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A multi-institutional experience in Vascular Ehlers Danlos Syndrome diagnosis

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Abstract

OBJECTIVE.—Vascular Ehlers-Danlos syndrome (vEDS) is a rare disorder and one of 13 types of Ehlers-Danlos syndrome (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 (ICD)-9 and 10-CM codes for EDS (756.83 and Q79.6). 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 (35.3% male, 56.6% Caucasian) individuals with vEDS. Of those, 11 (9.8%) had non-pathogenic 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 to 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 pneumo/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

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. As not all *COL3A1* variants are pathogenic, interpretation of the genetic testing results by an individual trained in variant assessment is essential to confirm the diagnosis. Accurate diagnosis is critical and has serious implications for lifelong screening and treatment strategies for the affected individual and family members.

Table of Contents summary

In this multi institutional cross-sectional retrospective Vascular Low Frequency Disease Consortium study of 173 individuals diagnosed with vEDS based on clinical criteria, genetic testing for pathogenic *COL3A1* variants was used to confirm the final diagnosis in 86 patients.

Keywords

Vascular Ehlers-Danlos Syndrome; *COL3A1* mutation; Vascular genetic testing; Heritable arteriopathies

INTRODUCTION

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 Ehlers-Danlos syndrome (vEDS), previously called Ehlers-Danlos syndrome type IV, is one of the subtypes with an estimated frequency of 1/50,000 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 average life expectancy 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 due to its rarity and heterogeneous clinical presentation.^{8,9} The diagnosis is suspected based on clinical findings and confirmed by molecular testing. 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 (IRB) approval from their home institution. The IRBs waived the patient consent process due to minimal patient risk. Data were collected by each institution's respective investigator(s), transmitted in a de-identified 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 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, 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 spontaneous or with minimal trauma.
- Thin translucent skin that is especially noticeable on the chest and abdomen.
- Characteristic facial appearance⁸, 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 (PTX, HTX).
- Talipes equinovarus (clubfoot).
- Arteriovenous carotid-cavernous sinus fistula (CCF).

Method of vEDS diagnosis was also reviewed. This included whether the diagnosis was made clinically without molecular confirmation or diagnosis that included molecular confirmation: 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 (PHB) and classified for pathogenicity. *COL3A1* variants were considered pathogenic (causative) if they met any of the following criteria in keeping with the ACMG 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. This type of mutation leads to a haploinsufficiency mutations (null mutation) and results in production of 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 non-pathogenic 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 as 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, the number and pattern of censoring in the two groups is similar. All statistical tests were two-sided and a P-value < .05 was considered statistically significant. Statistical analysis was performed using Microsoft Excel 2007 software (Microsoft, Redmond, WA) and SPSS version 19 for Windows (SPSS, Inc., Chicago, IL).

RESULTS

A total of 173 (35.3% male, 56.6% Caucasian) individuals were identified by chart review as having a diagnosis of EDS during the study period at 11 institutions: 9 in the United States (n= 149) and one each Germany (n=5) and Italy (n=19). Non-pathogenic alterations in *COL3A1* were found in 11 (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 (Figure 1). Diagnosis of vEDS was made by clinical criteria only in 56 (34.5%) individuals. An additional 20 (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 this 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 (50.6%) individuals, and by skin biopsy in 5 (3.1%) individuals. There was no difference in the age of diagnosis between males and females (28.2 ± 17.4 years vs. 29.4 ± 15.1 years, $P=.748$). Individuals with a family history of vEDS (not including children with vEDS) were younger at the age of diagnosis compared to 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 to 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) (Figure 2).

Comparison of the clinical features between the two cohorts

The clinical diagnosis only cohort had a significantly higher number of females compared to 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%, $P<.001$), 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 pneumo/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 (Figure 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 from the time of diagnosis was 8.2 ± 8.28 years. There were 13 (15.1%) deaths at a mean age of 40.3 ± 15.5 . Causes of death were due to the following: 2 due to strokes, 1 due to a coronary artery dissection, 1 due to ventricular rupture, 2 related to aortic dissections, 1 related to hemothorax, 1 due to mesenteric arterial ruptures, and in the rest of the cases, the cause was unknown. In the cohort without molecular confirmation, the mean follow up from the time

of diagnosis was 9.8 ± 8.8 years. There were 6 (7.8%) deaths 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).

DISCUSSION

Due to its rarity and heterogeneous presentation, the diagnosis of vEDS can be difficult even for experienced clinicians. This contemporary experience from 11 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 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 while the major and minor diagnostic clinical criteria can be used to help raise the suspicion for a vEDS the diagnosis must be confirmed by genetic testing. In vEDS, the major 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 overlap with the other subtypes of EDS, there are clinical features overlap with other heritable connective tissues disorders and genetically triggered arteriopathies that result from mutations in the genes involved in the transforming growth factor-beta (TGF- 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

As not all *COL3A1* variants are pathogenic, the interpretation of the genetic testing results by an individual trained in variant assessment in keeping with the ACMG 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 non-pathogenic *COL3A1* variants and with current criteria did not have vEDS. This, too, has substantial implications for the individuals and their family members as previously mentioned.

Accurate diagnosis of vEDS has research implications. An example derives from the BBEST celiprolol trial, which aimed to assess if celiprolol (a long acting β_1 adrenergic receptor

antagonist with partial β_2 adrenergic receptor agonist) would reduce the risk of arterial dissections and ruptures in treated individuals.²⁰ In this trial, the 53 enrolled individuals had the 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 reduced 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 beta-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 beta-blockers.

In this study, 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 has been previously demonstrated² and highlights an opportunity for early diagnosis.²³ However, the family history is frequently absent as 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, as this appears to present at a younger age with male predominance compared to 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 ICD-9 and 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 creation of separate ICD codes for the different subtypes by the patient advocacy groups.

The study is limited by the relative 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 is, in part, could be due to the identifications of individuals with a severe phenotype who are identified because of a complication. Additionally, due to the rarity of vEDS, few clinicians have encountered affected individuals and as such, this could lead to 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 is highly relevant as the clinical features of vEDS overlap with those of other forms of EDS and other genetically triggered arteriopathies. 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.

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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 vEDS 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 vEDS and genetic testing for pathogenic COL3A1 variants appears important. Accurate diagnosis is critical and has serious implications for lifelong screening, medical, and surgical management strategies for the affected individual and family members.

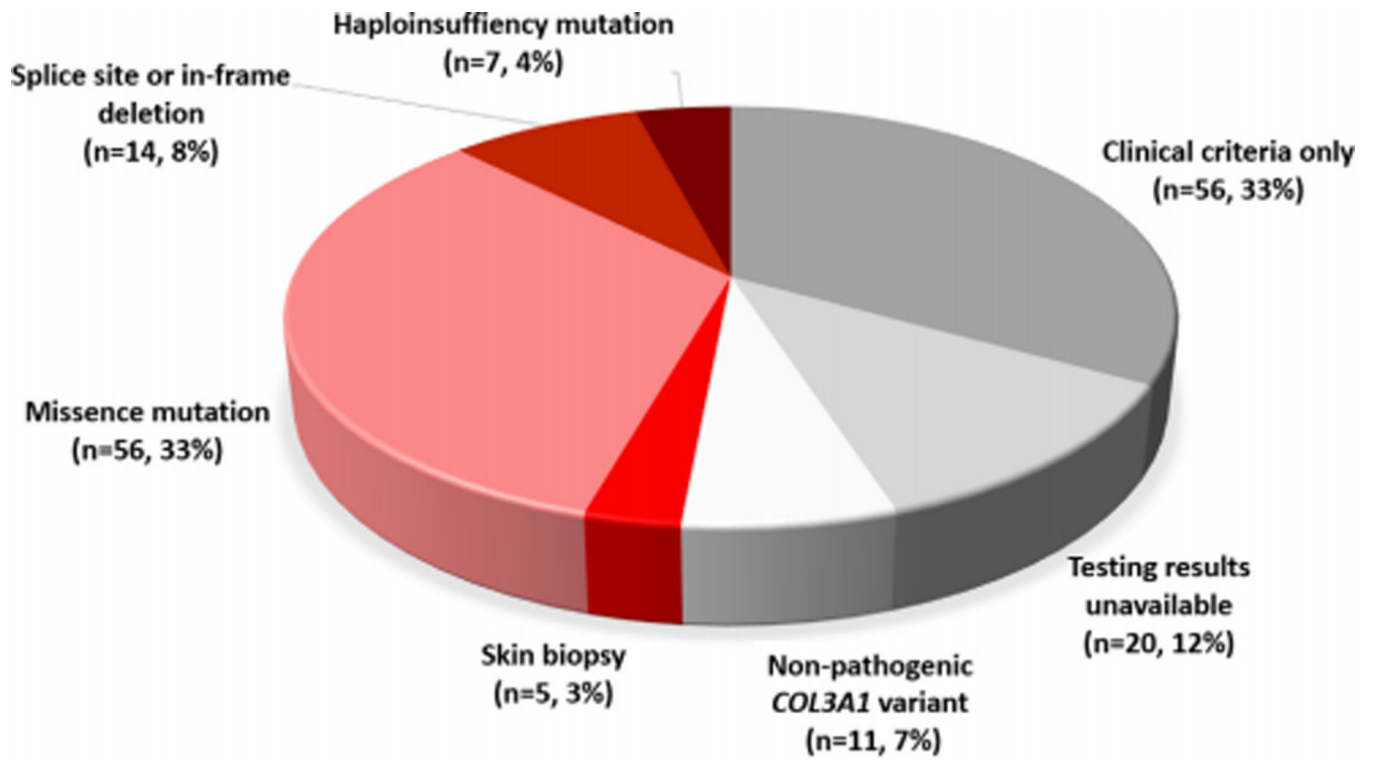


Fig 1. Method of diagnosis of 173 patients identified as having vascular Ehlers-Danlos syndrome (vEDS).

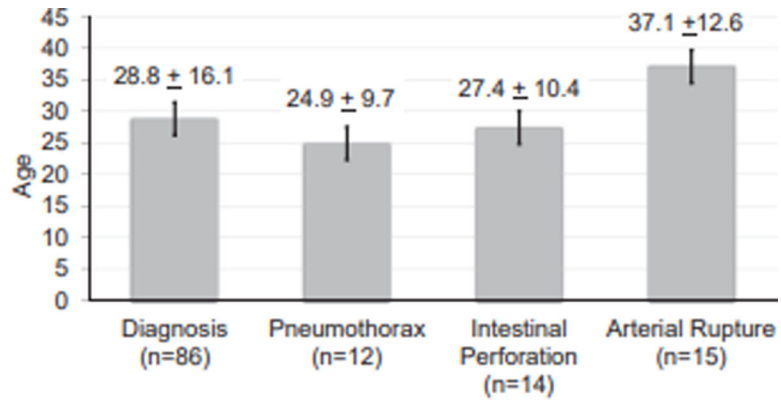
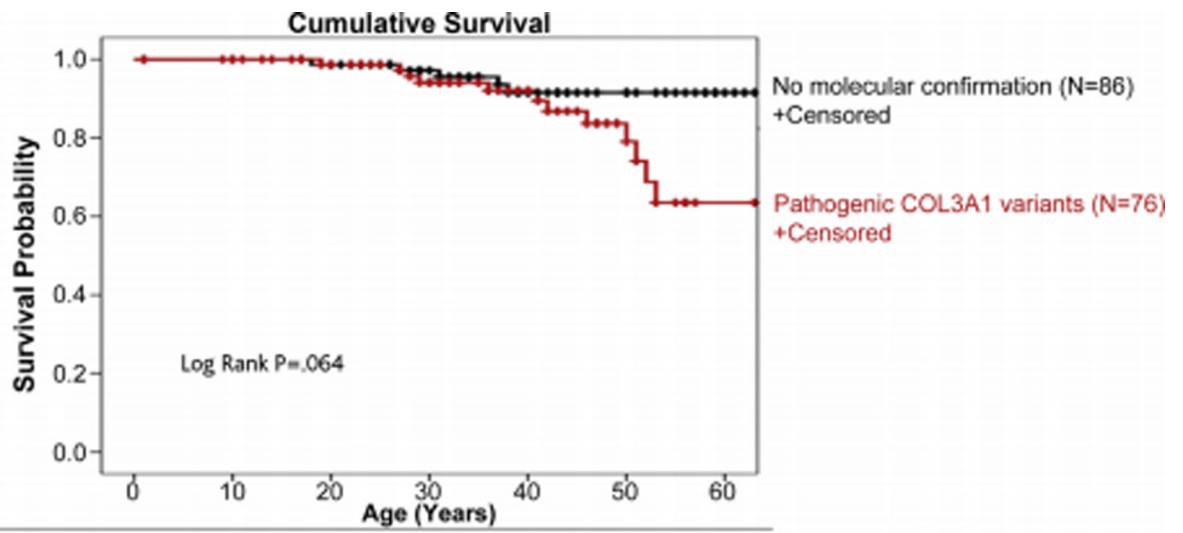


Fig 2. Mean \pm standard deviation age at diagnosis, at first spontaneous pneumothorax, at first intestinal perforation, and at first arterial rupture among individuals with vascular Ehlers-Danlos syndrome (vEDS) with confirmed pathogenic **COL3A1** variants.



	Numbers at risk						
	0	10	20	30	40	50	60
Pathogenic COL3A1 variants	86	82	72	58	37	17	6
No molecular confirmation	76	76	74	59	40	27	13

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.

Table I.

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

Variable	Pathogenic <i>COL3A1</i> variants (n – 86)	Clinical diagnosis of vEDS (n – 76)	P value
Age at diagnosis	28.8 ± 16.2	27.3 ± 12.5	0.636
Male	41 (47.7)	15 (19.7)	<0.001
Race			<0.001
Caucasian	74 (86)	15 (19.7)	
Multi-racial	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)	0.213
Deep vein thrombosis	13 (15.1)	6 (7.9)	0.154
Mitral valve prolapse	1 (1.2)	8 (10.5)	0.009
Smoker (past or current)	17 (19.8)	26.3 (20)	0.322
Family history ^a	42 (48.8)	33 (43.4)	0.490
Major complications			
Arterial rupture	15 (17.4)	7 (9.2)	0.127
Any arterial pathology ^c	53 (61.6)	16 (21.1)	<0.001
Intestinal perforation	14 (16.3)	3 (3.9)	0.011
Uterine rupture ^b	4 (18.2)	0	0.019
Minor Diagnostic Criteria			
Hypermobility of small joints	35 (40.7)	52 (68.4)	<0.001
Easy bruising	55 (64)	18 (23.7)	<0.001
Thin, translucent skin	42 (48.8)	13 (17.1)	<0.001
Characteristic facial features	27 (31.4)	6 (7.9)	<0.001
Skin hyperextensibility	12 (14)	10 (13.2)	0.883
Early-onset varicose veins	13 (15.1)	2 (2.6)	0.006
Tendon or muscle rupture	9 (10.5)	6 (7.9)	0.573
Spontaneous Pneumo/hemothorax	12 (14)	3 (3.9)	0.028
Carotid-cavernous fistula	4 (4.7)	1 (1.3)	0.221
Clubfoot	8 (9.3)	3 (3.9)	0.176
Follow up Duration post diagnosis	8.2 ± 8.2	9.8 ± 8.8	0.349
Mortality	13 (15.1%)	6 (7.9%)	0.154

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 *z* standard deviation.

^aFamily history of vEDS, aortic & arterial aneurysms and dissections, sudden death

^bOf 52 patients who had pregnancies.

^cDefined as defined as aortic/arterial aneurysm, pseudoaneurysm, dissection, fistula, or rupture.

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Table II.

Demographics of individuals with vascular Ehlers-Dantos syndrome 1 (vEDS) iMio experierved an arterial event.

Variable	Pathogenic <i>COL3A1</i> variants (n – 55)	Clinical diagnosis of vEDS (n – 16)	P value
Male	27 (50.9)	10 (62.5)	0.417
Caucasian	47 (88.7)	11 (68.8)	0.056
Comorbid conditions			
Hypertension	17 (32.1)	3 (18.8)	0.303
Deep vein thrombosis	12 (22.6)	3 (18.8)	0.741
Smoker (past or current)	12 (22.6)	2 (12.5)	0.377
Family history ^a	30 (56.6)	6 (37.5)	0.180
Minor Diagnostic Criteria			
Spontaneous Pneumo/hemothorax	7 (13.2)	2 (12.5)	0.941
Carotid-cavernous fistula	4 (7.5)	1 (6.3)	0.861
Hypermobility of small joints	17 (32.1)	10 (62.5)	0.029
Easy bruising	32 (60.4)	10 (62.5)	0.879
Thin, translucent skin	26 (49.1)	5 (31.3)	0.209
Characteristic facial features	16 (30.2)	6 (37.5)	0.582
Skin hyperextensibility	8 (15.1)	4 (25)	0.360
Early-onset varicose veins	8 (15.1)	2 (12.5)	0.796
Tendon or muscle rupture	5 (9.4)	2 (12.5)	0.722
Clubfoot	6 (11.3)	1 (6.3)	0.556
Age at first arterial diagnosis	34.6 ±12.5	35.1 ±12.5	0.877
Age at first arterial diagnosis (Males)	32.8 ±14.1	37.1 ±12.8	0.423
Age at first arterial diagnosis (Females)	34.4 ±11.7	32.2 ±12.6	0.674
Diagnosis established prior to index arterial pathology diagnosis	21 (38.9)	9 (56.3)	0.218
Arterial Rupture	15 (17.4)	7 (9.2)	0.127
Arterial pathology ^b			
Carotid and Vertebral arteries	19 (22.1)	3 (3.9)	0.001
Age	32.6 ±12.5	21.3 ±2.5	0.060
Aorta	17 (19.8)	2 (2.6)	0.001
Age	37.1 ±15.8	40 ±12.7	0.805
Mesenteric arteries	22 (25.6)	7 (9.2)	0.007
Age	40.1 ±11.3	34.2 ±12.2	0.216
Renal arteries	12 (14)	4 (5.3)	0.064
Age	38.3 ±9.4	32.8 ±10.5	0.417
Iliac arteries	17 (19.8)	5 (6.6)	0.014
Age	38.2 ±9.4	40 ±13.7	0.766
Follow up post first arterial pathology diagnosis (years)	7.2±6	6.2±7.2	0.579

The *P value* is for the comparisons between those with molecular confirmation showing pathogenic COL3AI variants and those diagnosed using clinical criteria without molecular confirmation. Values are presented as number (%) or mean *z* standard deviation.

^a of vEDS, aortic & arterial aneurysms and dissections, sudden death

^b Defined as defined as aortic/arterial aneurysm, pseudoaneurysm, dissection, fistula, or rupture..

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