Journal of the American Heart Association

ORIGINAL RESEARCH

Vertebral Tortuosity Is Associated With Increased Rate of Cardiovascular Events in Vascular Ehlers-Danlos Syndrome

Sara B. Stephens, MPH; Sherene Shalhub, MD, MPH; Nicholas Dodd, MD; Jesse Li, MD; Michael Huang, MD; Seitaro Oda, MD, PhD; Kalyan Kancherla, MD; Tam T. Doan, MD; Siddharth K. Prakash, MD, PhD; Justin D. Weigand, MD; Federico M. Asch, MD; Taylor Beecroft, MS, CGC; Alana Cecchi, MS, CGC; Teniola Shittu, MS, MPH; Liliana Preiss, MS; Scott A. LeMaire, MD; Richard B. Devereux, MD; Reed E. Pyeritz, MD, PhD; Kathryn W. Holmes, MD, MPH; Mary J. Roman, MD; Ronald V. Lacro, MD; Ralph V. Shohet, MD; Rajesh Krishnamurthy, MD; Kim Eagle, MD; Peter Byers, MD; Dianna M. Milewicz, MD, PhD; Shaine A. Morris, MD, MPH

BACKGROUND: Arterial tortuosity is associated with adverse events in Marfan and Loeys-Dietz syndromes but remains understudied in Vascular Ehlers-Danlos syndrome.

METHODS AND RESULTS: Subjects with a pathogenic *COL3A1* variant diagnosed at age <50 years were included from 2 institutions and the GenTAC Registry (National Registry of Genetically Triggered Thoracic Aortic Aneurysms and Cardiovascular Conditions). Height-adjusted vertebral artery tortuosity index (VTI-h) using magnetic resonance or computed tomography angiography was calculated. Associations between VTI-h and outcomes of (1) cardiovascular events (arterial dissection/rupture, aneurysm requiring intervention, stroke), or (2) hollow organ collapse/rupture at age <50 years were evaluated using receiver operator curve analysis (using outcome by age 30 years) and mixed-effects Poisson regression for incidence rate ratios. Of 65 subjects (54% male), median VTI-h was 12 (interquartile range, 8–16). Variants were missense in 46%, splice site in 31%, and null/gene deletion in 14%. Thirty-two subjects (49%) had 59 events, including 28 dissections, 5 arterial ruptures, 4 aneurysms requiring intervention, 4 strokes, 11 hollow organ ruptures, and 7 pneumothoraces. Receiver operator curve analysis suggested optimal discrimination at VTI-h ≥15.5 for cardiovascular events (sensitivity 70%, specificity 76%) and no association with noncardiovascular events (area under the curve, 0.49 [95% CI, 0.22–0.78]). By multivariable analysis, older age was associated with increased cardiovascular event rate while VTI-h ≥15.5 was not (incidence rate ratios, 1.79 [95% CI, 0.76–4.24], P=0.185). However, VTI-h ≥15.5 was associated with events among those with high-risk variants <40 years (incidence rate ratios, 4.14 [95% CI, 1.13–15.10], P=0.032), suggesting effect modification by genotype and age.

CONCLUSIONS: Increased arterial tortuosity is associated with a higher incidence rate of cardiovascular events in Vascular Ehlers-Danlos syndrome. Vertebral tortuosity index may be a useful biomarker for prognosis when evaluated in conjunction with genotype and age.

Key Words: arterial rupture ■ cardiovascular ■ dissection ■ genetics ■ VEDS

Correspondence to: Shaine A. Morris, MD, MPH, Texas Children's Hospital, 6651 Main St E1920, Houston, TX 77030. Email: shainem@bcm.edu

Supplemental Material is available at https://www.ahajournals.org/doi/suppl/10.1161/JAHA.123.029518

This manuscript was sent to Saket Girotra, MD, SM, Associate Editor, for review by expert referees, editorial decision, and final disposition.

For Sources of Funding and Disclosures, see page 13.

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

CLINICAL PERSPECTIVE

What Is New?

- Findings suggest that longitudinal imaging surveillance with vertebral tortuosity measurement for risk stratification can benefit children and adults with Vascular Ehlers-Danlos syndrome.

What Are the Clinical Implications?

- Risk associated with increased arterial tortuosity may be modified by the *COL3A1* genotype, regardless of age, with patients harboring a missense or splice site variant and height-adjusted vertebral tortuosity index ≥15.5 having 4.1 times the rate of cardiovascular events compared with those with a null variant and same height-adjusted vertebral tortuosity index range.

Nonstandard Abbreviations and Acronyms

CTA	computed tomography angiogram
GenTAC	National Registry of Genetically Triggered Thoracic Aortic Aneurysms and Cardiovascular Conditions
IRR	incidence rate ratio
KM	Kaplan–Meier
TCH	Texas Children's Hospital
VEDS	Vascular Ehlers-Danlos syndrome
VTI	vertebral tortuosity index
VTI-h	height-adjusted vertebral tortuosity index

Vascular Ehlers-Danlos syndrome (VEDS) is a rare hereditary disorder associated with spontaneous arterial dissection and rupture attributable to pathogenic variants in the *COL3A1* gene, which encodes the chains of type III collagen.[1] With a reported median life expectancy of 48 years and arterial rupture frequently occurring even in the absence of prior symptoms or aneurysm, VEDS is a high-risk connective tissue disorder, although the severity of disease is highly variable.[1–3] The true prevalence of VEDS is estimated to be ≈1 in 50 000, which is much less common than Marfan syndrome with a reported prevalence between 1 in 5000 to 1 in 10 000.[4]

Given the rarity of the condition and its variable presentation, risk stratification has been challenging. The primary variable that has been shown to be associated with earlier events is the nature of the *COL3A1* variant. For example, bulky substitutions for a glycine residue in the repeated Gly-X-Y triplet of the triple helical domain or splice site variants that result in exon skipping are associated with early clinical manifestations of disease compared with haploinsufficient variants.[5–7] Variants in *COL3A1* variants resulting in haploinsufficiency of *COL3A1* typically have a later presentation and on average longer survival.[5–7] However, even within families, severity and age at first event are highly variable, demonstrating that additional risk stratification tools are needed beyond genotype.[8] Understanding an individual's risk could improve management, including medical and surgical therapy, timing and type of imaging, and potential for guidance for lifestyle modifications.

Arterial tortuosity is a biomarker that has been associated with adverse health outcomes in patients with other connective tissue disorders, including Marfan syndrome and Loeys-Dietz syndrome, where increased tortuosity is associated with younger age at surgical intervention, aortic dissection, and death.[9–12] Increased arterial tortuosity has also been associated with arterial events in nonsyndromic populations.[13] However, the clinical utility of tortuosity in patients with VEDS has not been evaluated. Further investigations in this high-risk population will improve clinical management and surveillance strategies in VEDS.

In this study, we evaluated the association between arterial tortuosity and cardiovascular or extracardiac events in children and adults with VEDS. Specifically, we hypothesized that increased height-adjusted vertebral tortuosity index (VTI-h) would be associated with a higher incidence of life-threatening events compared with those with lower VTI-h.

METHODS

Study Population

Subjects with VEDS from 4 longitudinal cohorts were included: (1) subjects prospectively enrolled in a longitudinal study of VEDS in the pediatric and young adult population at Texas Children's Hospital (TCH), (2) patients clinically evaluated and followed at TCH not enrolled in the prospective study but part of a separate retrospective cohort study of aortopathy, (3) adult patients evaluated at the University of Texas Health Science Center at Houston, and (4) subjects enrolled in the GenTAC Registry (National Registry of Genetically Triggered Thoracic Aortic Aneurysms and Cardiovascular Conditions). The GenTAC protocol has been reported.[14,15] Cores for both archived imaging and phenotypic information were used to ensure proper classification. The same phenotypic parameters and imaging measurements were used for all subjects. Subjects in cohorts 1 and 4 provided written informed consent as approved by their respective institutional review board committees. Subjects in cohorts 2 and 3 were included as part of a retrospective study under an institutional review board-approved

protocol with a waiver of consent. Approval for studies of all cohorts was provided by Baylor College of Medicine's institutional review board, and approval for the study of cohort 3 was also approved by the Committee for the Protection of Human Subjects at University of Texas Health Science Center at Houston. Inclusion criteria for all cohorts included: (1) age <50 years at time of VEDS diagnosis, (2) available clinical genetic testing records that identified a pathogenic or likely pathogenic *COL3A1* variant, and (3) at least 1 magnetic resonance angiogram (MRA) or computed tomography angiogram (CTA) that included the vertebral arteries and was available for review for VTI ascertainment. Subjects were excluded if all CTA/MRAs had fields of view covering <2.5 cm of both vertebral arteries or were of poor quality. The study was limited to ages <50 years given evidence of arterial tortuosity as a biomarker in young populations but with less discrimination in older subjects because of development of tortuosity with aging.[9,16] Subjects being evaluated at TCH for the retrospective cohort (cohort 2) were identified using an electronic medical record query identifying patients seen between January 1, 1990, and December 1, 2021. Among patients in cohort 2, those who were contactable were consented prospectively and recruited for additional evaluation, including a research history and physical exam (and were included with those in cohort 1).

Medical records were reviewed for cohorts 1 to 3, including *COL3A1* genotype, genetics-focused physical examination findings, serial cardiovascular imaging (echocardiography and MRA/CTA studies), history of cardiovascular or organ-specific events, and surgical interventions. For subjects in cohort 4, the same information was collected from the GenTAC database, which has fields of all these same data, and data fields were harmonized. Because of the retrospective waiver of consent allowing for inclusion of patients in this study, as well as the possibility of unintentionally sharing information that could be used to reidentify patients with this rare condition, data that support this study's findings are not directly available for request.

Imaging

For all cohorts, cardiovascular and extracardiac imaging was performed at the discretion of patients' physicians based on institutional surveillance protocols and at times of clinical concern. MRA/CTA images from cohort 1 to 3 were measured at TCH. Images for GenTAC subjects (cohort 4) were deidentified at participating sites and transferred to the imaging core (MedStar Health Research Institute, Washington, DC) for analysis.[17] Imaging analysis for both cohorts used a standard protocol for all modalities, including blinding to clinical outcomes and characteristics. Commercially available software was used for CTA/MRA post-processing (TCH: Philips Extended MR WorkSpace version 2.6 or Vitrea systems; GenTAC: Osirix for MRA and FujiPACS for CTA).

Assessment of Arterial Tortuosity

The vertebral artery tortuosity index was calculated from available MRA and CTA imaging (Data S1). At both core imaging sites, readers were trained by S.A.M. in VTI measurement, and all VTI measurements were read in a core laboratory fashion. VTI was measured as previously described and shown to be highly reproducible.[9] In prior longitudinal evaluation of patients with Marfan syndrome, we observed that the VTI in children decreases as height increases during childhood (likely because of "stretching" of the vertebral artery with growth) and plateaus in late adolescence once growth has stopped.[18] Using the prior data, we were able to discern the relationship between height and VTI to calculate height-adjusted VTI (VTI-h) using the formula (VTI-h=e$^{(\log(\text{VTI})+0.00532\ *\ (\text{height in cm})\ -\ 0.266)}$).[18] For patients with >1 exam, the primary VTI and VTI-h were calculated from the exam covering the longest portion of the vertebral arteries. If multiple exams successfully imaged both vertebral arteries in their entirety, the primary VTI and VTI-h were calculated from the first study. Typically, patients in the study were followed either with serial MRI or serial computed tomography (CT), but rarely both, especially that which included neck imaging. To ensure the largest possible sample size, we used VTI-h from either CT or MRI. Given these modalities measure the same vessels and the relative shape, not dimension, we did not anticipate a major difference in VTI-h between modalities.

Aortic Measurements

Although traditionally aortic dilation is not commonly present in VEDS, we evaluated associations between aortic root size and cardiovascular outcomes, given strong associations in other disorders. For cohorts 1, 2, and 4, echocardiographic measurements were performed in systole using an inner-edge-to-inner-edge technique, as per the American Society of Echocardiography pediatric guidelines and per GenTAC imaging protocols for all ages.[17,19] For the TCH cohort (cohorts 1 and 2), echocardiographic measurements were taken from clinical reports, given lack of universal availability of images before 2006 and standard measurement methods in the TCH laboratory using the guidelines above for >30 years. For cohort 3, aortic root measurements were made using the standard adult method, in diastole, measuring from leading edge to leading edge. Studies have shown minimal difference in measurements using these 2 different methodologies.[20] For cohort 4 (GenTAC), aortic measurements were performed in the core laboratory. Aortic

root measurements performed after an aortic root replacement for aneurysm or type A aortic dissection were excluded.

For CT and magentic resonance (MR) imaging in all cohorts, all aortic root and ascending aortic measurements were performed using double oblique views, measuring the maximum distance from inner edge to inner edge. For gated studies, the measurement was performed in systole. For all cohorts, CT measurements were obtained from the CTA. For cohorts 1 and 2, MR aortic root measurements were performed on gated MRI cine steady-state free-precession images of the root when available. Otherwise, measurements were collected from the MRA images in the same fashion as CT.

Aortic root and ascending aortic z-scores for subjects under the age of 18 years at the time of echocardiography were calculated using the Boston Children's Hospital Z-Scores.[21,22] For aortic z-scores measured by MR/CT in subjects ages 0 to 17.99 years, methods published by Kaiser et al were used.[23] Z-scores for subjects ages 18 years and older were calculated using published methodologies by Campens et al for echocardiology and Lin et al for MR/CT measurements.[24,25] All z-score calculations required availability of height and weight at time of imaging. If height at the time of imaging study was not available, the height at time of other imaging within 3 years before or after was extrapolated if the patient was postpubertal. For those with multiple height measurements available at alternative dates available, the height at the date closest to the original imaging study within 6 months was used for extrapolation. If a height measurement using this criteria or weight at time of imaging study was not available, echo z-score could not be calculated. A subject was considered to have aortic dilation if the calculated z-score was >2 on any imaging with aortic measurements available.

Covariates

Covariates included sex, age at diagnosis, *COL3A1* genotype, aortic root and ascending aortic z-scores, race, and ethnicity. *COL3A1* variant classification was informed by prior literature, where subjects were classified into high-risk genotype (either glycine substitutions for large amino acids such as valine, aspartate, glutamate, or arginine, or alternatively, splice site variant), or low-risk genotype (null variant or *COL3A1* deletion resulting in haploinsufficiency).[6,26–28] For glycine to serine and glycine to alanine substitutions, the expected effect is predicted to be milder because of the small residue of the substituted amino acids and observed longevity in patients harboring this type of glycine substitution.[5] Therefore, these variants were also classified as low-risk. Classification of each individual variant was confirmed by a board-certified clinical geneticist (P.B.). Because of a limited number of non-Hispanic Black and Asian subjects in the cohort, analyses of race and ethnicity were limited to non-Hispanic White and Hispanic subjects.

Outcomes

Three different event types (any cardiovascular or organ event, any cardiovascular event exclusively, or any organ event exclusively) were evaluated in 2 different ways: any event (dichotomized) before age 50 years or number of events (count variable) before age 50 years. Cardiovascular events were defined as arterial dissection or rupture, aneurysm requiring surgical intervention, or stroke. Organ events were defined as a pneumothorax or a perforation/rupture of a hollow organ. For all analysis incorporating age at event, outcomes were limited to those with a reported age at event.

Statistical Analysis

The primary independent variable was VTI-h, first evaluated as a continuous variable. We first examined the association between VTI-h and each event using Cox regression. The relationship between VTI-h and the occurrence of an event before the age of 30 years was then evaluated using receiver operator curve (ROC) analysis to identify any potentially clinically relevant cut-off value for VTI-h identifying higher risk for early events. A VTI-h cutoff was selected to optimize both sensitivity and specificity. VTI-h measurements greater than or equal to the cut-off discerned through ROC analysis were defined as a "high" VTI-h while those lower than the cut-off were considered to be "low."

Evaluating Time to First Event

All statistical analyses described below evaluated VTI-h as a dichotomized cut-off value based on ROC analysis. Time to first event by dichotomized VTI-h and other potential covariates (race and ethnicity, genotype group, and sex) was evaluated using Kaplan–Meier (KM) analyses with log-rank test. Outcomes for the KM analysis were (1) time to first event, (2) time to first cardiovascular event, and (3) time to first organ event, with time zero defined as birth. Survival times were censored at the time of first event for subjects who had a first event or last follow-up or death for those who did not.

Evaluating Multiple Events

Event incidence rates were calculated using number of events (including repeated events in the same individual) as the numerator and summed age at last follow-up as the denominator and were reported as number

of events per 100 person-years. Event incidence rates were compared by dichotomized VTI-h, age decile, race and ethnicity, genotype group, and sex. These incidence rates were first compared in unadjusted analyses, computing crude incidence rate ratio (IRR).

Mixed-effects Poisson regression was then used to examine the association between dichotomized VTI-h and outcomes in an adjusted model accounting for multiple events per individual and for the effect of increasing age. In this model, age decile was retained for all models as a fixed effect, given the known profound effect of age on outcomes. To reduce the degrees of freedom, the inverse transformation of decile was used as a continuous variable rather than decile as categories. Inverse transformation of age had a superior fit in relationship to event rate compared with other transformations such as log or square root. Patient study number served as a random effect. IRRs were calculated, with event number offset by number of person-years in each decile to account for differences in exposure time (log-transformed). IRRs for each outcome were evaluated for all covariates assessed in this model with age decile alone. Interaction terms were evaluated with their base covariates simultaneously in the model. A multivariable model was then created for all outcomes and cardiovascular outcomes. Age decile-adjusted and multivariable models were not created for organ outcomes, given the low number of events. For the multivariable models, covariates and interaction terms with a P value <0.2 in association with each outcome were included in an initial multivariable model. Backward elimination was then performed, retaining VTI-h dichotomized, inverse transformation of decile, risk-stratified genotype, and any other covariate maintaining a P value <0.05.

As an exploratory analysis, the resulting multivariable model was then applied limited to subjects with a high-risk genotype to evaluate for effect modification by the *COL3A1* genotype. Because of a low number or outcomes, this analysis could not be replicated among subjects with a low-risk genotype. For visualization, a KM curve and log-rank test were performed to evaluate the association between dichotomized VTI-h and time to first cardiovascular event among subjects with high-risk and low-risk genotypes. Of note, stratification was elected to evaluate effect measure modification as cell counts for low-risk genotype were too low to implement an interaction term. KM analyses suggested that time to cardiovascular event by dichotomized VTI-h converged near age 40 years. Therefore, we conducted a post hoc analysis repeating multivariable modeling of subjects with a high-risk variant limited to follow-up before age 40 years. A 2-tailed P value of <0.05 was considered statistically significant. All statistical analyses were performed using Stata statistical software (Stata/IC v16.1, College Station, TX).

RESULTS

Cohort Characteristics

Sixty-five subjects were eligible for inclusion (53.9% male, Table 1). These included 29 subjects from cohort 1, 1 subject in cohort 2, 23 subjects in cohort 3, and 12 subjects in cohort 4. Median age at genetic diagnosis was 10.5 years (interquartile range [IQR], 5.0–31.5 years). Median age at last follow-up was 19.4 years (IQR, 11.0–39.0 years). Thirty subjects (46%) were below the age of 18 years at time of last follow-up. The majority of subjects were non-Hispanic White (67.7%) or Hispanic (29.2%). By genotype risk group, 18 subjects (27.7%) had a low-risk variant while 40 subjects (61.5%) had a high-risk variant. One subject with a splice site variant predicted to be a frameshift mutation resulting in an incomplete protein was considered low-risk with an effectively null variant (Table S1). The remaining 7 (10.8%) subjects were classified as "unknown" and were excluded from risk-stratified genotype analysis. Nine subjects (9/54 with imaging on file, 16.7%) had aortic root dilation detected by any imaging modality, while 12 (12/54, 22.2%) had ascending aortic dilation (Table 1). Of note, 1 subject only had imaging following a type A dissection warranting aortic root replacement and was therefore excluded from analysis. Median VTI in the cohort was 7 (IQR, 5–10). After adjusting for height, median VTI-h was 12 (IQR, 8–16).

Outcomes

Overall, 32 subjects (49.2%) had at least 1 event with an overall sum of 59 events. This included 17 subjects (26.2%) with only cardiovascular event(s), 10 (15.4%) with only extravascular organ event(s), and 5 (7.7%) with both. A total of 27.7% of subjects had 1 event (n=18), while 12.3% and 9.2% had 2 and more than 2 events, respectively. The most frequent event type was arterial or aortic dissection (28 dissections in 19 subjects), followed by hollow organ rupture (11 ruptures in 10 subjects), pneumothorax (7 pneumothoraces in 6 subjects), aneurysm requiring surgical intervention (4 aneurysms in 4 subjects), arterial rupture (5 ruptures in 4 subjects), and stroke (4 strokes in 4 subjects, Figure S1). Events were fatal for 1 subject (Table S2). Six subjects (of 40 subjects who reached the outcome before 18 or reached age 18 years with no event, 15.0%) had an event before the age of 18 years.

When accounting for multiple events, overall incidence rate for all events was 3.25 events per 100 person-years (95% CI, 2.43–4.26, Table S3). Of note, age at event for 7 events was unknown and therefore these were not included in incidence rate calculations. The overall incidence rate for cardiovascular events was 2.38 per 100 person-years and 0.88 per 100 person-years for organ events (Table 2 and Table S3).

Table 1. Cohort Characteristics and Outcomes

Variable	Available	n (%) or median (IQR)
Male sex	65	35 (53.9%)
Age at diagnosis, y	64	10.5 (5.0 to 31.5)
Age at last follow-up, y	65	19.4 (11.0 to 39.0)
Race and Ethnicity	65	
Non-Hispanic White		44 (67.7%)
Hispanic		19 (29.2%)
Non-Hispanic Black		1 (1.5%)
Asian		1 (1.5%)
COL3A1 genotype	65	
Glycine substitution		30 (46.2%)
Splice site variant, donor		15 (23.1%)
Splice site variant, acceptor		5 (7.7%)
Haploinsufficient/null		9 (13.9%)
Other/unknown		6 (9.2%)
Categorized *COL3A1* genotype	65	
High-risk		40 (61.5%)
Low-risk		18 (27.7%)
Not classified		7 (10.8%)
Echo aortic measurements		
Max aortic root z-score	16	1.32 (−0.11 to 1.71)
Max ascending aortic z-score	23	1.74 (0.38 to 2.19)
Aortic root dilation on echo	16	3 (18.8%)
Ascending aortic dilation on echo	23	8 (34.8%)
Any aortic dilation by echo	26	11 (42.3%)
MRA/MRI/CTA aortic measurements		
Max aortic root z-score	37	0.92 (0.33 to 1.61)
Max ascending aortic z-score	30	0.83 (0.069 to 1.74)
Aortic root dilation on MRA/MRI/CTA	37	6 (16.2%)
Ascending aortic dilation on MRA/MRI/CTA	30	4 (13.3%)
Any aortic dilation on CTA/MRA	40	9 (22.5%)
Aortic assessment by any imaging modality		
Max aortic root z-score	54	0.98 (0.24 to 1.65)
Max ascending aortic z-score	53	1.26 (0.13 to 1.98)
Aortic root dilation by any modality	54	9 (16.7%)
Ascending aortic dilation by any modality	54	12 (22.2%)
Any aortic dilation	54	19 (35.2%)
Imaging to ascertain VTI-h	65	
Computed tomography angiography		29 (44.6%)
Magnetic resonance angiography/imaging		34 (52.3%)
Unknown imaging type		2 (3.1%)
Median VTI (IQR)	65	7 (5 to 10)
Median VTI-h (IQR)	65	12 (8 to 16)
Dichotomized VTI-h	65	
VTI-h≥15.5		20 (30.8%)
VTI-h<15.5		45 (69.2%)
Any cardiovascular or organ event	65	31 (47.7%)
Event count by subject	65	
0 events		33 (50.8%)
1 event		18 (27.7%)
2 events		8 (12.3%)
>2 events		6 (9.2%)
Any cardiovascular event	65	22 (33.9%)
Any extravascular organ event	65	15 (23.1%)
Subjects with death following event	65	1 (1.5%)

All values expressed as n (%) or median (Q1, Q3). CTA indicates computed tomography angiography; IQR, interquartile range; MRA, magnetic resonance angiography; MRI, magnetic resonance imaging; VTI, vertebral tortuosity index; and VTI-h, height-adjusted vertebral artery tortuosity index.

VTI-h and Time to Event

As a continuous variable, VTI-h was not associated with time to first event of any kind on Cox regression (Table 3). However, ROC analysis evaluating association between VTI-h and any event before age 30 years demonstrated mild discriminatory ability, with area under the curve 0.64 (95% CI, 0.48–0.80, *P*=0.092, Figure 1A). When limiting the assessment to cardiovascular events before age 30 years, discriminative ability improved (area under the curve 0.71 [95% CI, 0.54–0.88], *P*=0.038, Figure 1B), while VTI-h had no discriminative ability for extravascular organ events before age 30 years (area under the curve 0.49 [95% CI, 0.22–0.76], *P*=0.937, Figure 1C). This suggested that the lower discriminative ability for all events was largely driven by the inclusion of noncardiovascular events. Based on these findings, subsequent analyses were performed focusing on the relationship of VTI-h and cardiovascular events.

Based on ROC analysis of cardiovascular events, the optimal cut point for VTI-h was ≥15.5, (sensitivity 70.0%, specificity 76.4%, 75.4% correctly classified for cardiovascular events). Among subjects, 45 (69.2%) had a low VTI-h and 20 (30.8%) had a high VTI-h. On KM analysis, survival curves suggested earlier cardiovascular events among subjects with a high VTI-h early in life; however, the curves converged near age 40 years (log-rank *P*=0.270, Figure 2). Those in the high VTI-h

Table 2. Incident Cardiovascular Event Rate Ratios by Characteristics

Variable	Incidence rate components						Model adjusted for decile alone[†]		Multivariable model[‡]	
	No. individuals	No. cardiovascular events	No. person-years	Incidence rate[*] (95% CI)	Crude IRR (95% CI)	*P* value	Adjusted IRR (95% CI)	*P* value	Adjusted IRR (95% CI)	*P* value
All	65	38	1600.01	2.38 (1.68–3.26)	…	…				
Age decile							N/A	0.003[¶]	N/A	0.01[¶]
0–9.9 y	65	1	600.96	0.17 (0.00–0.93)	Reference					
10–19.9 y	52	1	407.78	0.25 (0.01–1.37)	1.47 (0.02–115.68)	0.81				
20–29.9 y	32	10	292.32	3.42 (1.64–6.29)	20.56 (2.92–892.18)	<0.001[¶]				
30–39.9 y	27	12	221.14	5.43 (2.80–9.48)	32.61 (4.83–1394.0)	<0.001[¶]				
40–49.9 y	15	14	77.81	17.99 (9.84–30.19)	108.13 (16.45–4572.0)	<0.001[¶]				
Stratified VTI-h[§]										
High	20	22	582.42	3.78 (2.37–5.72)	2.40 (1.21–4.89)	0.01[¶]	1.78 (0.89–3.56)	0.10	1.79 (0.76–4.24)	0.19
Low	45	16	1017.59	1.57 (0.90–2.55)	Reference		Reference		Reference	
Sex										
Male	35	20	713.66	2.80 (1.71–4.33)	1.38 (0.69–2.77)	0.33	1.85 (0.97–3.53)	0.06	…	…
Female	30	18	886.35	2.03 (1.20–3.21)	Reference		Reference			
Race and Ethnicity										
Non-Hispanic White	44	34	1144.80	2.97 (2.06–4.15)	2.92 (1.04–11.32)	0.03[¶]	2.04 (0.69–6.09)	0.20	…	…
Hispanic	19	4	393.00	1.02 (0.28–2.61)	Reference		Reference			
Genotype										
Glycine substitution	30	14	854.39	1.64 (0.90–2.75)	0.57 (0.20–2.03)	0.30	2.42 (0.98–6.01)	0.06	…	…
Splice site variant	20	8	351.91	2.27 (0.98–4.48)	0.79 (0.23–3.109	0.68	1.98 (1.00–3.92)	0.049[¶]		
Null variant	9	5	174.79	2.86 (0.93–6.78)	Reference		Reference			
Genotype group										
High-risk	40	18	877.09	2.05 (1.22–3.24)	0.88 (0.39–2.03)	0.71	1.41 (0.71–2.81)	0.33	1.17 (0.53–2.59)	0.70
Low-risk	18	11	465.23	2.36 (1.18–4.23)	Reference		Reference		Reference	
Aortic root dilation[‖]										
Dilation	9	4	192.02	2.08 (0.57–5.33)	0.86 (0.22–2.42)	0.83	1.05 (0.33–3.34)	0.93	…	…
No dilation	45	34	1407.99	2.42 (1.67–3.37)	Reference		Reference			

(*Continued*)

Table 2. Continued

Variable	Incidence rate components				Crude IRR (95% CI)	*P* value	Model adjusted for decile alone†		Multivariable model‡	
	No. individuals	No. cardiovascular events	No. person-years	Incidence rate* (95% CI)			Adjusted IRR (95% CI)	*P* value	Adjusted IRR (95% CI)	*P* value
Ascending aortic dilation‖										
Dilation	12	5	193.11	2.59 (0.84–6.04)	1.10 (0.34–2.85)	0.80	3.08 (0.66–13.67)	0.14	…	…
No dilation	42	33	1406.90	2.35 (1.62–3.29)	Reference		Reference			
Any aortic dilation‖										
Dilation	19	7	341.14	2.05 (0.83–4.23)	0.83 (0.31–1.93)	0.69	1.59 (0.54–4.64)	0.40	…	…
No dilation	35	31	1258.87	2.46 (1.67–3.50)	Reference		Reference			

For incidence rate ratio calculations, patients classified as Asian or Black race or other/unknown genotype were excluded from analyses of genotype. Calculations involving aortic dilation were limited to subjects with aortic measurements, height, and weight available to calculate aortic z-scores. IRR indicates incidence rate ratio.

*Per 100 person-years.

†Adjusted only for decile (inverse transformed) and repeated measures, using Poisson regression.

‡Adjusted for decile (inverse transformed) and repeated measures, using Poisson regression, and listed variables.

§High height-adjusted vertebral tortuosity index was defined as ≥15.5. Low height-adjusted vertebral tortuosity index was defined as 15.5 or less.

‖Because aortic dilation was ascertained using different imaging modalities (echo vs computed tomography/magentic resonance) and different techniques (adult vs pediatric), aortic root and ascending dilation was excluded from statistical modeling.

¶*P* values indicate statistical significance at a 95% confidence level.

Table 3. Cox Regression for VTI-h and Outcomes

Event type	HR (95% CI)	*P* value
Any event	1.00 (0.98–1.03)	0.97
Cardiovascular event	1.00 (0.97–1.03)	0.90
Extravascular organ event	0.98 (0.96–1.01)	0.28

HR indicates hazard ratio; and VTI-h, height-adjusted vertebral tortuosity index.

group had median age at first cardiovascular event at age 33.9 years compared with the low VTI-h group, in which median age at first event was 41.6 years, and no cardiovascular event in the low VTI-h group occurred before age 20 years. Freedom from cardiovascular event by risk-stratified genotype showed a similar pattern, with separation early in life and convergence later around age 40 years (Figure 2). Males started having cardiovascular events earlier than females, but this did not meet statistical significance (*P*=0.081, Table 4). No significant differences in freedom from event were detected by ethnicity. Comparisons of KM curves for all outcomes and organ events can be found in Figure S2.

Further, on assessment of subjects stratified by high-risk and low genotype, analysis suggested earlier time to cardiovascular event among subjects with a high VTI-h in those with a high-risk genotype, although this did not meet significance (Figure 3, log-rank *P*=0.064). This pattern was not evident among subjects with a low-risk genotype (*P*=0.492).

VTI-h and Event Rate

When evaluating multiple events, IRRs were noted to increase by person-decile for all events, cardiovascular events, and organ events (Table 2; Table S3). VTI-h ≥15.5 was associated with a higher crude event rate for cardiovascular events (3.78 versus 1.57, *P*=0.008). Non-Hispanic White race and ethnicity was also associated with a higher cardiovascular event rate than in Hispanic patients (2.97 versus 1.02, *P*=0.026). On crude analysis, neither presence of aortic root dilation nor ascending aortic dilation was associated with higher event rates. Event rates and comparisons for all events and extracardiac events are noted in Table S3. The effect of age on cardiovascular events appeared to be significantly modified by high versus low VTI-h (Figure 4), with a slower increase in risk with aging among those with low VTI-h versus high.

Backward elimination for cardiovascular outcomes yielded a final multivariable model containing age decile, dichotomized VTI-h, and risk-stratified *COL3A1* genotype (Table 2). After controlling for age and genotype, subjects with a high VTI-h had more than 1.79 times the rate of incident cardiovascular events compared with subjects with a low VTI-h, although this did not meet significance criteria (*P*=0.185). Risk-stratified

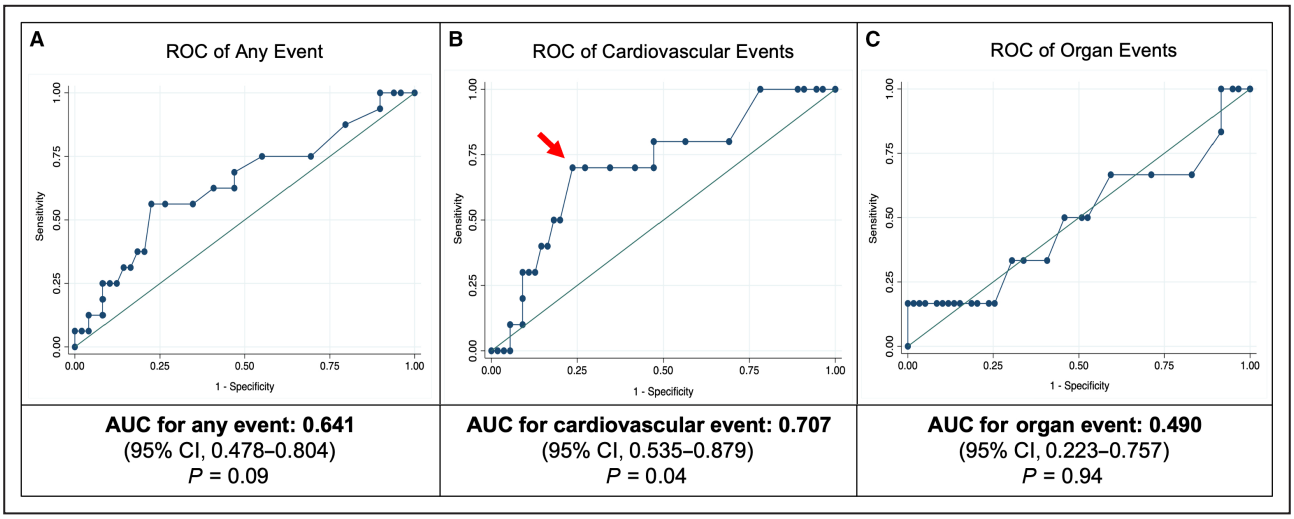

Figure 1. ROC for height-adjusted vertebral tortuosity index and event before age 30 years.
A, ROC of any event; **B**, ROC of cardiovascular events; **C**, ROC of organ events. ROC analysis of height-adjusted vertebral tortuosity index and having an event before age 30 years demonstrated mild discriminatory value with an area under the curve of 0.64. However, predictive ability increased when limiting to cardiovascular events before age 30 years (area under the curve=0.71), comparatively higher than the ROC for organ events before age 30 (area under the curve=0.49). Based on ROC analysis of cardiovascular events alone, an optimal cut point was selected at 15.5 (sensitivity 70.0%, specificity 76.4%). This optimal cut point is denoted by the red arrow. AUC indicates area under the curve; and ROC, receiver operator curve.

genotype group did not meet significance criteria to be associated with rate of cardiovascular event on multivariable analysis (IRR=1.17 [95% CI, 0.53–2.59], *P*=0.699). When multivariable analysis was limited to subjects with a high-risk genotype, the association was magnified (IRR for high VTI-h versus low VTI-h 3.25 [95% CI, 0.98–10.78], *P*=0.054) and more so when limited to the first 4 decades of life and high-risk variants (IRR=4.14 [95% CI, 1.13–15.10], *P*=0.032) (Table 5). Incidence rates of cardiovascular events by VTI-h and person-decade can be found in Figure 4.

DISCUSSION

In this study, nearly one-half of patients with VEDS experienced at least 1 life-threatening event before age 50 years. Findings demonstrated that increasing age is strongly associated with events and, further, that height-adjusted VTI may be independently associated with increased rate of incident cardiovascular events among patients with VEDS, especially among those with a high-risk genotype under age 40 years. While height-adjusted VTI did not meet significance threshold on multivariable analysis, this may have been a by-product of limited statistical power in this study and warrants further investigation. Given the low rate of events among subjects with a low-risk genotype, elevated VTI-h was most discriminating among those with high-risk genotypes. Although sex did not meet significance criteria, males tended to have earlier cardiovascular events. Further, findings indicated that neither aortic root dilation nor ascending aortic dilation was associated with any type of event rate. This study suggests that height-adjusted tortuosity may be another useful tool for risk stratification in young people with VEDS and that the risk conferred by degree vertebral tortuosity may be modified by *COL3A1* variant type and age. These findings are helpful as risk stratification can guide medical therapy, activity guidance, and timing of intervention.[28,29]

The reproducibility of vertebral artery tortuosity index as a biomarker of risk for cardiovascular events has been noted in other prior investigations, as arterial tortuosity has been characterized in other genetic arteriopathies such as Loeys-Dietz syndrome, Marfan syndrome, and familial thoracic aneurysm and aortic dissection.[9,11,30–32] The pathogenesis of increased arterial tortuosity in patients with aortopathy is not known, although several mechanisms are postulated. Arterial lengthening has been shown to reduce axial stress, so it may be an adaptation in response to perceived high stress in genetically mediated arteriopathy.[33] Another possibility is that genetically abnormal vessel walls actually have lower manifest axial tension, which has been shown in manipulated rabbit carotid arteries to result in increased tortuosity.[34]

Interestingly, the degree of vertebral artery tortuosity in VEDS is significantly lower than in other vasculopathies, with a VEDS median VTI of 7 compared with a Marfan median VTI of 26 and a Loeys-Dietz syndrome median VTI of 58.[9] VTI >15 is typically visible to the naked eye, and normal is median 4.5 with IQR, 3 to 6.[9] Given the significantly increased risk of adverse events

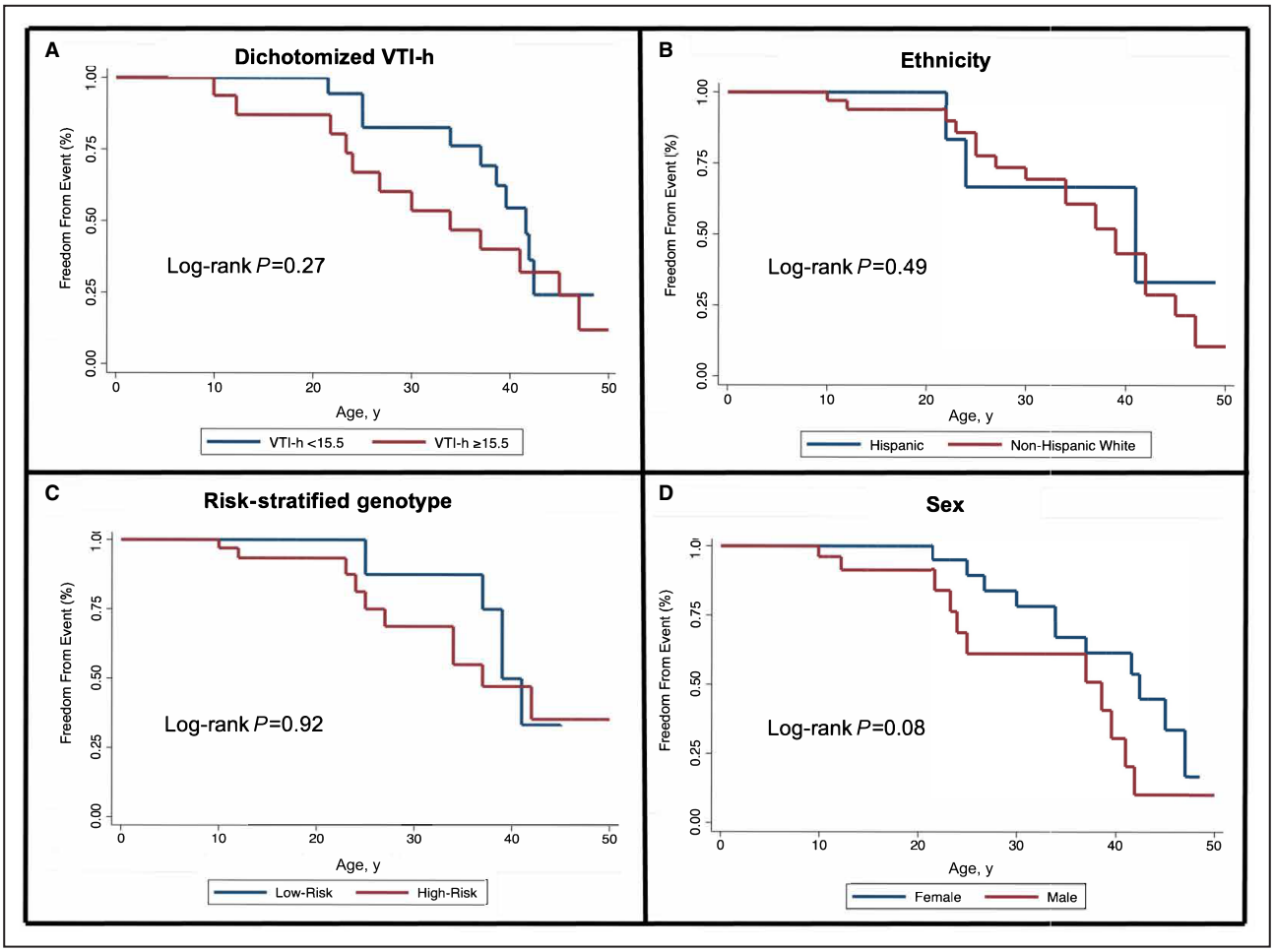

Figure 2. Freedom from cardiovascular event by characteristics.
A, By dichotomized VTI-h; **B**, By ethnicity; **C**, By risk-stratified genotype; **D**, By sex. All Kaplan–Meier graphs present freedom from event (1-hazard of event) on its y axis. KM indicates Kaplan–Meier; VTI-h, height-adjusted vertebral tortuosity index.

with age in VEDS, this study used VTI-h instead of VTI, as this adjusts for the mild progressive decline in VTI seen in childhood thought to occur as the body lengthens without equal lengthening of the vertebral arteries.[18] VTI-h is approximately equivalent to what the VTI would be at birth and has a median in controls of 7.8 with 25th to 75th percentiles of 5.3 to 10.0. The median VTI-h in this study (12) was just above the 75th percentile to the established "normal" VTI-h denoted among patients without aortopathy, suggesting that most patients with VEDS do not have visibly evident tortuosity, despite many of their events involving the arterial system.[9] The cutoff for concern for VTI-h is also much lower in VEDS than seen in Marfan syndrome, in that a VTI-h >68 is associated with increased risk of dissection at aortic diameters <5 cm.[35] With respect to its longitudinal trends, VTI-h changes minimally with age in other conditions, and we anticipate that this is similar in VEDS. Given this anticipated minimal change, it is possible that only 1 MRA or CTA of the vertebral arteries in full would be sufficiently informative. However, more definitive evaluations of the longitudinal changes in VTI and VTI-h in VEDS are needed to confirm this.

Differences in degree of aortic enlargement and tortuosity among patients with VEDS compared with other connective tissue disease cohorts are likely attributable to inherent differences in disease pathology between these conditions. While some studies cite aortic root dilation in 89% of patients with Marfan syndrome, this is much less common in VEDS.[36] Additionally, aortic dilation in VEDS is not as well associated with risk of dissection compared with other vasculopathies, whereas aortic size is one of the strongest predictors of dissection risk in Marfan and Loeys-Dietz syndromes.

This study was limited in its ability to statistically model aortic root and ascending aortic dilation because of heterogeneity in imaging modalities, although the associations between aortic size and outcomes did not appear to be strong. There was an elevated IRR between ascending aortic dilation and cardiovascular outcomes, but this was driven by a single case.

Table 4. Subject Characteristics Associated With Time-to-First Event

Variable	n	Any event (n=32)	*P* value	Cardiovascular event (n=22)	*P* value	Organ event (n=15)	*P* value
Stratified VTI-h							0.59
VTI-h≥15.5	20	30.0 (21.8–42.0)	0.53	33.9 (23.3–45.0)	0.27	‡ (42.0–‡)	
VTI-h<15.5	45	38.5 (25.0–41.9)		41.6 (37.0–42.4)		‡ (35.3–‡)	
Sex							
Male	35	36.2 (23.3–39.3)	0.11	38.6 (24.0–41.0)	0.08	42.0 (41.9–‡)	0.77
Female	30	33.9 (25.0–45.0)		42.4 (33.9–47.0)		‡ (33.9–‡)	
Race and ethnicity*							
Non-Hispanic White	44	33.9 (25.0–41.9)	0.44	38.6 (26.8–45.0)	0.49	‡ (35.3–‡)	0.55
Hispanic	19	36.2 (21.8–41.0)		41.0 (24.0–‡)		‡ (36.2–‡)	
Genotype†							
Glycine substitution	30	38.5 (24.0–45.0)	0.29	41.6 (25.0–‡)	0.23	‡ (38.5–‡)	0.29
Splice site variant	20	26.8 (18.0–36.2)		37.0 (26.8–47.0)		27.2 (18.0–36.2)	
Null variant	9	38.6 (37.0–39.3)		38.6 (37.0–39.6)		‡ (‡ – ‡)	
Genotype group†							
High-risk	40	24.0 (9.9–32.0)	0.40	26.0 (23.0–34.0)	0.92	27.0 (9.0–34.0)	0.02§
Low-risk	18	38.9 (37.8–41.5)		39.0 (37.0–42.0)		38.9 (38.9–‡)	

For each characteristic, median age at first event in years and its interquartile range are reported. *P* values were computed using univariable Cox proportional hazard regression by time to event for each respective section. For statistics with a median but no interquartile range presented, only 1 individual in the group had an event and, therefore, because of no variation in age at event, all percentiles are the median of the 1 individual. VTI-h indicates height-adjusted vertebral tortuosity index.

*Statistical analyses of race and ethnicity excluded Black and Asian subjects because of low counts (n=2).

†Analyses by genotype and genotype group excluded patients classified as "other/unknown" (n=6) or of unknown risk (n=7).

‡Unable to calculate because of failure to reach this percentile of events.

§*P* values indicate statistical significance.

Therefore, future prospective studies evaluating VTI-h using uniform measurement techniques of the aortic segments by CT and MRI/MRA will allow for more robust evaluation of arteriopathy as a covariate. For this analysis, we included VTI-h measured from both MRA and CTA, assuming these were similar within a

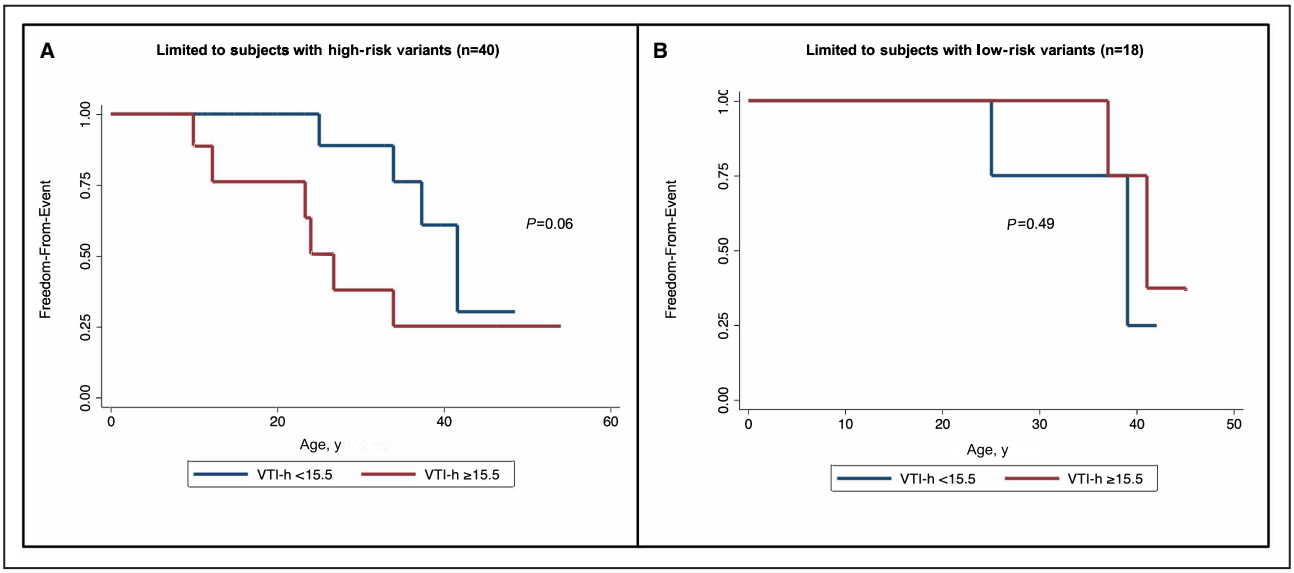

Figure 3. Freedom from cardiovascular event by dichotomized height-adjusted vertebral tortuosity index and risk-stratified COL3A1 genotype.

A, Limited to subjects with high-risk variants (n=40); **B**, Limited to subjects with low-risk variants (n=18). All Kaplan–Meier graphs present freedom from event (1-hazard of event) on its y axis. KM indicates Kaplan–Meier; and VTI-h, height-adjusted vertebral tortuosity index.

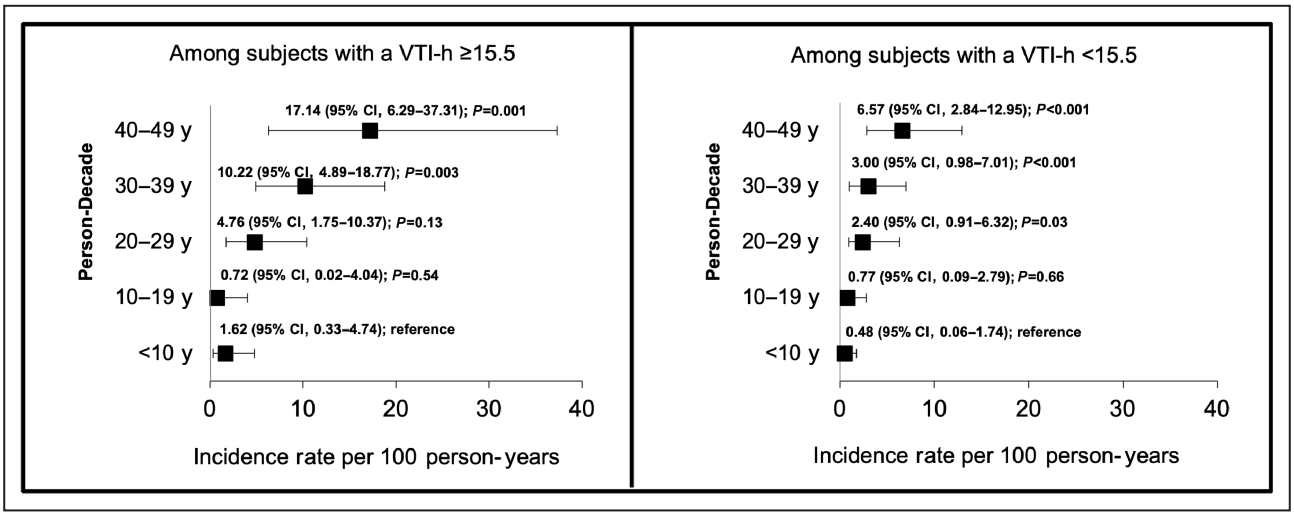

Figure 4. Incidence rate of events by dichotomized height-adjusted vertebral tortuosity index and person-decade.
Incidence rates per 100 person-years and corresponding 95% CIs are presented for each decade of life. *P* values denote whether or not the incidence rate ratio computed between the rate of any event by each person-decade and event rate within the first decade of life, within each respective group stratified by height-adjusted vertebral tortuosity index, are significantly different. VTI-h indicates height-adjusted vertebral tortuosity index.

patient. Additional studies are needed to formally compare VTI-h between imaging modalities.

While analysis of the *COL3A1* genotype did not reach statistical significance in the overall group, we postulate that this may be because of limited statistical power in this study, which is a key limitation of this study. Because of this limited power, we were also unable to perform multivariable analysis using >3 to 4 predictors or to investigate subjects with a low-risk genotype alone. Additionally, nearly the entire cohort was White, reflecting a critical need for more racially and ethnically diverse cohorts of patients with VEDS. There is possible selection bias and medical surveillance bias as patients included were followed by cardiac specialists and therefore may possess more resources to access specialized care, motivation to participate in research, or have a more severe phenotype given their diagnosis or family history of VEDS. Further studies with increased sample sizes are needed to investigate risk stratification among these patients with VEDS.

Table 5. Multivariable Modeling of Cardiovascular Events in Subjects With High-Risk *COL3A1* Genotype

Variable	Multivariable model	
	Adjusted IRR (95% CI)	*P* value
Model 1: Follow-up time<50 y		
Age, y (inverse transformed)	N/A	0.01*
Stratified VTI-h[1]		
VTI-h≥15.5	3.25 (0.98–10.78)	0.054
VTI-h<15.5	Reference	
Model 2: Follow-up time<40 y		
Age (inverse transformed)	N/A	0.02*
Stratified VTI-h[1]		
VTI-h≥15.5	4.14 (1.13–15.10)	0.03*
VTI-h<15.5	Reference	

IRR indicates incidence rate ratio; and VTI-h, height adjusted vertebral tortuosity index.
**P* values indicate statistical significance.

CONCLUSIONS

Height-adjusted vertebral artery tortuosity may be independently associated with increased rate of cardiovascular events among patients with VEDS, although findings suggested that the association between tortuosity and event rates is modified by *COL3A1* genotype and age and particularly magnified among patients <40 years of age, with a *COL3A1* variant conferring a higher risk of events. VTI did not predict extracardiac events involving hollow organ collapse or rupture. Prospective studies that include more racially and ethnically diverse subjects are needed to evaluate VTI's predictive ability in this high-risk population.

ARTICLE INFORMATION

Received April 5, 2023; accepted July 26, 2023.

Affiliations

Division of Cardiology, Department of Pediatrics, Baylor College of Medicine, Texas Children's Hospital, Houston, TX (S.B.S., T.T.D., J.D.W., T.B., T.S., S.A.M.); Department of Epidemiology, Human Genetics & Environmental Sciences, School of Public Health, The University of Texas Health Science Center at Houston, Houston, TX (S.B.S.); Division of Vascular and Endovascular Surgery, Department of Surgery, Oregon Health & Science University, Portland, OR (S.S.); Memorial Health University Medical Center, Savannah, GA (N.D.); Division of Medical Genetics, Department of Internal Medicine, The University of Texas Health Science Center, Houston, TX

(J.L., M.H., S.K.P., A.C., D.M.M.); Department of Diagnostic Radiology, Faculty of Life Sciences, Kumamoto University, Kumamoto, Japan (S.O.); National Registry of Genetically Triggered Thoracic Aortic Aneurysms and Cardiovascular Conditions (GenTAC) (S.O., K.K., F.M.A., K.E.); MedStar Heart and Vascular Institute, Washington, DC (K.K., F.M.A.); CHI St. Vincent, Little Rock, AR (K.K.); RTI International, Research Triangle Park, NC (L.P.); Division of Cardiothoracic Surgery, Michael E. DeBakey Department of Surgery, Baylor College of Medicine, Houston, TX (S.A.L.); Department of Medicine, Weill Cornell Medicine, New York, NY (R.B.D., M.J.R.); Division of Translational Medicine and Human Genetics, Perelman School of Medicine at the University of Pennsylvania, PA (R.E.P.); Section of Cardiology, Department of Pediatrics, Oregon Health & Science University and OHSU Doernbecher Children's Hospital, Portland, OR (K.W.H.); Department of Cardiology, Boston Children's Hospital, Boston, MA (R.V.L.); John A. Burns School of Medicine, Honolulu, HI (R.V.S.); Department of Radiology, Nationwide Children's Hospital, Columbus, OH (R.K.); Division of Cardiovascular Medicine, Frankel Cardiovascular Center, Department of Internal Medicine, Michigan Medicine, University of Michigan, Ann Arbor, MI (K.E.); and Department of Laboratory Medicine and Pathology, Department of Medicine (Medical Genetics) (P.B.), University of Washington, Seattle, WA .

Acknowledgments

S.B.S. and S.A.M. had full access to the data in the study and take responsibility for the integrity and accuracy of the data analysis. The authors are grateful for the valuable contributions of the patients and families in the Vascular Ehlers-Danlos community who contributed their time and energy to help us with this research. We would also like to thank the VEDS patient advocacy groups, including but not limited to the VEDS Movement and Marfan Foundation, for their partnership in disseminating new findings and research opportunities for enrollment within the VEDS community.

Sources of Funding

Research reported in this publication was supported by the National Heart, Lung, and Blood Institute of the National Institutes of Health under award number K23HL127266 and by the Lauren Ovelgonne Tenney Memorial Foundation. Dr LeMaire's work is supported in part by the Jimmy and Roberta Howell Professorship in Cardiovascular Surgery at Baylor College of Medicine.

Disclosures

Drs Morris and Shalhub served as scientific advisors and consultants for Aytu Biopharma for the AR101 Enzastaurin Clinical Trial. Dr Asch directs an academic echocardiographic core laboratory with institutional funding from Abbott, Edwards, Boston Scientific, Medtronic, Livanova, GDS, Neovasc, and MVRx. Dr LeMaire discloses financial relationships with Terumo Aortic, Cerus, and CytoSorbants. Dr Eagle receives support from the Marfan Foundation and W.L. Gore & Associates. The remaining authors have no disclosures to report.

Supplemental Material

Data S1
Tables S1–S3
Figures S1–S2

REFERENCES

1. Byers PH. Vascular Ehlers-Danlos syndrome. In: Adam MP, Mirzaa GM, Pagon RA, Wallace SE, Lora JHB, Gripp KW, Amemiya A, eds. *GeneReviews®*. Seattle (WA): University of Washington, Seattle; 1993-2023. https://www.ncbi.nlm.nih.gov/books/NBK1494/; 1999.
2. Pepin M, Schwarze U, Superti-Furga A, Byers PH. Clinical and genetic features of Ehlers–Danlos syndrome type IV, the vascular type. *N Engl J Med*. 2000;342:673–680. doi: 10.1056/NEJM200003093421001
3. Milewicz DM, Reid AJ, Cecchi AC. Vascular Ehlers–Danlos syndrome. *Circ Cardiovasc Genet*. 2018;7:5–7. doi: 10.1161/CIRCGENETICS.114.000507
4. Dietz H. FBN1-Related Marfan Syndrome. Accessed December 31, 2022. https://www.ncbi.nlm.nih.gov/books/NBK1335/.
5. Pepin MG, Schwarze U, Rice KM, Liu M, Leistritz D, Byers PH. Survival is affected by mutation type and molecular mechanism in vascular Ehlers–Danlos syndrome (EDS type IV). *Genet Med.* 2014;16:881–888. doi: 10.1038/gim.2014.72
6. Frank M, Albuisson J, Ranque B, Golmard L, Mazzella J-M, Bal-Theoleyre L, Fauret A-L, Mirault T, Denarié N, Mousseaux E, et al. The type of variants at the COL3A1 gene associates with the phenotype and severity of vascular Ehlers–Danlos syndrome. *Eur J Hum Genet.* 2015;23:1657–1664. doi: 10.1038/ejhg.2015.32
7. Okamoto O, Ando T, Watanabe A, Sato F, Mimata H, Shimada T, Fujiwara S. A novel point mutation in type III collagen gene resulting in exon 24 skipping in a case of vascular type Ehlers-Danlos syndrome. *Arch Dermatol Res.* 2008;300:525–529. doi: 10.1007/s00403-008-0884-8
8. Jørgensen A, Fagerheim T, Rand-Hendriksen S, Lunde PI, Vorren TO, Pepin MG, Leistritz DF, Byers PH. Vascular Ehlers–Danlos syndrome in siblings with biallelic COL3A1 sequence variants and marked clinical variability in the extended family. *Eur J Hum Genet.* 2015;23:796–802. doi: 10.1038/ejhg.2014.181
9. Morris SA, Orbach DB, Geva T, Singh MN, Gauvreau K, Lacro RV. Increased vertebral artery tortuosity index is associated with adverse outcomes in children and young adults with connective tissue disorders. *Circulation.* 2011;124:388–396. doi: 10.1161/CIRCULATIONAHA.110.990549
10. Welby JP, Kim ST, Carr CM, Lehman VT, Rydberg CH, Wald JT, Luetmer PH, Nasr DM, Brinjikji W. Carotid artery tortuosity is associated with connective tissue diseases. *Am J Neuroradiol.* 2019;40:1738–1743. doi: 10.3174/ajnr.a6218
11. Franken R, El Morabit A, de Waard V, Timmermans J, Scholte AJ, van den Berg MP, Marquering H, Planken NRN, Zwinderman AH, Mulder BJM, et al. Increased aortic tortuosity indicates a more severe aortic phenotype in adults with Marfan syndrome. *Int J Cardiol.* 2015;194:7–12. doi: 10.1016/j.ijcard.2015.05.072
12. Chu LC, Haroun RR, Beaulieu RJ, Black JH, Dietz HC, Fishman EK. Carotid artery tortuosity index is associated with the need for early aortic root replacement in patients with Loeys-Dietz syndrome. *J Comput Assist Tomo.* 2018;42:747–753. doi: 10.1097/RCT.0000000000000764
13. Kim BJ, Yang E, Kim N-Y, Kim M-J, Kang D-W, Kwon SU, Kim JS. Vascular tortuosity may be associated with cervical artery dissection. *Stroke.* 2018;47:2548–2552. doi: 10.1161/STROKEAHA.116.013736
14. Eagle KA; Consortium for the G. Rationale and design of the National Registry of genetically triggered thoracic aortic aneurysms and cardiovascular conditions (GenTAC). *Am Heart J.* 2009;157:319–326. doi: 10.1016/j.ahj.2008.10.005
15. Kroner BL, Tolunay HE, Basson CT, Pyeritz RE, Holmes KW, Maslen CL, Milewicz DM, LeMaire SA, Hendershot T, Desvigne-Nickens P, et al. The national registry of genetically triggered thoracic aortic aneurysms and cardiovascular conditions (GenTAC): results from phase I and scientific opportunities in phase II. *Am Heart J.* 2011;162:627–632.e1. doi: 10.1016/j.ahj.2011.07.002
16. Ohnishi T, Suda K, Komatsu M, Harmon SM, Watanabe T, Asukai M, Kadoya K, Takahata M, Iwasaki N, Minami A. Morphologic changes in the vertebral artery subsequent to cervical spine degeneration and aging: analyses by computed tomography angiography using multiplanar and 3-dimensional reconstructions. *World Neurosurg.* 2021;150:e686–e695. doi: 10.1016/j.wneu.2021.03.080
17. Asch FM, Yuriditsky E, Prakash SK, Roman MJ, Weinsaft JW, Weissman G, Weigold WG, Morris SA, Ravekes WJ, Holmes KW, et al. The need for standardized methods for measuring the aorta multimodality: core lab experience from the GenTAC registry. *JACC Cardiovasc Imaging.* 2016;9:219–226. doi: 10.1016/j.jcmg.2015.06.023
18. Morris SA, Payne WA, Sami S, Wang Y, LeMaire SA, Tyson JE, Krishnamurthy R, Milewicz DM. Arterial tortuosity and change with age in young patients with aortopathy. *J Cardiov Magn Reson.* 2015;17:P403. doi: 10.1186/1532-429X-17-S1-P403
19. Lopez L, Colan SD, Frommelt PC, Ensing GJ, Kendall K, Younoszai AK, Lai WW, Geva T. Recommendations for quantification methods during the performance of a pediatric echocardiogram: a report from the pediatric measurements writing Group of the American Society of echocardiography pediatric and congenital heart disease council. *J Am Soc Echocardiog.* 2010;23:465–495. doi: 10.1016/j.echo.2010.03.019
20. Servato ML, Teixidó-Turá G, Sabate-Rotes A, Galian-Gay L, Gutiérrez L, Valente F, Fernandez-Galera R, Casas G, López-Sainz A, González-Alujas MT, et al. Are aortic root and ascending aorta diameters measured by the pediatric versus the adult American Society of Echocardiography guidelines interchangeable? *J Clin Medicine.* 2021;10:5290. doi: 10.3390/jcm10225290
21. Sluysmans T, Colan SD. Structural measurements and adjustment for growth. In: Lai WW, Cohen MS, Geva T, Mertens L, eds.

Echocardiography in Pediatric and Congenital Heart Disease. West Sussex: Wiley-Blackwell; 2009:51–62.
22. Z-Score Calculator. Accessed May 21, 2022. https://zscore.chboston.org/
23. Kaiser T, Kellenberger CJ, Albisetti M, Bergsträsser E, Buechel ERV. Normal values for aortic diameters in children and adolescents–assessment in vivo by contrast-enhanced CMR-angiography. *J Cardiov Magn Reson*. 2008;10:56. doi: 10.1186/1532-429X-10-56
24. Campens L, Demulier L, Groote KD, Vandekerckhove K, Wolf DD, Roman MJ, Devereux RB, Paepe AD, Backer JD. Reference values for echocardiographic assessment of the diameter of the aortic root and ascending aorta spanning all age categories. *Am J Cardiol*. 2014;114:914–920. doi: 10.1016/j.amjcard.2014.06.024
25. Lin FY, Devereux RB, Roman MJ, Meng J, Jow VM, Jacobs A, Weinsaft JW, Shaw LJ, Berman DS, Gilmore A, et al. Assessment of the thoracic aorta by multidetector computed tomography: age- and sex-specific reference values in adults without evident cardiovascular disease. *J Cardiovasc Comput*. 2008;2:298–308. doi: 10.1016/j.jcct.2008.08.002
26. Leistritz DF, Pepin MG, Schwarze U, Byers PH. COL3A1 haploinsufficiency results in a variety of Ehlers-Danlos syndrome type IV with delayed onset of complications and longer life expectancy. *Genet Med*. 2011;13:717–722. doi: 10.1097/GIM.0b013e3182180c89
27. Schwarze U, Schievink WI, Petty E, Jaff MR, Babovic-Vuksanovic D, Cherry KJ, Pepin M, Byers PH. Haploinsufficiency for one COL3A1 allele of type III procollagen results in a phenotype similar to the vascular form of Ehlers-Danlos syndrome, Ehlers-Danlos syndrome type IV. *Am J Hum Genetics*. 2001;69:989–1001. doi: 10.1086/324123
28. Byers PH, Belmont J, Black J, Backer JD, Frank M, Jeunemaitre X, Johnson D, Pepin M, Robert L, Sanders L, et al. Diagnosis, natural history, and management in vascular Ehlers-Danlos syndrome. *Am J Med Genet C Semin Med Genet*. 2017;175:40–47. doi: 10.1002/ajmg.c.31553
29. Bergqvist D, Björck M, Wanhainen A. Treatment of vascular Ehlers-Danlos syndrome. *Ann Surg*. 2013;258:257–261. doi: 10.1097/SLA.0b013e31829c7a59
30. Morris SA. Arterial tortuosity in genetic arteriopathies. *Curr Opin Cardiol*. 2015;30:587–593. doi: 10.1097/HCO.0000000000000218
31. Loeys BL, Schwarze L, Holm T, et al. Aneurysm syndromes caused by mutations in the TGF-beta receptor. *J Vasc Surg*. 2006;44:1374–1375. doi: 10.1016/j.jvs.2006.10.011
32. Lindsay ME, Schepers D, Bolar NA, Doyle JJ, Gallo E, Fert-Bober J, Kempers MJE, Fishman EK, Chen Y, Myers L, et al. Loss-of-function mutations in TGFB2 cause a syndromic presentation of thoracic aortic aneurysm. *Nat Genet*. 2012;44:922–927. doi: 10.1038/ng.2349
33. Humphrey JD, Eberth JF, Dye WW, Gleason RL. Fundamental role of axial stress in compensatory adaptations by arteries. *J Biomech*. 2009;42:1–8. doi: 10.1016/j.jbiomech.2008.11.011
34. Jackson ZS, Dajnowiec D, Gotlieb AI, Langille BL. Partial off-loading of longitudinal tension induces arterial tortuosity. *Arterioscler Thromb Vasc Biol*. 2005;25:957–962. doi: 10.1161/01.ATV.0000161277.46464.11
35. Morris SA, Dodd NA, Oda S, Kancherla K, Asch FM, Challman M, Andreas SS, Payne W, Preiss L, LeMaire S, et al. Abstract 14983: arterial tortuosity and cardiovascular outcomes in Marfan syndrome and Loeys-Dietz syndrome. *Circulation*. 2018;134. doi: 10.1161/circ.134.suppl_1.14983
36. Roman MJ, Devereux RB, Preiss LR, Asch FM, Eagle KA, Holmes KW, LeMaire SA, Maslen CL, Milewicz DM, Morris SA, et al. Associations of age and sex with Marfan phenotype. *Circ Cardiovasc Genet*. 2018;10:e001647. doi: 10.1161/CIRCGENETICS.116.001647