting candidate variants were further assessed using the these guidelines.[22] Resulting classifications were then reviewed by an American Board of Medical Genetics and Genomics certified clinical molecular geneticist.

Validation of Variant Calling

We successfully validated 100% (78/78) of variants, including both germline and somatic, selected for re-testing with a second technology using Multiple Independent Primer PCR Sequencing (MIPP-Seq).[23] We followed the standard method of MIPP-Seq which involves single-step barcoded polymerase chain reaction, pooled sequencing on Ion Torrent S5, and variant calling using custom scripts with Samtools mPileup.

Statistical Analysis

The variant contributions of rare germline and somatic events were assessed for all predicted damaging variants identified in the cohort with TAA and compared against those from age-matched individuals without TAA. Statistical testing of variant contributions was performed as follows. First, the odds ratio (OR), SE, and 95% CIs were calculated using the approach described by Altman 1991 where $\mathrm{OR} = \frac{ad}{bc}$, where a = number of cases with variants, b = number of cases without variants, c = number of controls with variants, and d = number of controls without variants. The SEs of the log OR (SE{ln(OR)}) were calculated using the following formula: $\mathrm{SE}\{\ln(\mathrm{OR})\} = \sqrt{\frac{1}{a}+\frac{1}{b}+\frac{1}{c}+\frac{1}{d}}$. The 95% CIs were determined using $95\%\,\mathrm{CI} = \exp(\ln(\mathrm{OR}) - 1.96 \times \mathrm{SE}\{\ln(\mathrm{OR})\})$. P values of the ORs were calculated under the assumption of the deviation from a normal distribution, using: $z - \mathrm{value} = \ln(\mathrm{OR}) / \mathrm{SE}\{\ln(\mathrm{OR})\}$. Furthermore, when possible, gene-level burdens were calculated using the same approach. However, due to the small sample size, most genes were affected by variants in a single individual, thus lacking the power to assess their enrichment in individuals with TAA.

An additional comparison of the variant contributions in individuals with TAA was performed using whole genome sequencing (WGS)-derived variants extracted from the gnomAD v2.11 database. Although gnomAD may contain individuals with later onset disorders such as TAA, we do not expect it to be enriched for such variants. The ascertainment differential, the difference between the burden of variants in individuals

with and without TAA, represents the estimated fraction of variants that contribute to TAA.[19,24] The fraction of observed variants contributing to TAA is then calculated as ascertainment differential divided by the total variant burden in individuals with TAA.

RESULTS

Cohorts of Individuals With and Without TAA

One hundred eighty-one consecutive, nonsyndromic patients with TAA (54 female, 127 male), and who met entry criteria, formed the cohort with TAA. Patient demographics are detailed in Table 1. The median age was 51 years (interquartile range: 44–56 y) (Table 1). Of the cohort with TAA, 90% were sporadic in nature. The other 10% of the cohort with TAA had a documented family history of aortic aneurysms from the medical record. The control cohort consisted of 354 individuals who were matched by age, sex, and self-reported race and ethnicity to the individuals with TAA. The race and ethnicity of individuals in the TAA cohort were extracted from the EPIC electronic medical record category "race." Deep targeted gene sequencing was performed on both individuals with TAA and those without TAA (control).

No Contribution of Copy Number Variants Affecting TAA Genes

Copy number variants are known to result in TAA in the literature.[25,26] Therefore, copy number variant detection was performed with use of a custom densely tiled exonic comparative genomic hybridization microarray that targeted 615 genes, including both known and candidate TAA-associated genes, and genes involved in the TGF-β, MAPK, and RAS signaling pathways (Data S1). All individuals in the cohort with TAA were compared against a single sex-matched Agilent male or female control DNA. The ability to detect copy number variants in TAA-associated genes was confirmed with use of positive control DNA samples with known deletion events. Surprisingly, no copy number variants, including small exonic changes, were detected within candidate or known TAA genes in our cohort.

Table 1. Demographics of Individuals With TAA (n=181)

Sex	
Female	54 (29.8%)
Male	127 (70.2%)
Median age, y (interquartile range)	51 (44–56)
Women	50 (43–50)
Men	52 (44–57)
Family history of aortic aneurysm* (n=174)†	18 (10.3%)
Cardiovascular risk factors (n=174)†	
Body mass index>25, kg/m^2 (n=118)	84 (71%)
History of hypertension	101 (58.8%)
Type 2 diabetes	6 (3.4%)
History of smoking	43 (24.7%)
Former	39 (22.4%)
Current	4 (2.3%)
Coronary artery disease	30 (17.2%)
Stroke	19 (10.9%)
Both coronary artery disease and stroke	5 (2.8%)

*Aortic aneurysm site was unspecified in clinical chart.
†Clinical data were not available for 7 patients.
TAA indicates thoracic aortic aneurysm.

Contribution of Germline Variants Across TAA Genes

There are 11 core genes known to cause TAA, though clinical testing often includes at least 26 genes. Therefore, we first assessed the impact of the core genes in the TAA cohort by comparing the prevalence of variants (v, calculated as number of variants [n_v] divided by the number of individuals [n] in a cohort) in our cohort with TAA against that of our age- and sex-matched cohort of individuals without TAA. As expected, we found an increased frequency of likely damaging variants in the core TAA genes in the cohort with TAA (v=0.12), versus individuals without TAA (v=0.05, OR, 2.58 [95% CI, 1.35–4.95], P=0.004). Interestingly, the other 15 associated genes from TAA clinical testing panels exhibited a slight excess that did not reach significance (v_{TAA}=0.14, $V_{control}$=0.09, OR, 1.73 [95% CI, 0.99–3.04], P=0.06). Overall, we found a significant increase in the frequency of likely damaging variants across the 26 gene set in the cohort with TAA (v=0.31), versus individuals without TAA (v=0.16, OR, 2.44 [95% CI, 1.59–3.73], P=4.4×10^{-5}, Figure 1A) The genetic contribution, estimated from the ascertainment differential or difference in the variant frequencies between individuals with and without TAA (0.15), suggests that germline variants in known TAA genes account for ≈15% of individuals with TAA.

Genetic Contribution of Candidate TAA Genes in Known TAA Functional Pathways

Next, we sought to determine if the 88 additional genes in known TAA functional pathways, without known association to TAA, might contribute to disease. Individuals with TAA were more likely to carry predicted damaging germline variants (LOF and predicted damaging missense, NsynD) in the 88 candidate genes than individuals without TAA (OR, 1.55 [95% CI, 1.04–2.34], P=0.03), suggesting that a portion of genetic contribution to TAA in this cohort arises from outside of the 26 genes typically assessed by clinical testing. To ascertain the total genetic contribution in our TAA cohort, we investigated the collective 114 genes, where we find a greater prevalence of rare damaging events

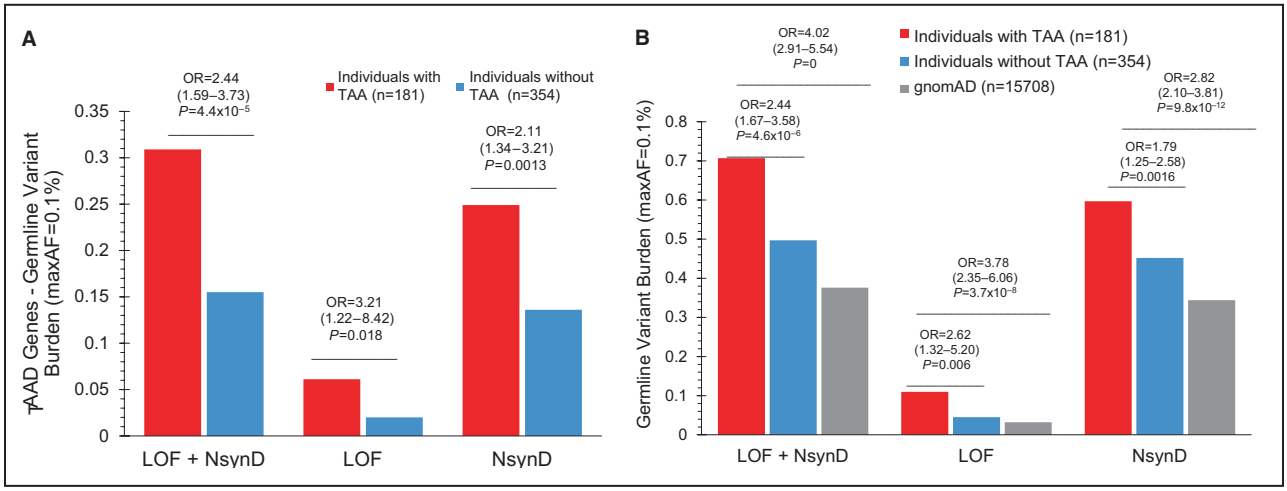

Figure 1. Germline variant burden analysis in cohort with TAA.
A, Individuals with TAA, when compared with individuals without TAA, were enriched for likely damaging variants overall in the 26 known TAA genes. This is true both of LOF variants and NsynD variants. **B**, Individuals with TAA, when compared with individuals without TAA and gnomAD, were enriched for damaging variants overall in the panel of 114 candidate genes. This is true both of LOF variants and NsynD variants. gnomAD indicates Genome Aggregation Database; LOF, loss-of-function; maxAF, maximum population allele frequency; NsynD, missense; OR, odds ratio; TAA, thoracic aortic aneurysm; and TAAD, thoracic aortic aneurysm and dissection.

in individuals with TAA diagnoses compared with individuals without TAA (OR, 2.44 [95% CI, 1.67–3.58], P=4.6×10^{-6}) (Figure 1B). The excess of variants in the cohort with TAA remained significant, even when comparing against WGS data from >15 000 individuals from the gnomAD database (gnomAD v2.1.1, OR, 4.02 [95% CI, 2.91–5.454], P=0, Figure 1B). The ascertainment differential (0.21) suggested a genetic cause of up to 21% of the individuals with TAA. Therefore, an additional 6% of individuals would be found to have a genetic cause with the expanded TAA panel, as opposed to using the classic TAA gene panel alone. In other words, this expanded list of 114 candidate genes results in a potential 40% increase (6/15) in diagnostic yield. See Table S3 for candidate variants.

Most Germline Genetic Contribution in TAA Arises From Partial Loss-of-Function and Missense Variants

Damaging germline variants found in our cohort ranged from more severe (ie, classic LOF) that result in the loss of functional protein, to missense (ie, NsynD) that may in some instances retain residual protein activity. To determine whether a particular class of alleles accounts for greater disease risk in our cohort, we assessed the genetic contribution across each variant category (LOF versus NsynD). LOF variants were more prevalent in individuals with TAA (OR, 2.62 [95% CI, 2.35–6.06], P=0.006, Figure 1B) accounting for an estimated 6.5% of the total genetic contribution. Interestingly, 74% of the genetic contribution arose from splice-altering variants whereas classic LOF variants (eg, stop-gain) accounted for only 26%. The genetic contributions of LOF variants in our cohort with TAA remained highly significant when compared with the rates extracted from gnomAD (OR, 3.78 [95% CI, 2.35–6.06], P=3.6×10^{-8}), with no enrichment detected in individuals without TAA versus gnomAD (OR, 1.44 [95% CI, 0.86–2.40], P=0.16). Even more, NsynD alleles exhibited the greatest genetic contribution with an estimated 14.5% of individuals with TAA (ascertainment differential 0.6 versus 0.45) (OR, 1.79 [95% CI, 1.25–2.58], P=0.0016) (Figure 1B) suggesting that less severe, potentially pathogenic variants have the greatest genetic contribution in our nonsyndromic cohort with milder TAA.

To further understand the apparent depletion of classic LOF events in the cohort with TAA, we compared the distribution of variant classes (eg, stop-gain, missense, splicing) within our cohort against all published damaging variants extracted from the Human Gene Mutation Database (HGMD; Figure 2). We find a nearly 6-fold depletion of stop-gain and frameshift LOF variants (OR, 0.13 [95% CI, 0.026–0.417], P=1.6×10^{-5}) compared with reported variants in patients with TAA in the HGMD cohort (Figure 2). Instead, missense variants were significantly enriched in our cohort compared with those in HGMD, comprising the largest genetic contribution for the cohort with TAA (OR, 1.99 [95% CI, 1.02–4.03], P=0.04) (Figure 2).

Ultra-Rare, Likely Private, Variants Have the Highest Genetic Contribution in TAA

Given the preponderance of sporadic, later-onset TAA in the cohort, it is likely that much of the genetic cause

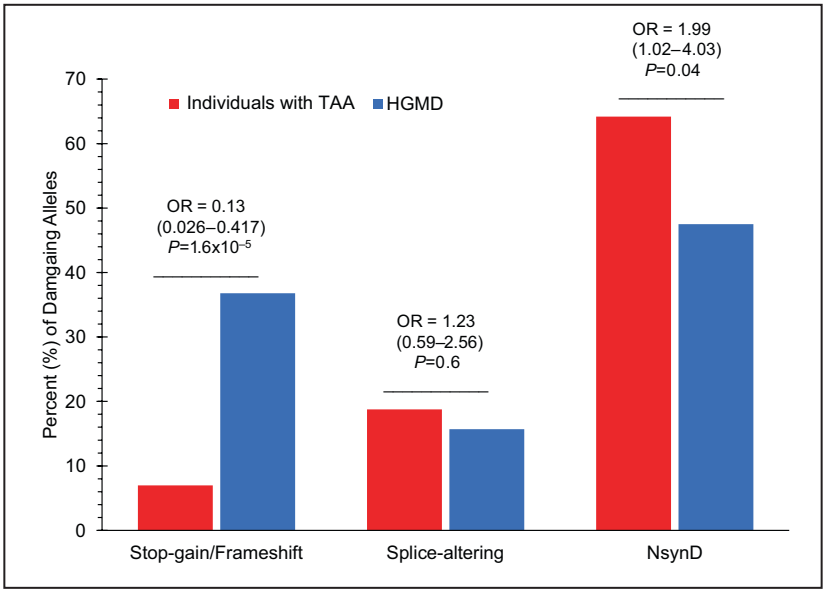

Figure 2. Types of damaging variants in patients with TAA.
When compared with alleles reported in HGMD, individuals with TAA were depleted for LOF (stop-gain and frameshift) and enriched for NsynD variants. HGMD indicates Human Gene Mutation Database; LOF, loss-of-function; NsynD, missense; OR, odds ratio; and TAA, thoracic aortic aneurysm.

identified would be ultra-rare or de novo variants compared with rare inherited alleles. To assess the impact of the candidate variants on inherited versus nonsyndromic TAA, we investigated the impact of likely private alleles that are absent from public databases as a proxy for de novo allele given the proband-only nature of the cohort. We find that damaging, private variants are significantly enriched in individuals with TAA (n_v=41 v=0.227) compared with individuals without TAA (n_v=25, v=0.071), suggesting a genetic contribution in 15.6% of individuals in the cohort (OR, 3.85 [95% CI, 2.26–6.58], P=7.8×10^{-7}). This suggests that of those in the cohort with a genetic cause of TAA, 73% of individuals (15.6/21%) had damaging private variants. This is consistent with the HGMD cohort where 72% of all reported variants within the targeted genes were also private.

Contribution of Somatic Variants

The increased prevalence of ultra-rare and private variants in TAA suggests that additional genetic contribution might arise from somatic variants. Individuals with TAA were nominally enriched for damaging somatic variants (LOF and predicted damaging missense, NsynD) compared with individuals without TAA (OR, 4.71 [95% CI, 1.20–18.43], P=0.026, Figure 3, Table S4), though this enrichment was not significant after Bonferroni correction for multiple comparisons, presumably reflecting our modest cohort size. As expected, the rates of likely benign mosaic variants (eg, synonymous and intronic at nonconserved sites) were similar in individuals with and without TAA (OR, 1.03 [95% CI, 0.07–6.29], P=0.95).

Gene-Level Analysis

To examine individual gene contributions to TAA in the cohort, we examined the gene-level variant enrichments with a 2-step approach. First, we identified 6 genes with suggestive evidence of enrichment in TAA compared with individuals without TAA (ie, OR>2, P<0.1, and n_m>2). The resulting 6 genes include 5 known TAA genes (*COL3A1*, *COL5A1*, *FBN1*, *FLNA*, *NOTCH1*) and, intriguingly, the known cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) gene *NOTCH3*. These 6 genes collectively harbor 34 possibly damaging variants in our cohort with TAA and account for ~75% of the total genetic contribution to TAA in our cohort. Due to the limited size of our cohort of individuals without TAA, the significance of the variant enrichments in these genes were tested by comparison against WGS data in gnomAD. Although all 6 genes exhibited significant raw P values with ORs>2, likely due to our cohort size, only 2 genes, *FLNA* and *NOTCH3*, remained significantly enriched after correction for multiple hypothesis testing. However, caution should be noted for bias with use of gene panels for discovery of novel gene-disease associations, such as for *NOTCH3* in our study. Therefore, the identification in our cohort of *NOTCH3*, known to cause CADASIL,

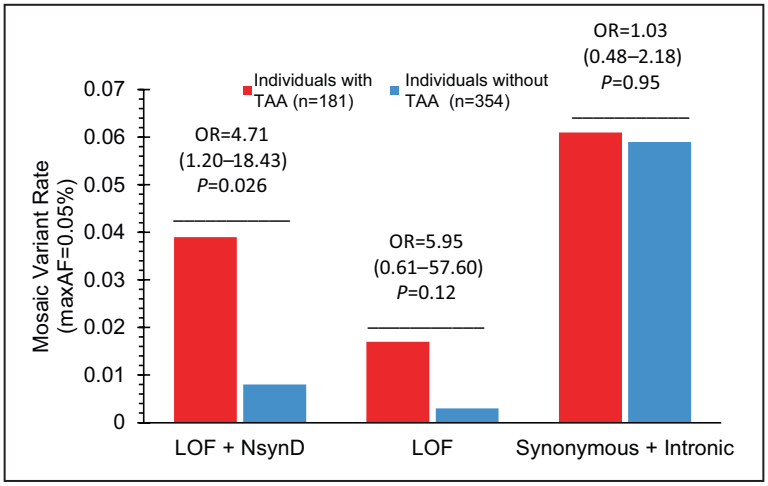

Figure 3. Contribution of somatic variants in cohort with TAA.
When compared with individuals without TAA, individuals with TAA were enriched for likely damaging variants overall in the panel of 114 candidate genes, but due to the cohort size, did not pass significance after Bonferroni corrections. This trend is not present for likely benign events such as synonymous and noncoding intronic variants. LOF indicates loss-of-function; maxAF, maximum population allele frequency; NsynD, missense; OR, odds ratio; and TAA, thoracic aortic aneurysm.

which can include TAA as a syndromic feature, warrants future large-scale whole genome studies to assess its association to TAA.

Established TAA Genes

COL3A1 (n_v=4)

Dominant variants in the *COL3A1* gene are known to cause vascular Ehlers–Danlos syndrome, with at least 786 variants reported as disease variants in HGMD, of which 52% (412/786) are missense and 42% (332/786) are splice-altering variants.[27] We found an enrichment of damaging variants of *COL3A1* (OR, 3.36, *P*=0.01) in individuals with TAA (4/181, *v*=0.022) compared with individuals without TAA (1/354, *v*=0.003) and gnomAD WGS (105/15708, *v*=0.007) (Table 2). Variants in *COL3A1* accounted for 9% of the genetic contribution in our cohort with 4 individuals carrying variants, including 2 NsynD3, 1 LOF3 (ie, a predicted damaging missense with a predicted splicing impact), and 1 LOF4 (ie, intronic splice-altering variant). Interestingly, 2 of the 3 missense variants altered a glycine residue, which is the most common pathogenic amino acid change observed in *COL3A1* and accounts for 97% of reported alleles in Ehlers–Danlos syndrome (HGMD).[28] Interestingly, the intronic variant, c.2446-10T>C, has not been previously reported in the literature but is present in ClinVar as a variant of unknown significance (Variation ID: 404292) and is predicted to reduce normal splicing (Maximum Entropy Distribution [MaxEnt]: –18.3%, Neural Network Splice Site Prediction [NNSPLICE]: –11.4%, Splice Site Finder [SSF]: –4.1%). Of note, other variants at other –10 splicing acceptor sites in *COL3A1* are reported in Ehlers–Danlos syndrome.[28]

COL5A1 (n_v=6)

Dominant variants in the *COL5A1* gene are known to cause classical Ehlers–Danlos syndrome, with at least 157 variants reported as disease variants in HGMD, with predicted damaging missense variants accounting for 26% of alleles (41/157). Predicted damaging variants in *COL5A1* were nominally enriched (OR, 2.53, *P*=0.018) in individuals with TAA (6/181, *v*=0.033) compared with both our cohort of individuals without TAA (4/354, *v*=0.011) and gnomAD WGS (210/15708, *v*=0.013) (Table 2). These variants accounted for 10.5% of the genetic contribution in our cohort with 6 predicted damaging missense variants identified in our cohort. Interestingly, 3 of these variants reside within the collagen triple helix domain, where 63% of HGMD reported disease-associated missense alleles are located.[29,30]

FBN1 (n_v=6)

Variants in *FBN1* are well known to be associated with TAA, either in individuals with syndromic features such as Marfan syndrome (PMID: 1852208)[31] or in nonsyndromic individuals, with at least 2500 reported alleles for Marfan syndrome (HGMD) including

Table 2. Damaging Variants Identified in Genes of Interest (*COL3A1*, *COL5A1*, *FBN1*, *FLNA*, *NOTCH1*, *NOTCH3*)

Gene	Individual ID	Variant	Zygosity	Classification	Damaging predictions
COL3A1 (NM_000090.3)	568	c.916G>A p.G306S	Het.	NsynD	13/14
	417	c.1885A>G p.K629E	Het.	NsynD	10/15
	563	c.1979G>T p.G660V	Het.	LOF-3	16/16
	107	c.2446-10T>C	Het.	LOF-4	N/A
COL5A1 (NM_000093.4)	480	c.997G>A p.V333I	Het.	NsynD	2/15
	66	c.1842T>G p.S614R	Het.	NsynD	6/14
	368	c.3898G>A p.G1300S	Het.	NsynD	15/15
	298	c.4697C>T p.P1566L	Het.	LOF-3	13/14
	414	c.5468C>T p.A1823V	Het.	NsynD	5/13
	575	c.5468C>T p.A1823V	Het.	NsynD	5/13
FBN1 (NM_000138.4)	109	c.7496T>C p.L2499P	Het.	NsynD	10/12
	436	c.7082C>T p.S2361L	Het.	NsynD	11/12
	468	c.7082C>T p.S2361L	Het.	NsynD	11/12
	60	c.4437C>G p.D1479E	Het.	NsynD	11/12
	496	c.1910G>A p.C637Y	Het.	NsynD	12/12
	273	c.116C>G p.A39G	Het.	NsynD	8/13
FLNA (NM_001456.3)	49	c.2725G>A p.V909I	Hemi.	NsynD	7/13
	309	c.2657-5C>T	Het.	LOF-4	N/A
	332	c.4022C>T p.P1341L	Het.	NsynD	13/13
	547	c.6839G>A p.R2280H	Het.	NsynD	7/14
	555	c.7904G>A p.R2635H	Hemi.	NsynD	4/14
NOTCH1 (NM_017617.4)	534	c.7610C>T p.S2537F	Het.	NsynD	13/15
	321	c.7426G>A p.V2476M	Het.	NsynD	2/16
	281	c.7150C>G p.Q2384E	Het.	NsynD	4/15
	301	c.6724C>G p.L2242V	Het.	NsynD	3/15
	309	c.6235G>A p.V2079M	Het.	NsynD	7/16
	49	c.646G>T p.E216X	Het.	LOF-1	N/A
NOTCH3 (NM_000435.2)	274	c.6239G>A p.R2080Q	Het.	NsynD	6/13
	384	c.6097C>A p.P2033T	Het.	NsynD	11/13
	41	c.5281C>T p.R1761C	Het.	NsynD	10/13
	381	c.3383C>T p.S1128F	Het.	NsynD	9/14
	1	c.3718G>A p.G1240S	Het.	NsynD	15/15
	548	c.2786G>A p.S929N	Het.	NsynD	6/14
	426	c.1505C>T p.S502F	Het.	NsynD	11/14

LOF-1 indicates loss of function by frameshift or stop gain. LOF3: predicted damaging missense variant located within the first or last 2 bases of an exon with a potential impact on splicing. LOF-4 indicates a variant with predicted splicing impact located outside the canonical regions. NSynD indicates predicted damaging missense variant.

nearly equal contributions from LOF and predicted damaging missense alleles.[32,33] Predicted damaging variants in *FBN1* were enriched (OR, 2.35, P=0.04) in individuals with TAA (6/181, v=0.033) compared with both our cohort of individuals without TAA (3/354, v=0.008) and gnomAD WGS (226/15708, v=0.014). Variants in *FBN1* accounted for 12% of the total genetic contribution in the cohort, with 6 patients harboring ultra-rare predicted damaging missense variants (Table 2). Interestingly, several identified variants, p.Cys637Tyr, p.Ala39Gly, and p.Ser2361Leu, were previously reported in familial or less severe or incomplete Marfan diagnoses.[34–40]

FLNA (n_v=7)

Variants in *FLNA* are associated with a range of diagnoses, classically periventricular nodular heterotopia, cardiac valvular dystrophy, and otopalatodigital syndrome, but TAA has been shown to be surprisingly common in individuals with *FLNA* variants.[41] Predicted damaging variants in *FLNA* were

enriched (OR, 7.06, P=1.02×10^{-6}) in individuals with TAA (7/181, v=0.028) compared with both individuals without TAA (4/354, v=0.011) and gnomAD WGS (89/15708, v=0.006). Surprisingly, variants in *FLNA* accounted for 13% of the genetic contribution in our cohort and were more common in individuals with TAA than *FBN1* or *COL5A1* variants (Table 2). Additional manual curation of these alleles with use of American College of Medical Genetics guidelines suggested that 2 of 7 identified variants are more likely to be benign (ID 49, NM_001456.3; c.2725G>A and ID 279, c.2405-4G>A), matching our estimated genetic contribution when comparing the prevalence in individuals with TAA versus individuals without TAA. None of the patients with *FLNA* variants had a history of epilepsy or neurological disorders as typically seen periventricular nodular heterotopia; all 5 candidate variants were missense, with no LOF alleles being identified in the cohort. The clinical histories of individuals with damaging *FLNA* variants are presented in Table 3.

NOTCH1 (n_v=6)

Variants in *NOTCH1* are well-known to be associated with cardiovascular disorders such as TAA both in syndromic and nonsyndromic individuals.[31] There are at least 143 reported alleles (HGMD), with more than 60% of reported alleles being missense. Predicted damaging variants in NOTCH1 were enriched (OR, 2.26, P=0.05) in individuals with TAA (6/181, v=0.033) compared with both individuals without TAA (0/354, v=0) and gnomAD WGS (235/15708, v=0.018). NOTCH1 accounted for 16% of the total genetic contribution in our cohort, with 5 individuals with ultra-rare predicted damaging missense alleles and 1 individual with a LOF variant (Table 2).

NOTCH3 (n_v=7) to TAA

Damaging variants in *NOTCH3* typically cause cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), with more than 350 reported alleles (HGMD), of which

Table 3. Patients With TAA With Damaging *FLNA* Variants (n=5, 3 Women, 2 Men)

Sex	Age at last review	*FLNA* variant (NM_001456.3)	Classification	Maximum population frequency (gnomAD)	Affected *FLNA* domain	Clinical history
Male	49 y	c.2725G>A p.V909I	NsynD	0.0016	8 Vimentin binding	• Heart murmur as a child, ventricular septal defect that closed without intervention. • Experienced shortness of breath and "heartburn"-like chest pressure with exertion • Aneurysm of the ascending thoracic aorta: 6.8 cm • Age 33 y: Underwent replacement of the aortic valve, ascending aorta, and hemiarch *Note: Patient also has NOTCH1 variant.*
Female	44 y	c.2657-5C>T	Splicing/ conserved intronic splicing region + sites with large splicing prediction (eg, branch sites)	Absent	Splice site	• Previously diagnosed with nonspecific atypical aortitis. • Age 38 y: Underwent TAA repair • Age 44 y: Hospitalized with left middle cerebral artery stroke in the setting of subtherapeutic international normalized ratio, treated, and discharged. • A few days later, hospitalized with a large left thalamic intraparenchymal hemorrhage with intraventricular extension and passed away 9 days later.
Female	54 y	c.4022C>T p.P1341L	NsynD	Absent	11 Actin	• History of intermittent mild chest pain, postprandial progressive chest pain • Age 54 y: Found to have TAA (4.2 cm). • Additional history of pericarditis and moderate aortic insufficiency. • Currently undergoing medical management.
Female	56 y	c.6839G>A p.R2280H	NsynD	0.000033 (6/181154)	21 Smad2 binding	• Dizziness, and a chest computed tomography angiogram showed a TAA measuring 5.1 cm. • Age 56 y: Underwent aortic replacement, hemiarch repair, and excision of a pericardial cyst.
Male	59 y	c.7904G>A p.R2635H	NsynD	Absent	24 Smad2 binding	• Age 59 y: Celiac artery dissection (which eventually resolved) and was also found to have a mildly dilated ascending aorta. • Currently being medically followed.

AD indicates Genome Aggregation Database, NsynD >1 damaging prediction+<5 benign; and TAA, thoracic aortic aneurysm.

Table 4. Patients With TAA With *NOTCH3* Variants (n=7, 6 Men, 1 Woman)

Sex	Age at latest review	*NOTCH3* variant (NM_000435.2)	Classification	Maximum population frequency (gnomAD)	Exon	Clinical history
Male	47 y	c.2786G>A:p.S929N	NsynD	Absent	17	• Age 44 y: Bilateral iliac artery dissections and left renal artery dissection. Prescribed antiplatelet medication. • No genetic contribution found. No history of stroke, seizure, or CADASIL diagnosis.
Male	61 y	c.3383C>T:p.S1128F	NsynD	Absent	21	• History of brain hyperventricular hypoattenuation, chronic microangiopathic change. • Age 50 y: severe aortic insufficiency and aortic root aneurysm (5.2), s/p AV replacement and s/p aortic root and ascending aortic graft. • History of pneumothorax. No history of stroke, seizure, or CADASIL diagnosis.
Male	56 y	c.3718G>A:p.G1240S	LOF3	Absent	Splice site	• Congenital AV stenosis status post valvotomy in infancy. • Age 20 y: AVR. • Age 53 y: Found to have TAA (6.3 cm). Underwent complex re-operative composite aortic root and total AV replacement. • Age 56 y: Pulmonary hypertension No history of stroke, seizure, or CADASIL diagnosis.
Male	53 y	c.6239G>A:p.R2080Q	NsynD	5.21×10^{-5} (8/153450)	33	• Bicuspid aortic valve. • Age 37 y: Found to have TAA (5.2 cm) and atrial fibrillation: s/p TAA repair. No history of stroke, seizure, or CADASIL diagnosis.
Male	47 y	c.6097C>A:p.P2033T	NsynD	4.44×10^{-5} (10/225350)	33	• Bicuspid aortic valve. • History of aortic coarctation and repair as child. • Age 34 y: TAA s/p AV replacement. Composite TAA and aortic hemiarch graft replacement. No history of stroke, seizure, or CADASIL diagnosis
Male	65 y	c.5281C>T:p.R1761C	NsynD	7.98×10^{-6} (2/250662)	29	• Bicuspid aortic valve. • Age 48 y: TAA and underwent AV replacement and thoracic aortic replacement. • Severe concentric left ventricular hypertrophy. • Mild carotid disease. • Family history of strokes No history of seizure or CADASIL diagnosis
Female	65 y	c.1505C>T:p.S502F	NsynD	7.48×10^{-5} (14/187230)	10	• Hypertensive w/atrial fibrillation. • Age 50 y: Found to have TAA. Subsequent TAA dissection and further thoracic aortic dilatation. • Age 53 y: Underwent total ascending aorta and aortic arch replacement. • Age 54 y: Descending TAA repair. Persistent distal abdominal aortic dissection extending to iliac arteries. • History of familial TAA dissection syndrome. No history of stroke, seizure, or CADASIL diagnosis.

None of the patients were noted to have connective tissue disease or joint hypermobility. AV indicates aortic valve; CADASIL, cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy; gnomAD, Genome Aggregation Database; LOF-3, exonic splicing sites (±2 bp); NsynD=>1 damaging prediction + <5 benign; and TAA, thoracic aortic aneurysm.

97% are missense variants. Although TAA can be present in individuals with CADASIL, severely damaging *NOTCH3* variants often result in diagnoses earlier in life with clinical features including stroke and seizure.[42,43] Surprisingly, of the 89 genes sequenced that were not previously associated with TAA, *NOTCH3* exhibited the greatest enrichment of damaging variants (OR, 3.00, P=0.005) in individuals with TAA (7/181, v=0.039) compared with individuals without TAA (4/354, v=0.011) and to gnomAD WGS (208/15708, v=0.013) (Table 2). In total, variants in *NOTCH3* accounted for 13% of the genetic contribution within the cohort, with variants identified in 7 individuals (Table 4). Interestingly, 5 of the 7 variants (71%), including 1 previously reported event altered highly conserved serine and arginine residues located within the epidermal growth factor receptor-like domains (Table 2).[44] Our data suggest that the *NOTCH3 gene* may play an important role in TAA, warranting additional assessment of its association to TAA by future large-scale genomic studies.

Somatic Variants

We identified 7 somatic events in individuals with TAA affecting several important genes such as *NOTCH1*, *ACVR1B*, *TGFB2*, *MED12*, and *LTBP1* (Table S4).

Compared with germline events, somatic variants trended toward more damaging impact (eg, LOF and splice-altering) and fewer missense. However, due to the limited number of somatic events detected in the small cohort, the damaging impact of these differences cannot be determined. Future studies should investigate the frequency of somatic variants in these genes with a larger sample size for greater statistical power.

DISCUSSION

We conducted a genetic analysis on a cohort of 181 nonsyndromic patients with TAA under the age of 60 years. A small percentage of individuals in the cohort with TAA were found to have a family history of aortic aneurysms, though medical records were not available for these family members. Therefore, despite the patients having known TAA, the locations of the aortic aneurysm in their family members were not always known. Using deep sequencing analysis, we established that (1) at least 24% of nonsyndromic patients with TAA are likely to have a genetic basis from germline (21%) and somatic (3%) variants; (2) 73% of the genetic basis arose from known TAA-associated genes, whereas yet to be associated genes account for 27% of TAA in our cohort; and (3) missense variants were especially enriched in the cohort. In addition, we found 2 genes, *FLNA* and *NOTCH3*, that are not typically considered as first-tier TAA genes, but which may warrant wider testing in TAA populations.

Of the individuals screened, we discovered that at least 24% of the cohort had a likely underlying genetic basis of their TAA diagnosis. Strikingly, 73% of the genetic cause arose from predicted damaging variants in genes already associated with familial or syndromic TAA, highlighting the potential importance of clinical genetic screening even in patients with nonsyndromic TAA. This is consistent with the empirical data where approximately 10% of early age sporadic aortic dissections had a variant in a TAA-associated gene.[45] To our knowledge, no studies have conducted deep-sequencing targeted sequence analysis of individuals with nonsyndromic, late onset TAA. In 2017, Guo et al. used whole exome sequencing to study patients with sporadic TAA below the age of 56 years and found 9.8% of the cohort had a pathogenic variant in a TAA-associated gene.[45] Our results confirm but further expand upon their findings. In 2017, the number of genes known to be associated with TAA increased from 11 to 29, then to 37 in 2019, suggesting that the original core 11 genes do not account for all of the genetic causes of TAA.[46,47] A recent study further clarified the genetic contribution to hereditary thoracic aortic aneurysm and dissection (TAAD) by classifying 53 genes based on the level of support in the literature.[48] Our findings underscore the growth of the field in recent years and highlights the need for greater genetic testing in this population for patients.

Additionally, the current study examined up- and downstream pathways of TAA-associated genes, reaffirming the important role of the TGF-ß pathway and identifying the potential importance of *NOTCH3* and *FLNA*.[3,6,49–51] When our panel was expanded to include these pathway genes, the proportion of the patient population with a likely causative variant increased 2-fold, highlighting that novel genetic risk factors have yet to be identified. Of note, we identified *FBN1* as significant after gene-level enrichment, confirming and further expanding findings from previous genome-wide association study studies by LeMaire that associated the 15q21.1 locus, which encompasses *FBN1*, with sporadic TAA. The results of this study further underscore the importance of screening the signaling pathways associated with TAA to provide further mechanistic data for improving clinical management.

Of the variants found, we discovered that patients with nonsyndromic TAA were enriched for missense variants, which accounts for 14.5% of individuals with TAA. Unlike LOF variants, missense alleles do not invariably cause complete loss of protein and therefore, can result in milder phenotypes and later presentation in a disease population as demonstrated in this cohort.[52] Conversely, LOF events are likely to result in more severe or classic phenotypes observed in familial TAA, for which clinicians would readily recognize and order clinical testing. Unsurprisingly, we found that our cohort of patients with TAA had a higher frequency of missense variants than typically reported in cohorts with TAA diagnosed in childhood and those with more severe presentations. In contrast, classic LOF variants were greatly depleted in the cohort, supporting the notion that LOF variants are typically associated with more severe clinical phenotypes, earlier presentation in life, and likely identified familial inheritance.[52] Our results may contribute further to the understanding of how missense and splicing variants affect the cause of adult-onset TAA disease.

Of the 114 genes screened, 2 genes emerged as significant after gene-level enrichment: *FLNA* and *NOTCH3*. Our findings established a much higher burden of *FLNA* than previously expected in our nonsyndromic population without neurological pathologies (OR, 7.06, $P=1.02\times10^{-6}$). Previous literature has delineated an association between TAA and *FLNA*, but always with the presence of classical periventricular nodular heterotopia or syndromic features such as a Marfan-like syndrome.[6,41,53] Consistent with this presentation, the *FLNA* variants previously described have primarily been inherited or de novo LOF variants.

In our cohort without classical *FLNA*-associated syndromes, only missense alleles were identified, consistent with the observation that missense variants may result in milder phenotypes. The high frequency of missense alleles could explain the later age presentation and lack of neurological disorders in our cohort. Surprisingly, variants in *FLNA* were as common as *FBN1* and *COL5A1* variants, genes well established to cause TAA, suggesting that *FLNA* may be a common potential candidate gene in nonsyndromic patients with TAA.[54,55]

The greatest enrichment of damaging variants was found in *NOTCH3* (OR, 3.00, *P*=0.005), accounting for 13% of the genetic contribution in our cohort. Previous literature has found associations between upregulated Notch3 protein levels and aortic aneurysm in vitro and in murine models.[56–58] Our study supports and expands the literature by (1) establishing a genetic association between *NOTCH3* and TAA in patients and (2) describing specific genetic variants in nonsyndromic patients with TAA.

The types of *NOTCH3* variants observed in our cohort were different from those observed in patients with CADASIL. *NOTCH3* variants associated with CADASIL primarily affect cysteine residues in the epidermal growth factor receptor-like domains of the protein.[59] In our cohort, we found that 71% of variants instead altered the highly conserved serine and arginine residues in the epidermal growth factor receptor-like domains. This finding suggests a potential genotype–phenotype correlation with *NOTCH3* and vascular disease, where certain variants in *NOTCH3* do not cause syndromes in the brain but rather manifest in the heart and vasculature. Future consideration of including *NOTCH3* in the genetic testing panels of patients with TAA, even patients displaying no CADASIL-like symptoms, deserves further study.

To our knowledge, no previous studies have systematically screened patients with TAA for somatic variants and have not established a somatic pathology of the disease. Though somatic variants were nominally enriched nonsyndromic patients with TAA, the difference did not survive correction for multiple hypothesis testing. Nonetheless, our pilot results suggest that the possibility of somatic variants contributing to the pathology of TAA deserves further study.

Limitations and Future Directions

The sample size was limited to sequential nonsyndromic patients from a single center and limited in definitive ascertainment of single-gene causality and did not involve analysis of family members. Even more, our analysis was restricted to dominant acting genes due to their prevalence in the population and knowledge in the current literature. With a larger sample size and more complete whole exome sequencing or WGS, germline variants in additional genes, including both dominant and recessively acting, may likely emerge as enriched. Furthermore, large whole exome sequencing cohorts for TAA would allow for more robust statistical analyses of rare variant risk across genes and variant class.[60] Because our retrospective study examined a preestablished cohort, the majority of findings were derived from medical history records and clinical cardiovascular screening. Despite these limitations, the high frequency of genetic variants in patients with nonsyndromic TAA warrants further examination and suggests the important role of genetics and even genetic testing in disease presenting in adulthood.

CONCLUSIONS

Our study highlights the potential clinical benefit of deep gene panel sequencing of nonsyndromic individuals with TAA. We demonstrate that at least 24% of these individuals had an unsuspected genetic cause of their disease at time of presentation, with most of the genetic contribution arising from missense instead of loss of function variants. Our results underscore correlations between severity of disease and type of variant found. Additionally, variants in *FLNA* and *NOTCH3*, usually associated with periventricular nodular heterotopia and CADASIL respectively, were highly enriched in our cohort, underscoring the importance of screening individuals with TAA despite the absence of typical neurologic or morphologic features. Our data suggest that both germline and somatic mosaic variants may play a much larger role even in nonsyndromic TAA than previously suspected, and that genetic testing and genetic counseling could be considered in all pediatric and adult patients who present with TAA.

ARTICLE INFORMATION

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Disclosures

None.

Supplemental Material

Data S1
Tables S1–S3

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