CONFLICT OF INTEREST DISCLOSURE

In connection with the presentation, I disclose COI with the following companies/organizations..

Joint research grant : AccuRna Inc.

Characteristic findings of vascular Ehlers–Danlos syndrome for helpful of diagnosis

Shujiro Hayashi , Atsushi Hatamochi

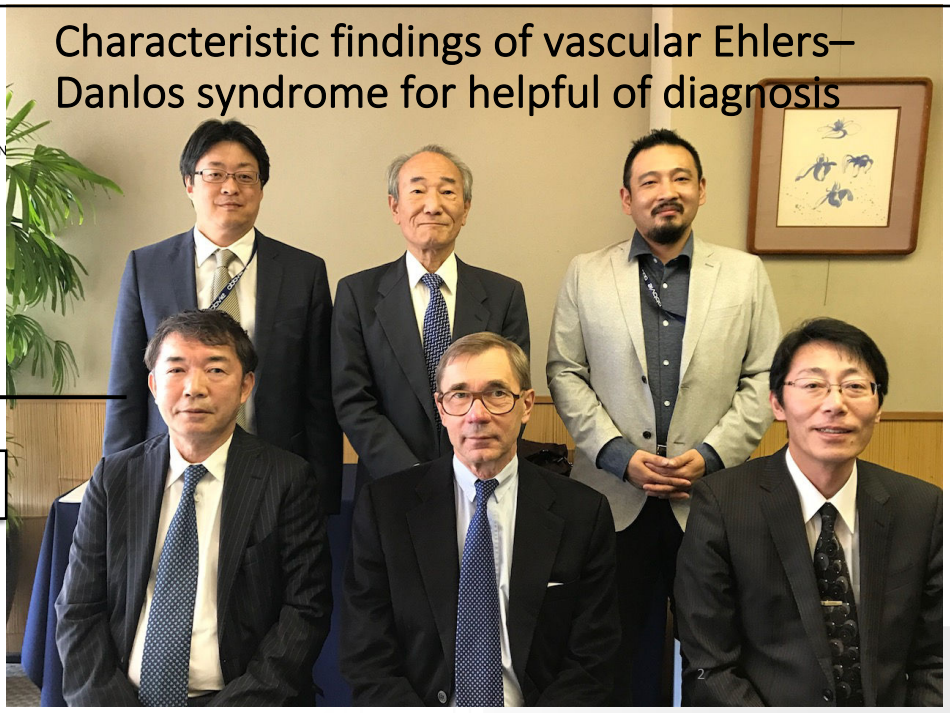
Department of Dermatology, Dokkyo Medical University, School of Medicine

SCIENTIFIC MEETING - RARER TYPES

TOKYO 2019

1

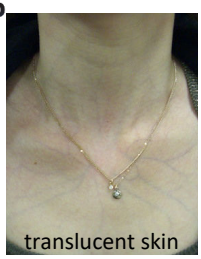
Characteristic findings of vascular Ehlers–Danlos syndrome for helpful of diagnosis

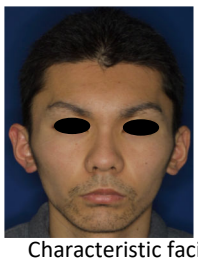


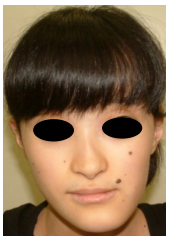
Prof,
Atsushi Hatamochi

2

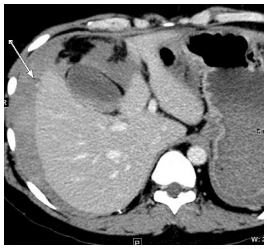
Clinical features of Vascular type Ehlers-Danlos syndrome (vEDS)


translucent skin




Characteristic facial appearance


Extensive bruising


Subcapsular bleeding

Subject

(Reference ; Beighton,P et al. Am J Med Genet, 1998)

***Major diagnostic criteria**

1) Thin, translucent skin
2) Arterial/intestinal/uterine fragility or rupture
3) Extensive bruising
4)Characteristic facial appearance

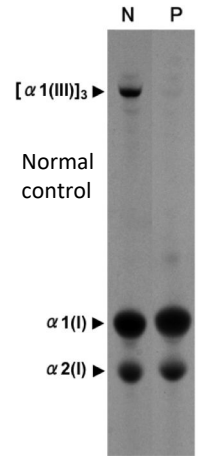
Minor diagnostic criteria

1) Acrogeria
2) Hypermobility of small joint
3) Tendon and muscles rupture
4) Talipes equinovarus (clubfoot)
5) Early-onset varicose veins
6) Arteriovenous, carotid-cavernous sinus fistula
7) Pneumothorax/ pneumohemothorax
8) Gingival recession
9) Positive family history, sudden death in (a) close relative(s).

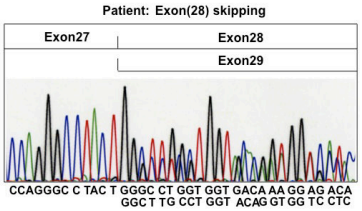
*The presence of any two or more of the major criteria is highly indicative of the diagnosis

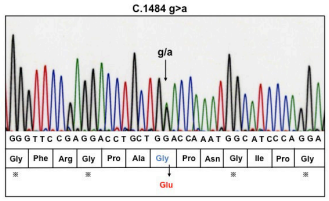
3

How to make definitive diagnosis with vEDS



- **Gene analyzing of *COL3a1***





British Journal of Dermatology 2010 163, pp704–710
Clinical and genetic features of 20 Japanese patients with vascular-type Ehlers–Danlos syndrome
Y. Shimaoka, S Hayashi, T. Kosho, A. Hatamochi, et al

- **Analysis of expression of collagen III expression from fibroblast.**

4

Problems in diagnosis for vEDS by clinical, gene/protein diagnostic method

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- Although vEDS is a **fatal disease**, vEDS has **similar symptoms to other connective tissue diseases** and is difficult to diagnose according to only clinical symptoms.
  American Journal of Medical Genetics 1998 77, pp31-37
- There are possibility of prevention for vascular events by β blocker (celiprolol), therefore **early diagnosis** would be important.
  Journal of the American College of Cardiology 2019 73, pp1948-1957
- Genetic and protein analysis can be done in the **limited facilities** and, **spent much time analyzing for COL3A1/type 3 collagen.**
  British Journal of Dermatology 2010 163, pp704–710
- In electronic microscope (EM) findings, **the collagen bundles were reported the various sizes,**
  Archives of disease in childhood 2015 100, pp 57-61

⇒Thus, We studied whether EM-findings could be applied to diagnosis.

5

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270 patients referred for diagnostic screening. (2004～2018)

Missing information (including electronic microscopy: EM test) n=194 (COL3A1 Variant n= 29)

Analyzed n=76 (no Relatives)

-No COL3A1 Variant with reduction of collagen 3-expression (n=0)

Pathogenic COL3A1 Variant with reduction of collagen 3-expression (n=21) **⇒vEDS**

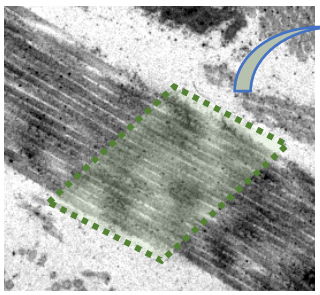
No COL3A1 Variant and no reduction of collagen 3-expression (n=55) **⇒control**

Mutation of *COL3A1* Glycine substitution n=15
Exon skip n=5 Incursion n=1

6

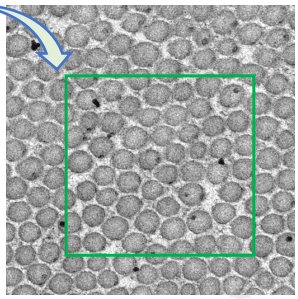
Comparing **coefficient of variation (COV)** between vEDS patients and controls in size of collagen bundles

Horizontal section for collagen bundles



(×10000)

Vertical sections for collagen bundles



(×50000)

・Measure the long diameter of collagen bundles present in 4 or more locations within 0.25μm^2 on 2 or more sections, more then 80 bundles, per patient. Average number of measurements per patient (± standard error)

・All samples were from upper arm with non-exposed area. ・ Measurement of COV was performed using ImageJ (version, 1.52 / 2018) , and analysis was performed using SPSS version 18 (SPSS, Inc., Chicago).

7

The size of the collagen fiber is biased depending on the observation site, age and gender. (n=6)

journal of cutaneous pathology:1984

Journal of Cutaneous Pathology 1984: **11**: 18–26

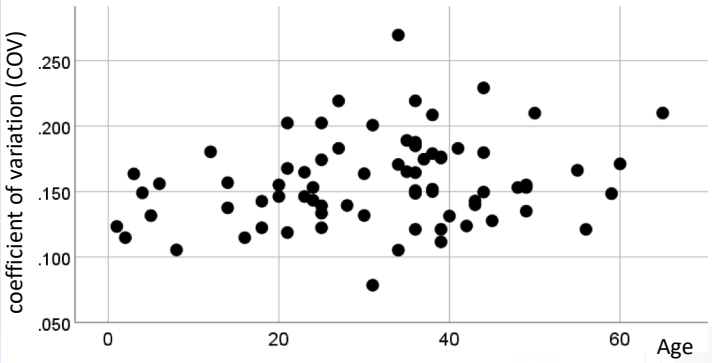
Measurement of collagen-fibre diameter in human skin

S. P. Barton, and R. Marks

Department of Medicine, Welsh National School of Medicine, Heath Park, Cardiff, Wales

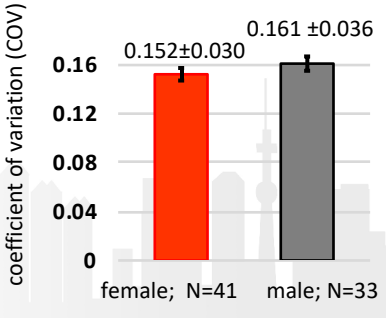
Age 1～65 mean 32 ± 14.3

correlation coefficient *P* value: 0.09 Nonparametric



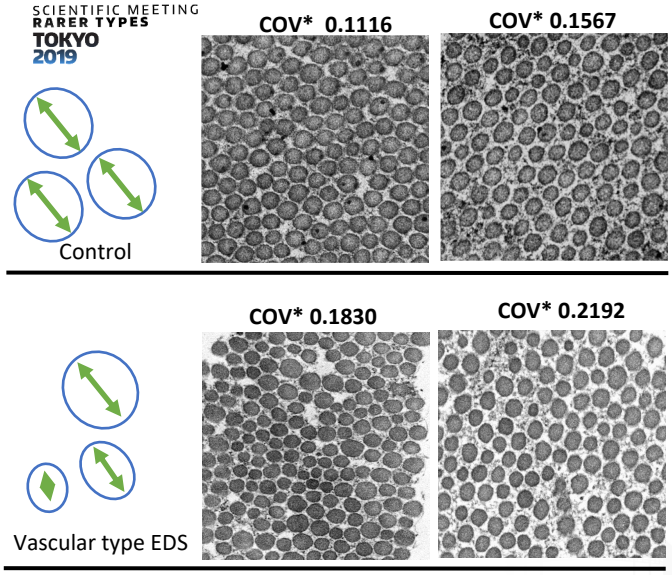
Gender

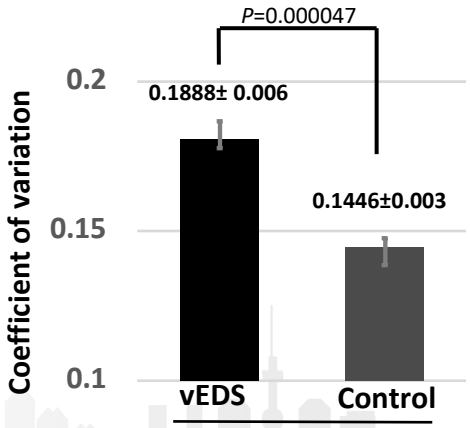
(female vs male) : *P* value 0.266



8

COV in vEDS are significantly higher than those in control

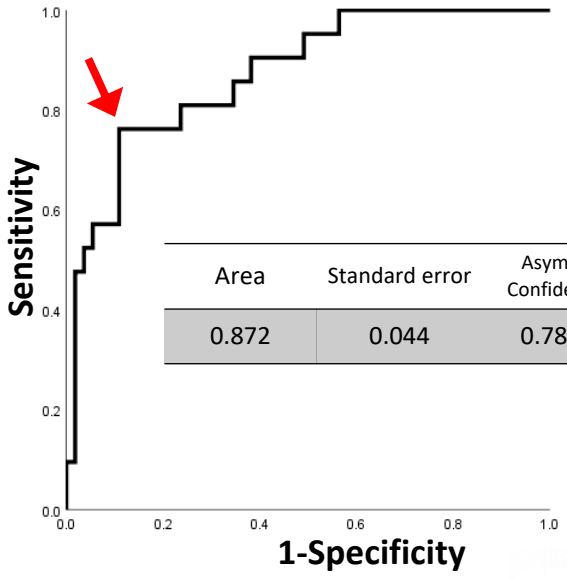




*coefficient of variation (COV)

9

Receiver Operating Characteristic (ROC)curve



Area	Standard error	Asymptotic 95% Confidence interval
0.872	0.044	0.786 - 0.958

COV* in the long diameter of collagen bundles	Sensitivity	Specificity
.166949	.762	.836
.169133	.762	.855
.170865	.762	.873
.172695	.762	.891
.174492	.714	.891
.175156	.667	.891
.176018	.619	.891

*Coefficient of variation

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RARER TYPES
TOKYO
2019

American Journal of Medical Genetics Part C (Seminars in Medical Genetics) 175C:8–26 (2017)

ARTICLE

The 2017 International Classification of the Ehlers–Danlos Syndromes

FRANSISKA MALFAIT,* CLAIR FRANCOMANO, PETER BYERS, JOHN BELMONT, BRITTA BERGLUND, JAMES BLACK, LARA BLOOM, JESSICA M. BOWEN, ANGELA F. BRADY, NIGEL P. BURROWS, MARCO CASTORI, HELEN COHEN, MARINA COLOMBI, SERWET DEMIRDAS, JULIE DE BACKER, ANNE DE PAEPE, SYLVIE FOURNEL-GIGLEUX, MICHAEL FRANK, NEETI GHALI, CECILIA GIUNTA, RODNEY GRAHAME, ALAN HAKIM, XAVIER JEUNEMAITRE, DIANA JOHNSON, BIRGIT JUUL-KRISTENSEN, INES KAPFERER-SEEBACHER, HANADI KAZKAZ, TOMOKI KOSHO, MARK E. LAVALLEE, HOWARD LEVY, ROBERTO MENDOZA-LONDONO, MELANIE PEPIN, F. MICHAEL POPE, EYAL REINSTEIN, LEEMA ROBERT, MARIANNE ROHRBACH, LYNN SANDERS, GLENDA J. SOBEY, TIM VAN DAMME, ANTHONY VANDERSTEEN, CAROLINE VAN MOURIK, NICOL VOERMANS, NIGEL WHEELDON, JOHANNES ZSCHOCKE, AND BRAD TINKLE

Major criteria

1. Family history of vEDS with documented causative variant in COL3A1
2. Arterial rupture at a young age
3. Spontaneous sigmoid colon perforation in the absence of known diverticular disease or other bowel pathology
4. Uterine rupture during the third trimester in the absence of previous C-section and/or severe peripartum perineum tears
5. Carotid-cavernous sinus fistula (CCSF) formation in the absence of trauma

Minor criteria

1. Bruising unrelated to identified trauma and/or in unusual sites such as cheeks and back
2. Thin, translucent skin with increased venous visibility
3. Characteristic facial appearance
4. Spontaneous pneumothorax
5. Acrogeria
6. Talipes equinovarus
7. Congenital hip dislocation

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1 *A family history of the disorder, arterial rupture, or dissection in individuals less than 40 years of age, unexplained sigmoid colon rupture, or spontaneous pneumothorax in the presence of other features consistent with vEDS should all lead to diagnostic studies to determine if the individual has vEDS.*

2 3 4

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But, reduction of collagen 3-expression
(n=0)

Pathogenic COL3A1 Variant

(n=21)

No COL3A1 Variant
and no reduction of collagen 3-expression

(n=55)

Family history of vEDS
Arterial rupture
Colon perforation
Pneumothorax

vEDS n=21

Mean number	Number of patient
0	2 (9.5%)
1	10
2	9
3	0
4	0

90.5% **Sensitivity**

Control n=55

Number of patient	
0	27 (49.1%) **Specificity**
1	23 (41.8%)
2	5 (9.1.%) 50.9%
3	0
4	0

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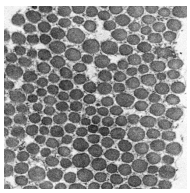
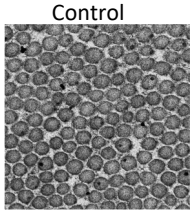
electronic microscope (EM)

vEDS



Control



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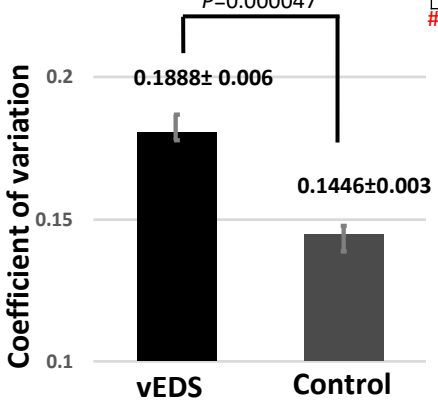
#Family history of vEDS #Arterial rupture #Colon perforation #Pneumothorax



COV* in the long diameter of collagen bundles
(N=76, vEDS:21, Control:55)

Coefficient of variation	vEDS n=21	Control n=55
case＜0.14	5% n=1	44% n=24
0.14≦ case＜0.17	19% n=4	42% n=23
0.17≦case＜0.2	24% n=5	13% n=7
0.2≦case	43% n=9	2% n=1
COV<0.17 n=52	vEDS n=5 (9.6%)	control **n=47 (90.4%)**

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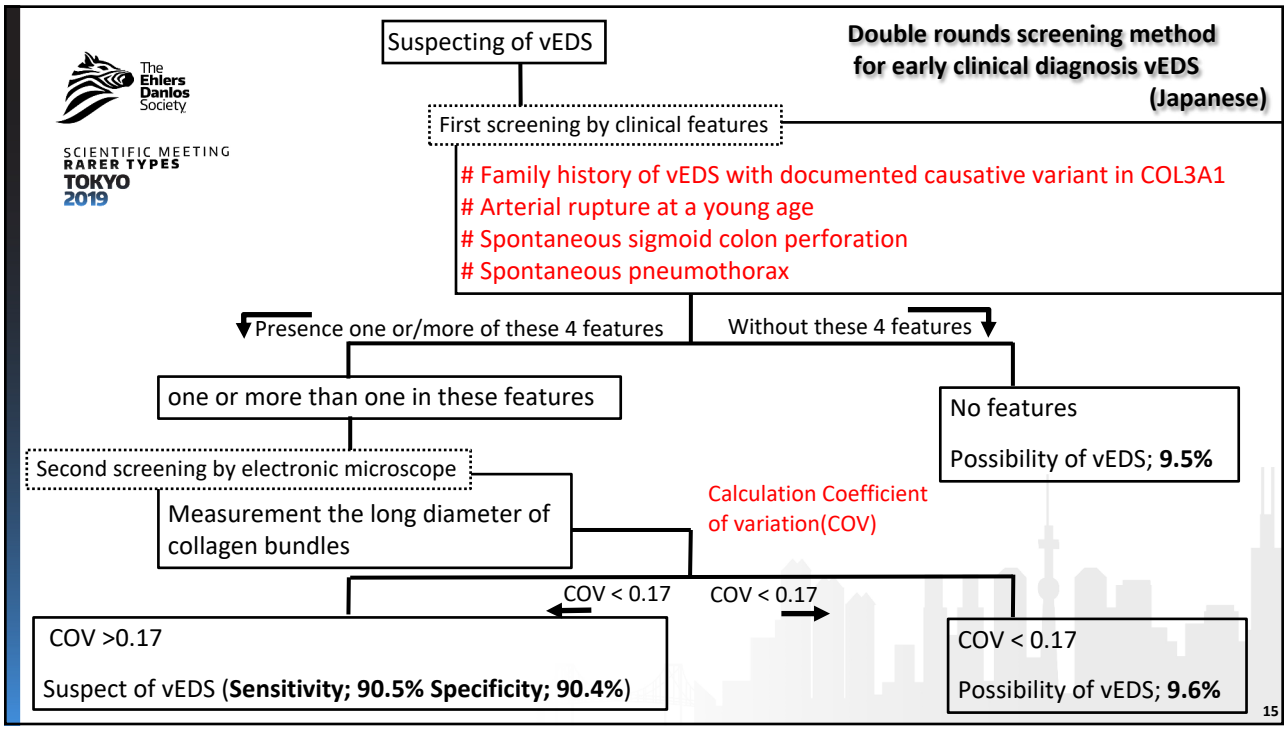

COV* in the long diameter of collagen bundles and Clinical findings (N=76, vEDS:21, Control:55)

*coefficient of variation (COV)

Beighton,P et al. Am J Med Genet, 1998

	Subjects of Clinical findings	None			Presence			*P* value	
		n	Average of COV	±SE	*n*	Average of COV	±SE	*P*<0.05	*P*<0.01
Major diagnostic criteria	Thin, translucent skin	41	0.147	0.028	35	0.168	0.035	0.00464	
	Arterial/intestinal/uterine fragility or rupture	41	0.150	0.035	35	0.165	0.029	0.00746	
	Extensive bruising	38	0.149	0.027	37	0.166	0.037	0.03929	
	Characteristic facial appearance	59	0.149	0.028	17	0.183	0.036	0.00012	
Minor diagnostic criteria	Acrogeria	71	0.156	0.033	5	0.173	0.031	0.25557	
	Hypermobility of small joint	29	0.156	0.036	47	0.157	0.032	0.84809	
	Tendon and muscles rupture	71	0.156	0.034	5	0.174	0.019	0.23139	
	Talipes equinovarus (clubfoot)	61	0.158	0.034	15	0.150	0.031	0.38771	
	Early-onset varicose veins	76	-	-	0	-	-		
	Arteriovenous, carotid-cavernous sinus fistula (CCSF)	71	0.155	0.031	5	0.189	0.047	0.02172	
	Pneumothorax/ pneumohemothorax	58	0.152	0.033	18	0.172	0.029	0.02700	
	Gingival recession	75	0.156	0.033	1	-	-	0.43066	
	Positive family history, sudden death in close relative(s).	70	0.156	0.034	6	0.166	0.022	0.46392	

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Coefficient of variation (COV) of collagen bundles and Clinical findings in patients with vEDS

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Beighton,P et al. Am J Med Genet, 1998

total *N*=21 (vEDS only)

*P<0.05

	Subjects of Clinical findings	None			Presence			
		n	Average of COV	±SE	n	Average of COV	±SE	*P* value
Major diagnostic criteria	Thin, translucent skin	6	0.169	0.022	15	0.197	0.031	0.049*
	Arterial/intestinal/uterine fragility or rupture	7	0.190	0.042	14	0.189	0.255	0.986
	Extensive bruising	7	0.188	0.026	14	0.195	0.032	0.219
	Characteristic facial appearance	9	0.187	0.028	12	0.190	0.034	0.874
Minor diagnostic criteria	Acrogeria	16	0.194	0.030	5	0.173	0.031	0.205
	Hypermobility of small joint	8	0.191	0.038	13	0.187	0.027	0.767
	Tendon and muscles rupture	19	0.188	0.032	2	0.193	0.014	0.856
	Talipes equinovarus (clubfoot)	17	0.191	0.031	4	0.180	0.034	0.528
	Early-onset varicose veins	21	-	-	0	-	-	-
	Arteriovenous, carotid-cavernous sinus fistula	17	0.186	0.028	4	0.201	0.046	0.561
	Pneumothorax/ pneumohemothorax	11	0.192	0.035	10	0.185	0.027	0.642
	Gingival recession	20	0.189	0.032	1	0.183	-	-
	Positive family history, sudden death in close relative(s)	16	0.196	0.031	5	0.168	0.024	0.072
	Skin hyperextension**	18	0.186	0.0321	3	0.206	0.0154	0.139

**Common symptom in inheritable connective tissue diseases

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Significantly correlation between COV of collagen bundles and Collagen 3 expression

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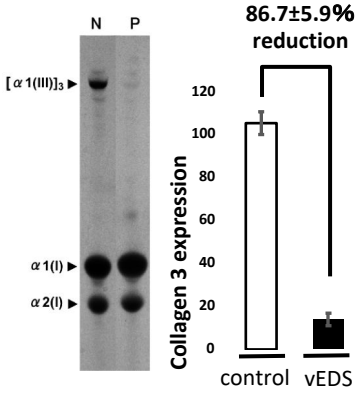

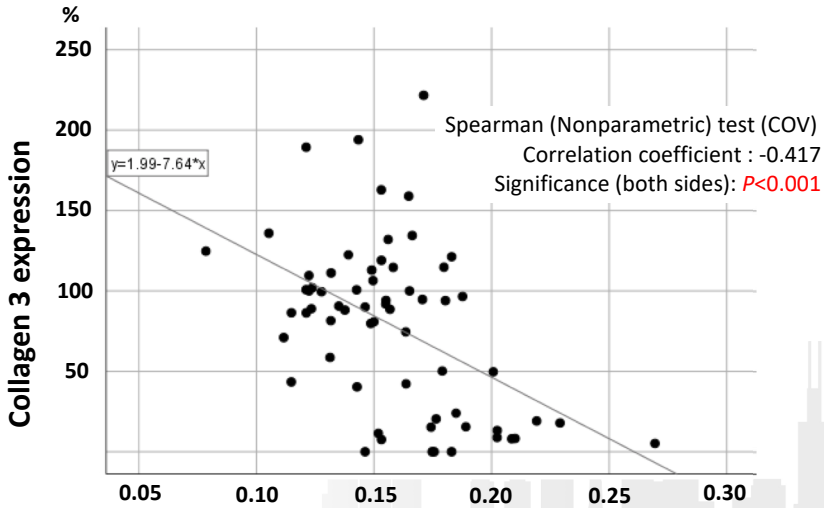

Measurement of density in COL3α1(III) / COLα1(I)+COLα1(I) was performed using ImageJ (version, 1.52 / 2018) , and analysis was performed using SPSS version 18 (SPSS, Inc., Chicago). Normalization is to normal healthy human.

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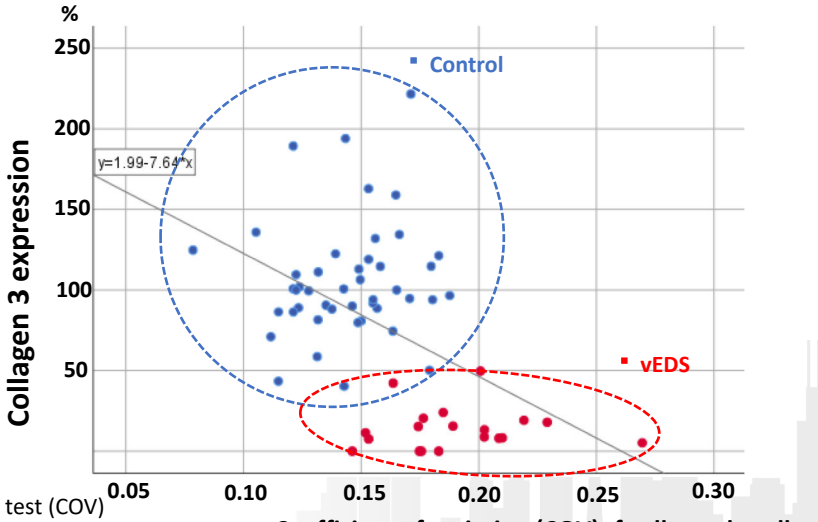
No significantly correlation between COV of collagen bundles and Collagen 3 expression in vEDS or control



Control
Spearman (Nonparametric) test (COV)
Correlation coefficient : 0.160
Significance (both sides): *P*=0.29

vEDS (COV)
Spearman (Nonparametric) test (COV)
Correlation coefficient : 0.181
Significance (both sides): *P*=0.46

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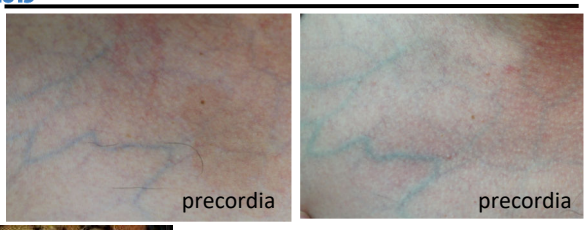
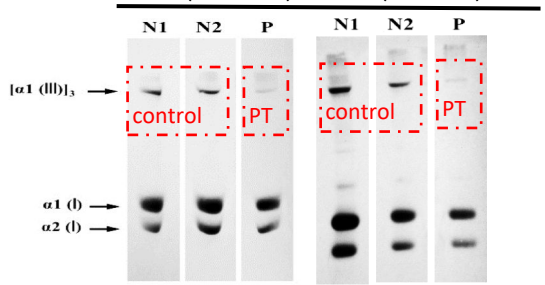
Case is 38-year-old men was diagnosed vEDS in 2015 (C. 1493 g>a, Gly→Glu).

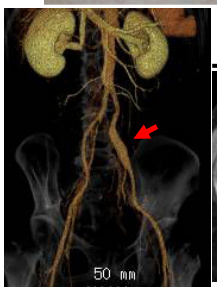
He started to take celiprolol after diagnosed then underwent re-skin-biopsy 3 years later.

Translucent skin

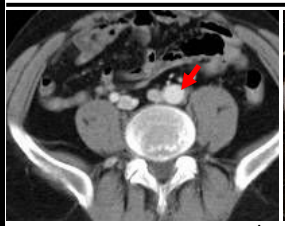


After 3 years

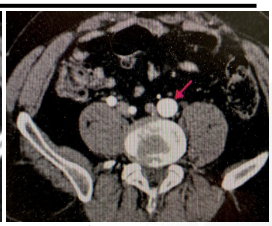




Aneurysm in Iliac artery

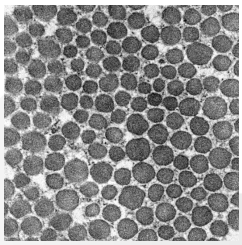


After 3 years

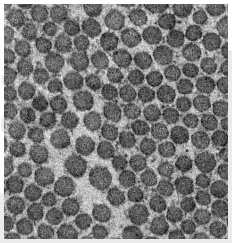


computed tomography; **CT**

COV: 0.189



After 3 years, COV: 0.145



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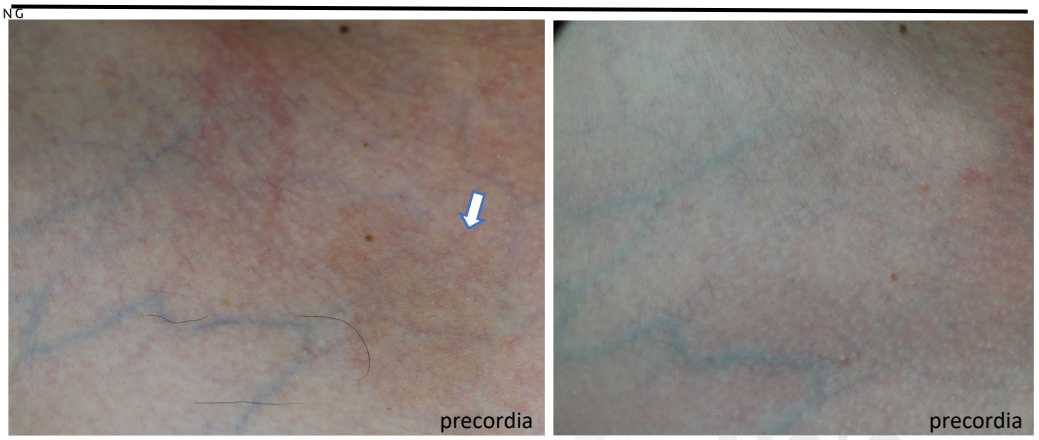
Translucent skin might be improved by celiprolol

Before celiprolol | After 3 years



These photos are from same angles among -/+ (3 y) taking celiprolol.
The pigmentation (arrow) due to internal bleeding, are diapered after 3 yaears.
Subcutaneous veins might be developing, but other small capillaries and Small wrinkles are disappeared,

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Summary

If setting cutoff to 0.172 of COV in size of collagen bundles with electronic microscope (EM) finding for diagnosis vEDS, sensitivity is 0.76, specificity is 0.89.

Double rounds screening method for early clinical diagnosis vEDS, vEDS can be suspected, Sensitivity; 90.5% Specificity; 90.4%.

Translucent skin are significantly correlation with *COV among only vEDS patients.

"Decreasing of COV" and "improvement of translucent skin" might be an indicator of the therapeutic effect of celiprolol.

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*Coefficient of variation: COV

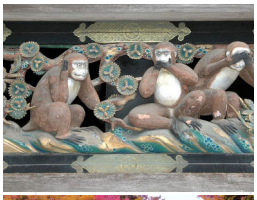
The Ehlers Danlos Society

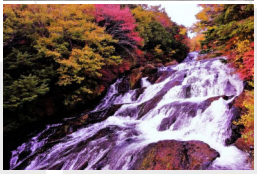
SCIENTIFIC MEETING
RARER TYPES
TOKYO
2019

Dokkyo Medical University, Department of Dermatology ,where is 100km north of Tokyo and close to NIKKO.

Thank you for your attention!

World Heritage!





日光 （Nikko）