From the Society for Clinical Vascular Surgery

A multi-institutional experience in vascular Ehlers-Danlos syndrome diagnosis

Sherene Shalhub, MD, MPH,[a] Peter H. Byers, MD,[b] Kelli L. Hicks, BS,[a] Dawn M. Coleman, MD,[c] Frank M. Davis, MD,[c] Giovanni De Caridi, MD, PhD,[d] K. Nicole Weaver, MD,[e] Erin M. Miller, MS, LGC,[f] Marc L. Schermerhorn, MD,[g] Katie Shean, MD,[g] Gustavo Oderich, MD,[h] Mauricio Ribeiro, MD, PhD,[i] Cole Nishikawa, MD,[j] Kristofer Charlton-Ouw, MD,[k] Christian-Alexander Behrendt, MD,[l] E. Sebastian Debus, MD, PhD,[m] Yskert von Kodolitsch, MD,[m] Devin Zarkowsky, MD,[n] Richard J. Powell, MD,[o] Melanie Pepin, MS, CGC,[b] Dianna M. Milewicz, MD, PhD,[p] Ellen S. Regalado, MS, CGC,[p] Peter F. Lawrence, MD,[q] *and* Karen Woo, MD, MS,[q] *Seattle, Wash; Ann Arbor, Mich; Messina, Italy; Cincinnati, Ohio; Boston, Mass; Rochester, Minn; São Paulo, Brazil; Sacramento, San Francisco, and Los Angeles, Calif; Houston, Tex; Hamburg, Germany; and Lebanon, NH*

ABSTRACT

Objective: Vascular Ehlers-Danlos syndrome (vEDS) is a rare disorder and 1 of 13 types of EDS. The syndrome results in aortic and arterial aneurysms and dissections at a young age. Diagnosis is confirmed with molecular testing via skin biopsy or genetic testing for *COL3A1* pathogenic variants. We describe a multi-institutional experience in the diagnosis of vEDS from 2000 to 2015.

Methods: This is a multi-institutional cross-sectional retrospective study of individuals with vEDS. The institutions were recruited through the Vascular Low Frequency Disease Consortium. Individuals were identified using the *International Classification of Diseases*-9 and 10-CM codes for EDS (756.83 and Q79.6). A review of records was then performed to select individuals with vEDS. Data abstraction included demographics, family history, clinical features, major and minor diagnostic criteria, and molecular testing results. Individuals were classified into two cohorts and then compared: those with pathogenic *COL3A1* variants and those diagnosed by clinical criteria alone without molecular confirmation.

Results: Eleven institutions identified 173 individuals (35.3% male, 56.6% Caucasian) with vEDS. Of those, 11 (9.8%) had nonpathogenic alterations in *COL3A1* and were excluded from the analysis. Among the remaining individuals, 86 (47.7% male, 68% Caucasian, 48.8% positive family history) had pathogenic *COL3A1* variants and 76 (19.7% male, 19.7% Caucasian, 43.4% positive family history) were diagnosed by clinical criteria alone without molecular confirmation. Compared with the cohort with pathogenic *COL3A1* variants, the clinical diagnosis only cohort had a higher number of females (80.3% vs 52.3%; $P < .001$), mitral valve prolapse (10.5% vs 1.2%; $P = .009$), and joint hypermobility (68.4% vs 40.7%; $P < .001$). Additionally, they had a lower frequency of easy bruising (23.7% vs 64%; $P < .001$), thin translucent skin (17.1% vs 48.8%; $P < .001$), intestinal perforation (3.9% vs 16.3%; $P = .01$), spontaneous pneumothorax/hemothorax (3.9% vs 14%, P.03), and arterial rupture (9.2% vs 17.4%; $P = .13$). There were no differences in mortality or age of mortality between the two cohorts.

From the Division of Vascular Surgery, Department of Surgery,[a] and Departments of Pathology and Medicine (Medical Genetics),[b] University of Washington, Seattle; the Section of Vascular Surgery, University of Michigan, Ann Arbor[c]; the Department of Cardiovascular and Thoracic Sciences, University of Messina, Messina[d]; the Division of Human Genetics, Cincinnati Children's Hospital Medical Center,[e] and the Divisions of Cardiology and Human Genetics, University of Cincinnati School of Medicine and Cincinnati Children's Hospital Medical Center,[f] Cincinnati; the Division of Vascular and Endovascular Surgery, Beth Israel Deaconess Medical Center, Boston[g]; the Mayo Clinic Division of Vascular Surgery, Rochester[h]; the Division of Vascular and Endovascular Surgery, Department of Surgery and Anatomy, Medical School of Ribeirão Preto, University of São Paulo, São Paulo[i]; the Department of Surgery, University of California, Davis Medical Center, Sacramento[j]; the Department of Cardiothoracic and Vascular Surgery, University of Texas Health Science Center at Houston, Houston[k]; the Department of Vascular Medicine,[l] and Department of Cardiology,[m] University Heart Center Hamburg, University Medical Center Hamburg-Eppendorf, Hamburg, Germany; the Division of Vascular and Endovascular Surgery, Department of Surgery, University of California San Francisco, San Francisco[n]; the Division of Vascular Surgery, Dartmouth-Hitchcock Medical Center, Lebanon[o]; the Division of Medical Genetics, Department of Internal Medicine, The University of Texas Health Science Center at Houston[p]; and the Division of Vascular Surgery, University of California Los Angeles, Los Angeles.[q]

Supported in part the National Center for Advancing Translational Sciences of the National Institutes of Health under Award Number UL1TR000423 (S.S.), in part by funds from the Freudmann Fund for Translational Research in Ehlers-Danlos syndrome at the University of Washington (P.H.B.), and in part by the National Institutes of Health (NIDDK 1K08DK107934) (K.O.W). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Author conflict of interest: none.

Presented at the Forty-fifth Annual Symposium of the Society for Clinical Vascular Surgery, Lake Buena Vista, Fla, March 18-22, 2017.

Correspondence: Sherene Shalhub, MD, MPH, Division of Vascular Surgery, Department of General Surgery, The University of Washington, 1959 N.E. Pacific St, Box 356410, Seattle, WA 98195 (e-mail: shalhub@uw.edu).

The editors and reviewers of this article have no relevant financial relationships to disclose per the JVS policy that requires reviewers to decline review of any manuscript for which they may have a conflict of interest.

0741-5214

https://doi.org/10.1016/j.jvs.2019.04.487

Conclusions: This study highlights the importance of confirming vEDS diagnosis by testing for pathogenic *COL3A1* variants rather than relying on clinical diagnostic criteria alone given the high degree of overlap with other forms genetically triggered arteriopathies. Because not all *COL3A1* variants are pathogenic, the interpretation of the genetic testing results by an individual trained in variant assessment is essential to confirm the diagnosis. An accurate diagnosis is critical and has serious implications for lifelong screening and treatment strategies for the affected individual and family members. (J Vasc Surg 2019;■:1-9.)

Keywords: Vascular Ehlers-Danlos syndrome; *COL3A1* mutation; Vascular genetic testing; Heritable arteriopathies

The Ehlers-Danlos syndromes (EDS) are a group of 13 heritable connective tissue disorders generally characterized by joint hypermobility, skin hyperextensibility, and tissue fragility.[1] Vascular EDS (vEDS), previously called EDS type IV, is one of the subtypes with an estimated frequency of 1 in 50,000 and accounts for 5% of all EDS cases.[2,3] The syndrome results from heterozygous mutations in the *COL3A1,* which encodes type III collagen.[4] The effect of the pathogenic *COL3A1* variants includes the production of defective type III collagen or the reduction in the quantity of type III collagen. The clinical manifestations include spontaneous arterial dissections, aneurysms, and rupture at a young age. Additionally, affected individuals are at increased risk for spontaneous intestinal perforation and pregnancy-related uterine rupture.[2,5,6] Consequently, individuals with vEDS have been reported to have an average life expectancy of less than 50 years with mortality usually related to arterial rupture or complications related to arterial repairs in these circumstances.[2,7]

Establishing the diagnosis in vEDS is challenging owing to its rarity and heterogeneous clinical presentation.[8,9] The diagnosis is suspected based on clinical findings and confirmed by molecular resting. Molecular testing includes biochemical testing via a skin biopsy and genetic testing for pathogenic *COL3A1* variants (the latter is the predominant contemporary mode of diagnosis).[1,2] We describe a multi-institutional experience in the diagnosis of vEDS and compare the demographics and clinical characteristics of individuals diagnosed using clinical diagnostic criteria only and those in whom the diagnosis was confirmed via molecular testing.

METHODS

This is a multi-institutional retrospective cohort study of individuals diagnosed with vEDS between January 1, 2000, and December 31, 2015. The institutions were recruited through the Vascular Low Frequency Disease Consortium (University of California-Los Angeles Division of Vascular Surgery).[10] A call for participation to institutions was sent through electronic mail. Each participating center obtained institutional review board approval from their home institution. The institutional review boards waived the patient consent process owing to minimal patient risk. Data were collected by each institution's respective investigator(s), transmitted in a deidentified fashion to the University of Washington, and stored using a password-encrypted database maintained by the University of Washington.

Individuals were identified with *International Classification of Diseases* (ICD)-9-CM code 756.83 or ICD-10-CM code Q79.6 for EDS as coded in the electronic medical records. This diagnostic code includes vEDS and an additional 12 subtypes of EDS.[1] A review of these case records was then performed to select individuals with vEDS based on the documentations in the medical records by providers caring for the patients. Individuals with the other 12 forms of EDS were excluded. Data regarding demographics, current age, age at diagnosis, and family history (defined as a family history of vEDS, aortic or arterial aneurysms and dissections, and/or sudden death) were abstracted.

The major and minor clinical diagnostic criteria for the cohort were reviewed. The major diagnostic criteria for vEDS include arterial rupture, intestinal rupture, uterine rupture during pregnancy, and family history of vEDS.[1,11] All arterial events (defined as aortic/arterial aneurysm, pseudoaneurysm, dissection, fistula, or rupture) were reviewed and noted. The minor diagnostic criteria include the following[1,11]:

- Hypermobility of small joints.
- Easy bruising that occurs spontaneously or with minimal trauma.
- Thin translucent skin that is especially noticeable on the chest and abdomen.
- Characteristic facial appearance,[8] which includes thin lips and philtrum, small chin, thin nose, large eyes, and attached ear lobes.[8]
- Skin hyperextensibility.
- Early-onset varicose veins.
- Tendon or muscle rupture.
- Pneumothorax/hemothorax.
- Talipes equinovarus (clubfoot).
- Arteriovenous carotid-cavernous sinus fistula.

Method of vEDS diagnosis was also reviewed. This review included whether the diagnosis was made clinically without molecular confirmation or a diagnosis that included molecular confirmation, namely, biochemical testing of a skin biopsy confirming disruption of the type III collagen fibrils, and genetic testing for pathogenic mutations in *COL3A1.* Genetic testing results including the *COL3A1* variant and protein effect were

reviewed by a single geneticist (P.H.B.) and classified for pathogenicity. *COL3A1* variants were considered pathogenic (causative) if they met any of the following criteria in keeping with the American College of Medical Genetics guidelines[3,7,12]:

- The variant resulted in substitution for a glycine residue in the Gly-X-Y repeats of the triple helical domain, thus disrupting the type III collagen folding process and leading to production of a minimal amount of normal collagen. This type of mutation is called a missense mutation and is the most common type of mutation affecting *COL3A1*.
- The variant altered the canonical splice acceptor site (-1G, -2A) or donor site (+1G, +2T). This type of mutation can lead to exon skip and splice site mutations by creating a frameshift that result in exon(s) deletion leading to defective collagen production similar to missense mutations.
- The variant creates premature termination codon either directly or through a frameshift mutation. This type of mutation leads to a haploinsufficiency mutations (null mutation) and results in production of one-half the normal amount of type III collagen. The individuals affected by this type of mutation present at a later age and have milder arterial disease than those who have the missense or exon skipping mutations.

Variants were considered nonpathogenic if they created amino acid substitutions in the amino-terminal propeptide or altered X- or Y-position amino acids in the triple helical domain and as such are not pathogenic.[3,5,13]

Individuals were classified into two cohorts: pathogenic *COL3A1* variants cohort and clinical diagnosis only cohort (no molecular confirmation). The demographics, major and minor diagnostic criteria, and arterial events between in the two cohorts were compared. Age results are presented as means and standard deviation. Age means were compared in the two cohorts using the Student *t*-test. Categorical data, including sex, presence of specific comorbidities, and diagnostic criteria, were compared between the two cohorts using the Pearson χ^2 test. The comparison of all-cause mortality was performed using a Kaplan-Meier survival curve with log-rank test comparison of the curves. The assumptions of Kaplan-Meier analysis were met: the event status is mutually exclusive between censored and mortality, the survival time is precisely measured and recorded, left censoring is not occurring because the starting point of the survival time is clearly defined and recorded, censoring and the event (mortality) are independent, there is no secular trend over time that biases the mortality, and the number and pattern of censoring in the two groups is similar. All statistical tests were two-sided and a *P* value of less than .05 was considered statistically significant. Statistical analysis was performed using Microsoft Excel 2007 software (Microsoft, Redmond, Wash) and SPSS version 19 for Windows (SPSS, Inc, Chicago, Ill).

ARTICLE HIGHLIGHTS

- **Type of Research:** Multi-institutional descriptive cross-sectional study of the Vascular Low Frequency Disease Consortium
- **Key Findings:** Out of 173 individuals diagnosed with vascular Ehlers-Danlos syndrome based on clinical criteria, genetic testing for pathogenic *COL3A1* variants was used to confirm the final diagnosis in 86 patients.
- **Take Home Message:** Clinical criteria alone is inadequate to establish the diagnosis of vascular Ehlers-Danlos syndrome and genetic testing for pathogenic *COL3A1* variants appears important. Accurate diagnosis is critical and has serious implications for lifelong screening and medical and surgical management strategies for the affected individual and family members.

RESULTS

A total of 173 individuals (35.3% male, 56.6% Caucasian) were identified by chart review as having a diagnosis of EDS during the study period at 11 institutions: nine in the United States (n = 149) and one each Germany (n = 5) and Italy (n = 19). Nonpathogenic alterations in *COL3A1* were found in 11 individuals (9.8%) who underwent genetic testing and as such they did not have vEDS and were excluded from subsequent analysis (N-terminal propeptide amino acid substitutions in the amino-terminal propeptide [n = 3] and altered X- or Y-position amino acids in the triple helical domain [n = 8]). Among the remaining individuals, 86 (47.7% male, 68% Caucasian, 48.8% positive family history) had pathogenic *COL3A1* variants (Fig 1). A diagnosis of vEDS was made by clinical criteria only in 56 individuals (34.5%). An additional 20 individuals (12.3%) were noted to carry the diagnosis of vEDS, but data from the genetic testing were unavailable for review and could not be obtained. A sensitivity analysis was performed and demonstrated substantial differences between this group and the cohort with pathogenic *COL3A1* variants; thus, they were included in clinical diagnosis only cohort. In the end, 76 individuals (19.7% male, 19.7% Caucasian, 43.4% positive family history) were included in the clinical diagnosis only cohort

Cohort with pathogenic *COL3A1* variants. The diagnosis of vEDS was confirmed by genetic testing in 82 individuals (50.6%) and by skin biopsy in 5 individuals (3.1%). There was no difference in the age of diagnosis between males and females (28.2 ± 17.4 years vs

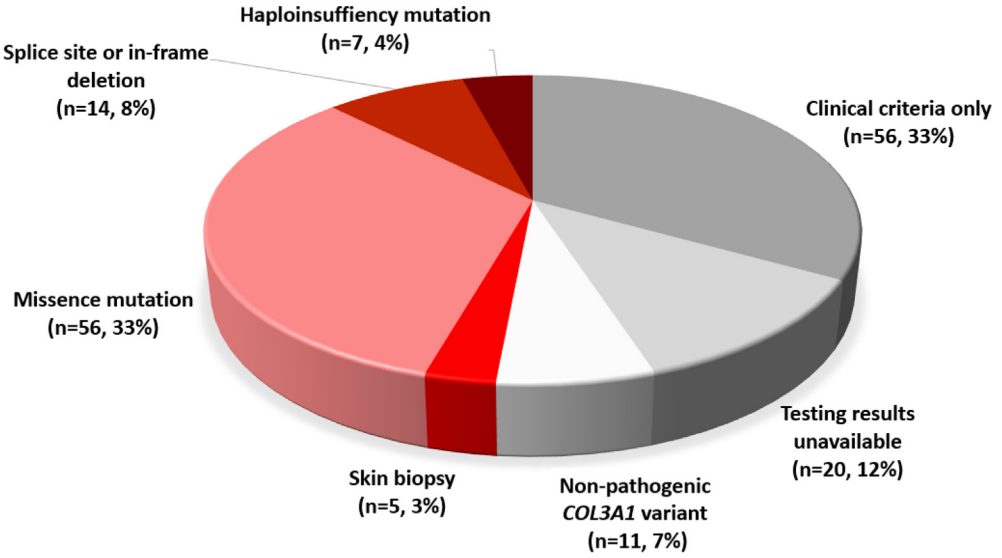

Fig 1. Method of diagnosis of 173 patients identified as having vascular Ehlers-Danlos syndrome (vEDS).

29.4 ± 15.1 years, respectively; $P = .748$). Individuals with a family history of vEDS (not including children with vEDS) were younger at the age of diagnosis compared with those without a family history (21.3 ± 13.6 years vs 30.2 ± 13.7 years; $P = .008$). In contrast, among those with a child diagnosed with vEDS (n = 11), the age of diagnosis was significantly older compared with the rest of the cohort (47.4 ± 18.8 years vs 26.5 ± 14.3 years; $P < .001$). There was the stepwise age increase with given complications so that spontaneous pneumothorax (n = 12, 66.7% male) occurring at a younger age than spontaneous intestinal perforation (n = 14, 42.3% male) and arterial rupture (n = 15, 33.3% male; Fig 2).

Comparison of the clinical features between the two cohorts. The clinical diagnosis only cohort had a significantly higher number of females compared with the cohort with pathogenic *COL3A1* variants (80.3% vs 52.3%; $P < .001$) and a significantly higher frequency of mitral valve prolapse (10.5% vs 1.2%; $P = .009$). There were also differences in the frequency of minor and major clinical diagnostic (Table I). Among the minor diagnostic criteria, there was a significantly higher frequency of joint hypermobility (68.4% vs 40.7%; $P < .001$) and a significantly lower frequency of easy bruising (23.7% vs 64%; $P < .001$) and thin translucent skin (17.1% vs 48.8%; $P < .001$). In terms of major diagnostic criteria, this cohort had a significantly lower frequency of arterial rupture, intestinal perforation, and spontaneous pneumothorax/hemothorax (Table I). The number of arterial events was significantly higher among those with pathogenic *COL3A1* variants (Table II).

Mortality. There were no differences in all-cause mortality (Fig 3) between the cohort with pathogenic COL3A1 variants and the cohort diagnosed without molecular confirmation (Table I). In the cohort with pathogenic *COL3A1* variants, the mean follow-up time from the time of diagnosis was 8.2 ± 8.28 years. There were 13 deaths (15.1%) at a mean age of 40.3 ± 15.5 years. Causes of death were two strokes, one coronary artery dissection, one ventricular rupture, two artic dissections, one hemothorax, and one mesenteric arterial rupture; in the rest of the cases, the cause was unknown. In the cohort without molecular confirmation, the mean follow-up time from the time of diagnosis was 9.8 ± 8.8 years. There were 6 deaths (7.8%) in this group (mean age, 35.8 ± 15.6 years). Causes of deaths were noted as a hemorrhagic stroke (n = 1), dissection and rupture without further detail (n = 1), complications from a perforated viscus (n = 1), and unknown (n = 3).

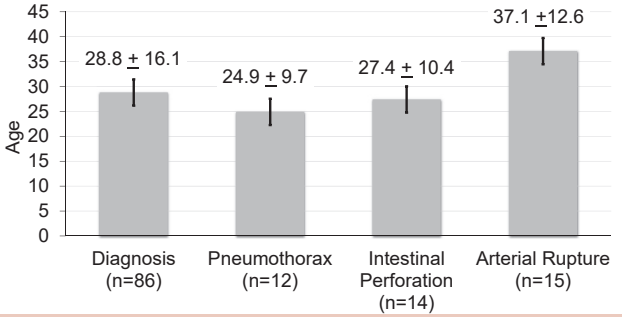

Fig 2. Mean ± standard deviation age at diagnosis, at first spontaneous pneumothorax, at first intestinal perforation, and at first arterial rupture among individuals with vascular Ehlers-Danlos syndrome (vEDS) with confirmed pathogenic *COL3A1* variants.

DISCUSSION

Owing to its rarity and heterogeneous presentation, the diagnosis of vEDS can be difficult even for experienced clinicians. This contemporary experience from 11

Table I. Demographics, major, and minor diagnostic criteria of 162 individuals with vascular Ehlers-Danlos (*vEDS*) syndrome at 11 institutions

Variable	Pathogenic *COL3A1* variants (n = 86)	Clinical diagnosis of vEDS (n = 76)	*P* value
Age at diagnosis	28.8 ± 16.2	27.3 ± 12.5	.636
Male	41 (47.7)	15 (19.7)	<.001
Race			<.001
Caucasian	74 (86)	15 (19.7)	
Multiracial	7 (8.1)	3 (3.9)	
African American	1 (0.9)	0	
Native American	1 (0.9)	0	
Unknown	3 (3.5)	58 (76.3)	
Comorbid conditions			
Hypertension	19 (22.1)	11 (14.5)	.213
Deep vein thrombosis	13 (15.1)	6 (7.9)	.154
Mitral valve prolapse	1 (1.2)	8 (10.5)	.009
Smoker (past or current)	17 (19.8)	26.3 (20)	.322
Family history[a]	42 (48.8)	33 (43.4)	.490
Major complications			
Arterial rupture	15 (17.4)	7 (9.2)	.127
Any arterial pathology[c]	53 (61.6)	16 (21.1)	<.001
Intestinal perforation	14 (16.3)	3 (3.9)	.011
Uterine rupture[b]	4 (18.2)	0	.019
Minor diagnostic criteria			
Hypermobility of small joints	35 (40.7)	52 (68.4)	<.001
Easy bruising	55 (64)	18 (23.7)	<.001
Thin, translucent skin	42 (48.8)	13 (17.1)	<.001
Characteristic facial features	27 (31.4)	6 (7.9)	<.001
Skin hyperextensibilty	12 (14)	10 (13.2)	.883
Early-onset varicose veins	13 (15.1)	2 (2.6)	.006
Tendon or muscle rupture	9 (10.5)	6 (7.9)	.573
Spontaneous pneumothorax/hemothorax	12 (14)	3 (3.9)	.028
Carotid cavernous fistula	4 (4.7)	1 (1.3)	.221
Clubfoot	8 (9.3)	3 (3.9)	.176
Follow-up duration after diagnosis	8.2 ± 8.2	9.8 ± 8.8	.349
Mortality	13 (15.1%)	6 (7.9%)	.154

The *P* value is for the comparisons between those with molecular confirmation showing pathogenic *COL3A1* variants and those diagnosed using clinical criteria without molecular confirmation. Values are presented as number (%) or mean ± standard deviation.
[a]Family history of vEDS, aortic and arterial aneurysms and dissections, sudden death.
[b]Of 52 patients who had pregnancies.
[c]Defined as defined as aortic/arterial aneurysm, pseudoaneurysm, dissection, fistula, or rupture.

institutions highlights the challenges related to an accurate diagnosis of vEDS. In this cohort, one-third of the individuals were given the diagnosis of vEDS based on clinical criteria alone without confirmatory molecular testing in the form of skin biopsy or genetic testing for pathogenic variants in *COL3A1*. The demographics and disease manifestations among this group make it clear that this was a different group than those with pathogenic *COL3A1* variants. In fact, their most common features were more consistent with hypermobile EDS or classical EDS than with vEDS (both in which skin hyperextensibility and generalized joint hypermobility are major diagnostic criteria).[1,14] This finding suggests that, at least for this cohort, the diagnosis of vEDS in the absence of confirmatory testing cannot be trusted. In the period that overlaps the time span for this study, gene discovery studies had identified more than a dozen genes associated with aneurysms and dissections. Some of those conditions overlap clinically with vEDS, such as other forms of EDS that result from mutations in *COL5A1* and *COL1A1*.[1] Thus, although the major and minor diagnostic clinical criteria can be used to help to raise the suspicion for a vEDS, the diagnosis must be confirmed by genetic testing. In vEDS, the major

Table II. Demographics of individuals with vascular Ehlers-Danlos syndrome (*vEDS*) who experienced an arterial event

Variable	Pathogenic *COL3A1* variants (n = 53)	Clinical diagnosis of vEDS (n = 16)	*P* value
Male	27 (50.9)	10 (62.5)	.417
Caucasian	47 (88.7)	11 (68.8)	.056
Comorbid conditions			
Hypertension	17 (32.1)	3 (18.8)	.303
Deep vein thrombosis	12 (22.6)	3 (18.8)	.741
Smoker (past or current)	12 (22.6)	2 (12.5)	.377
Family history[a]	30 (56.6)	6 (37.5)	.180
Minor diagnostic criteria			
Spontaneous pneumothorax/hemothorax	7 (13.2)	2 (12.5)	.941
Carotid-cavernous fistula	4 (7.5)	1 (6.3)	.861
Hypermobility of small joints	17 (32.1)	10 (62.5)	.029
Easy bruising	32 (60.4)	10 (62.5)	.879
Thin, translucent skin	26 (49.1)	5 (31.3)	.209
Characteristic facial features	16 (30.2)	6 (37.5)	.582
Skin hyperextensibilty	8 (15.1)	4 (25)	.360
Early-onset varicose veins	8 (15.1)	2 (12.5)	.796
Tendon or muscle rupture	5 (9.4)	2 (12.5)	.722
Clubfoot	6 (11.3)	1 (6.3)	.556
Age at first arterial diagnosis	34.6 ±12.5	35.1 ±12.5	.877
Age at first arterial diagnosis (males)	32.8 ±14.1	37.1 ±12.8	.423
Age at first arterial diagnosis (females)	34.4 ±11.7	32.2 ±12.6	.674
Diagnosis established before index arterial pathology diagnosis	21 (38.9)	9 (56.3)	.218
Arterial rupture	15 (17.4)	7 (9.2)	.127
Arterial pathology[b]			
Carotid and vertebral arteries	19 (22.1)	3 (3.9)	.001
Age	32.6 ±12.5	21.3 ±2.5	.060
Aorta	17 (19.8)	2 (2.6)	.001
Age	37.1 ± 15.8	40 ± 12.7	.805
Mesenteric arteries	22 (25.6)	7 (9.2)	.007
Age	40.1 ± 11.3	34.2 ± 12.2	.216
Renal arteries	12 (14)	4 (5.3)	.064
Age	38.3 ± 9.4	32.8 ± 105	.417
Iliac arteries	17 (19.8)	5 (6.6)	.014
Age	38.2 ± 9.4	40 ± 13.7	.766
Follow-up after first arterial pathology diagnosis, years	7.2 ± 6.0	6.2 ± 7.2	.579

The *P* value is for the comparisons between those with pathogenic *COL3A1* variants and those diagnosed using clinical criteria only without additional molecular testing. Values are presented as number (%) or mean ± standard deviation.
[a]Of vEDS, aortic and arterial aneurysms and dissections, sudden death.
[b]Defined as defined as aortic/arterial aneurysm, pseudoaneurysm, dissection, fistula, or rupture.

diagnostic criteria have high diagnostic specificity.[1] Even when these diagnostic clinical criteria are present, molecular testing is essential to confirm the diagnosis.

A confirmation of the diagnosis by molecular testing is critical for several reasons. Not only are there clinical features that overlap with the other subtypes of EDS, there are clinical features that overlap with other heritable connective tissues disorders and genetically triggered arteriopathies that result from mutations in the genes involved in the transforming growth factor-β signaling pathway, extracellular matrix production, and aortic smooth muscle cells development and function.[2,15-19] Establishing the correct diagnosis has serious implications of lifelong surveillance, medical and surgical management recommendations, and pregnancy planning for the patient and family members.[2,8] The diagnosis can be confirmed by obtaining a skin biopsy or by genetic testing for pathogenic variants in the *COL3A1*, but the latter is the contemporary approach to diagnosis.[2]

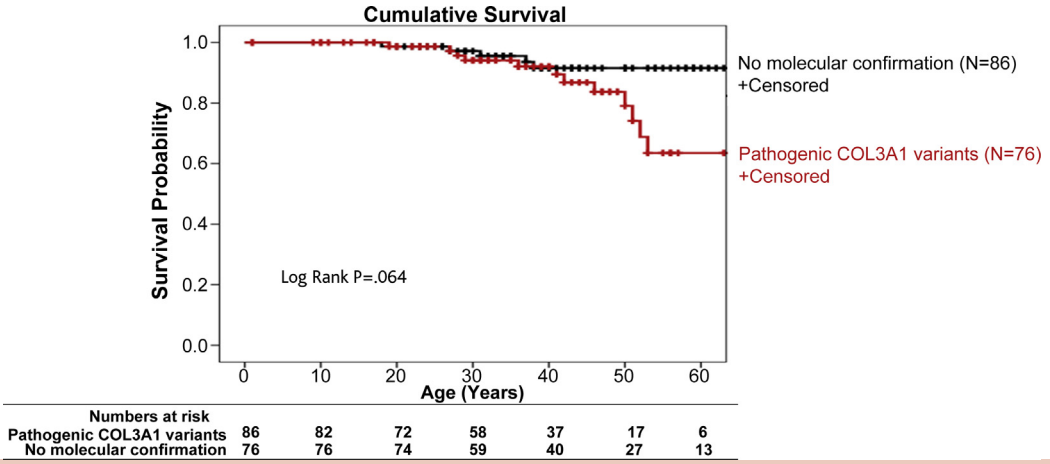

Fig 3. Kaplan-Meier estimate of cumulative survival in individuals with vascular Ehlers-Danlos syndrome (vEDS). Individuals with confirmed diagnosis showing pathogenic *COL3A1* variants are compared with individuals diagnosed using clinical criteria without additional molecular testing.

Because not all *COL3A1* variants are pathogenic, the interpretation of the genetic testing results by an individual trained in variant assessment in keeping with the American College of Medical Genetics guidelines[3,7,12] is essential to establishing the diagnosis. We found that nearly 10% of individuals in whom at least some of the clinical criteria for vEDS were met had nonpathogenic *COL3A1* variants and with current criteria did not have vEDS. This finding, too, has substantial implications for the individuals and their family members.

An accurate diagnosis of vEDS has research implications. An example derives from the BBEST celiprolol trial, which aimed to assess if celiprolol (a long-acting β1-adrenergic receptor antagonist with partial β2-adrenergic receptor agonist) would decrease the risk of arterial dissections and ruptures in treated individuals.[20] In this trial, the 53 enrolled individuals had a diagnosis of vEDS made by clinical criteria alone and only 33 were found to have a pathogenic *COL3A1* variant (52% in the celiprolol arm and 71% of the controls). The trial concluded that celiprolol decreased arterial rupture after extended treatment, based in part on the whole cohort. The presence of a misidentified disease process in those patients diagnosed by clinical criteria only suggests that the trial's experimental group was populated with patients at less (or more) risk for arterial events than the control group. Celiprolol is currently unavailable in the United States. Medical management in vEDS has been extrapolated from this study (use of β-blockers) as well as management of Marfan syndrome (use of atenolol and losartan) without additional trials in place.[20-22] A pragmatic trial to evaluate the role of anti-impulse therapy in patients with vEDS is still necessary to establish efficacy for celiprolol or other β-blockers.

In this study, a vEDS diagnosis was made at an earlier age in the setting of a family history of other family members with vEDS or a history of aortic or arterial aneurysms or dissections. This finding has been previously demonstrated[2] and highlights an opportunity for early diagnosis.[23] However, the family history is frequently absent, because 50% people in whom the diagnosis of vEDS is made have de novo mutations in *COL3A1* and the patient is the first in their family to have the mutation.[3,5] An additional window of opportunity for early diagnosis is at the time of presenting with a spontaneous pneumothorax, because this entity seems to present at a younger age with male predominance compared with individuals presenting with intestinal or arterial events, as demonstrated in this series and in a recent report on a separate cohort of patients with vEDS.[24]

This study demonstrates the limits related to the use of a diagnostic code when it does not discriminate among different bases for a common disorder. All 13 types of EDS are included in a single diagnostic code in both the ICD-9 and the ICD-10 (Q79.6). This creates confusion among clinicians and individuals with limited experience with vEDS and prevents the use of administrative data sets the study of vEDS. As a result, there is a call for the creation of separate ICD codes for the different subtypes by the patient advocacy groups.

This study is limited by the relatively small numbers of cases. The syndrome is rare; thus, accruing large numbers for analysis is challenging even in multi-institutional study designs. By virtue of the study design, it was not possible to ascertain systematically how these individuals in this cohort came to clinical attention and as such, this cohort may not be representative of the entire vEDS population. In this cohort, over half of the individuals in this cohort had an arterial event and the median age of death (38.5 years) was younger than was has been previously reported (51 years).[7,25] The younger death, in part, could be due to the identifications of individuals

with a severe phenotype who are identified because of a complication. Additionally, owing to the rarity of vEDS, few clinicians have encountered affected individuals and, as such, this could lead to the under-recognition of the milder phenotypes. Despite these limitations, the study adds to our understanding of vEDS and the importance of accurate diagnosis.

CONCLUSIONS

This study highlights the importance of confirming vEDS diagnosis by testing for pathogenic *COL3A1* variants rather than making the diagnosis by using clinical diagnostic criteria alone. This finding is highly relevant, because the clinical features of vEDS overlap with those of other forms of EDS and other genetically triggered arteriopathies. The interpretation of the testing results by an individual trained in variant assessment is essential to confirm the diagnosis given that not all variants of *COL3A1* are pathogenic. Accurate diagnosis is critical and has serious implications for lifelong screening and treatment strategies for the affected individual and family members as well as for research to identify treatments, genetic modifiers, and outcomes.

The authors acknowledge Binod Shrestha (Department of Cardiovascular and Vascular Surgery, at the University of Texas Health Science Center at Houston) for assisting with data collection at the University of Texas Health Science Center at Houston.

AUTHOR CONTRIBUTIONS

Conception and design: SS, KH
Analysis and interpretation: SS, PB
Data collection: SS, KH, DC, FD, GC, KW, EM, MS, KS, GO, MR, CN, KC, CB, ED, YK, DZ, RP, MP, DM
Writing the article: SS, PB
Critical revision of the article: SS, PB, KH, DC, FD, GC, KW, EM, MS, KS, GO, MR, CN, KC, CB, ED, YK, DZ, RP, MP, DM
Final approval of the article: SS, PB, KH, DC, FD, GC, KW, EM, MS, KS, GO, MR, CN, KC, CB, ED, YK, DZ, RP, MP, DM
Statistical analysis: SS, KW,
Obtained funding: SS, KW, PB
Overall responsibility: SS

REFERENCES

1. Malfait F, Francomano C, Byers P, Belmont J, Berglund B, Black J, et al. The 2017 international classification of the Ehlers-Danlos syndromes. Am J Med Genet C Semin Med Genet 2017;175:8-26.
2. Byers PH, Belmont J, Black J, De Backer J, Frank M, Jeunemaitre X, et al. Diagnosis, natural history, and management in vascular Ehlers-Danlos syndrome. Am J Med Genet C Semin Med Genet 2017;175:40-7.
3. 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;342:673-80.
4. Pope FM, Martin GR, Lichtenstein JR, Penttinen R, Gerson B, Rowe DW, et al. Patients with Ehlers-Danlos syndrome type IV lack type III collagen. Proc Natl Acad Sci U S A 1975;72:1314-6.
5. Pepin MG, Murray ML, Byers PH. Vascular Ehlers-Danlos syndrome. In: Adam MP, Ardinger HH, Pagon RA, et al., editors. Seattle, WA: GeneReviews(R); 1993. Available at: https://www.ncbi.nlm.nih.gov/books/NBK1494/.
6. Murray ML, Pepin M, Peterson S, Byers PH. Pregnancy-related deaths and complications in women with vascular Ehlers-Danlos syndrome. Genet Med 2014;16:874-80.
7. 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;16:881-8.
8. Shalhub S, Black JH 3rd, Cecchi AC, Xu Z, Griswold BF, Safi HJ, et al. Molecular diagnosis in vascular Ehlers-Danlos syndrome predicts pattern of arterial involvement and outcomes. J Vasc Surg 2014;60:160-9.
9. Eagleton MJ. Arterial complications of vascular Ehlers-Danlos syndrome. J Vasc Surg 2016;64:1869-80.
10. Harlander-Locke MP, Lawrence PF. The current state of the vascular low-frequency disease consortium. Ann Vasc Surg 2017;38:8-9.
11. Beighton P, De Paepe A, Steinmann B, Tsipouras P, Wenstrup RJ. Ehlers-Danlos syndromes: revised nosology, Villefranche, 1997. Ehlers-Danlos National Foundation (USA) and Ehlers-Danlos Support Group (UK). Am J Med Genet 1998;77:31-7.
12. 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;13:717-22.
13. Schwarze U, Schievink WI, Petty E, Jaff MR, Babovic-Vuksanovic D, Cherry KJ, et al. 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 Genet 2001;69:989-1001.
14. Brady AF, Demirdas S, Fournel-Gigleux S, Ghali N, Giunta C, Kapferer-Seebacher I, et al. The Ehlers-Danlos syndromes, rare types. Am J Med Genet C Semin Med Genet 2017;175:70-115.
15. Milewicz DM, Regalado E. Heritable thoracic aortic disease overview. In: Pagon RA, Adam MP, Ardinger HH, et al., editors. Seattle, WA: GeneReviews(R); 1993. Available at: https://www.ncbi.nlm.nih.gov/books/NBK1120/.
16. Brownstein AJ, Kostiuk V, Ziganshin BA, Zafar MA, Kuivaniemi H, Body SC, et al. Genes associated with thoracic aortic aneurysm and dissection: 2018 update and clinical implications. Aorta (Stamford) 2018;6:13-20.
17. Karimi A, Milewicz DM. Structure of the elastin-contractile units in the thoracic aorta and how genes that cause thoracic aortic aneurysms and dissections disrupt this structure. Can J Cardiol 2016;32:26-34.
18. Regalado ES, Mellor-Crummey L, De Backer J, Braverman AC, Ades L, Benedict S, et al. Clinical history and management recommendations of the smooth muscle dysfunction syndrome due to ACTA2 arginine 179 alterations. Genet Med 2018;20:1206-15.
19. Milewicz D, Hostetler E, Wallace S, Mellor-Crummey L, Gong L, Pannu H, et al. Precision medical and surgical management for thoracic aortic aneurysms and acute aortic dissections based on the causative mutant gene. J Cardiovasc Surg (Torino) 2016;57:172-7.

20. Ong KT, Perdu J, De Backer J, Bozec E, Collignon P, Emmerich J, et al. Effect of celiprolol on prevention of cardiovascular events in vascular Ehlers-Danlos syndrome: a prospective randomised, open, blinded-endpoints trial. Lancet 2010;376:1476-84.
21. Lacro RV, Dietz HC, Sleeper LA, Yetman AT, Bradley TJ, Colan SD, et al. Atenolol versus losartan in children and young adults with Marfan's syndrome. N Engl J Med 2014;371:2061-71.
22. Milleron O, Arnoult F, Ropers J, Aegerter P, Detaint D, Delorme G, et al. Marfan Sartan: a randomized, double-blind, placebo-controlled trial. Eur Heart J 2015;36:2160-6.
23. Hicks KL, Byers PH, Quiroga E, Pepin MG, Shalhub S. Testing patterns for genetically triggered aortic and arterial aneurysms and dissections at an academic center. J Vasc Surg 2018;68:701-11.
24. Shalhub S, Sanchez D, Dua A, Arellano N, McDonnell NB, Milewicz DM. Spontaneous pneumothorax and hemothorax frequently precede the Arterial and Intestinal complications of Vascular Ehlers Danlos syndrome. Am J Med Genet A 2019;179:797-802.
25. Frank M, Albuisson J, Ranque B, Golmard L, Mazzella JM, Bal-Theoleyre L, 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;23:1657-64.

Submitted Sep 7, 2018; accepted Apr 24, 2019.