# Clinical Sequencing for Vascular Ehlers-Danlos Syndrome Using Panel-Based Next-Generation Sequencing

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## The authors declare no conflict of interests

## Introductions

**Vascular Ehlers-Danlos Syndrome (vEDS)**

- An autosomal dominant disorder caused by pathogenic variants in *COL3A1*, with the estimated prevalence as 1/50,000-1/200,000

- A serious type that can causes fatal complications such as arterial dissection, aneurysm, or rupture, spontaneous sigmoid colon rupture, or uterine rupture during the third trimester

- Genetic testing is required for diagnosis, which enables appropriate surveillance and intervention including celiprolol therapy

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At present, 970 cases with *COL3A1* variants are registered in the Human Gene Mutation Database (HGMD) Professional 2019.1

| Variant type | Number of cases | |
|---|---|---|
| Missense variants (Gly substitutions) | 573 | 59.1% |
| Missense variants (non-Gly substitutions) | 1 | 0.1% |
| Missense variants outside the triple helix domain | 11 | 1.1% |
| Splice site alterations | 264 | 27.2% |
| Insertions/deletions | 75 | 7.7% |
| Nonsense variants | 30 | 3.1% |
| Stop-loss | 2 | 0.2% |
| Multi-exon deletions | 14 | 1.4% |
| **Total** | 970 | |

The majority of disease-causing variants in *COL3A1* are Gly substitutions and in-frame splice site alterations in the triple helix domain

Only about 36 Japanese cases were registered

## Materials and Methods

### Study subjects

Patients with suspected hereditary connective tissue disorders (HCTDs) referred for genetic analysis between April 2013 and May 2019 to the Center for Medical Genetics, Shinshu University Hospital

### Clinical sequencing strategy

NGS : Ion Torrent™ system

Panel: Ion AmpliSeq™ custom panel

consisting of causative genes for various HCTDs

e.g. 13 subtypes of EDS

Marfan syndrome

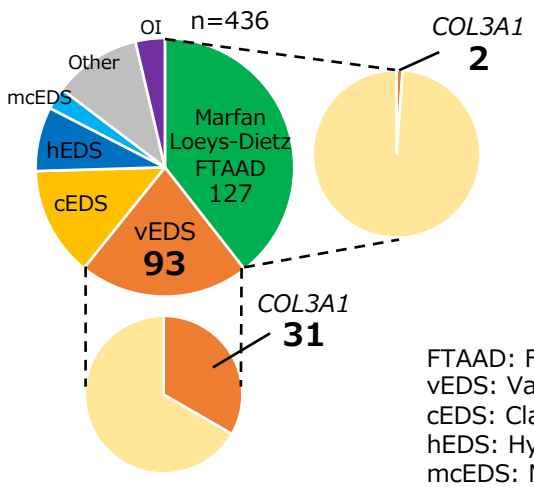
Loeys-Dietz syndrome

Familial thoracic aortic aneurysm and dissection (FTAAD)

CNV : CNV visualization method for Ion AmpliSeq data

(Nishio et al., Mol Genet Genomic Med 6:678-86, 2018)

## Results




- A total of 436 unrelated patients with suspected HCTDs have been analyzed
- In 93 patients clinically suspected as vEDS, 31 were found to have heterozygous variants in *COL3A1* (33.3%)
- Additionally, one with a clinical diagnosis of Loeys-Dietz syndrome and another with a clinical diagnosis of FTAAD were found to have heterozygous variants in *COL3A1*

FTAAD: Familial thoracic aortic aneurysm and dissection
vEDS: Vascular EDS
cEDS: Classical EDS
hEDS: Hypermobile EDS
mcEDS: Musculocontractural EDS
OI: Osteogenesis imperfecta

**A comprehensive list of variants in *COL3A1* detected in this study**

| Patient ID | Variants | Exon | HGMD Pro. (number of cases) | ClinVar | EDS Variant Database | ACMG guideline 2015 | Criteria for classifying pathogenic | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | PVS | PS | PM | PP |
| 80 | c.547G>A:p.Gly183Ser | 7 | Reported (20) | Pathogenic | Pathogenic | | | | | |
| 305 | c.547G>A:p.Gly183Ser | 7 | Reported (20) | Pathogenic | Pathogenic | | | | | |
| 157 | c.556G>A:p.Gly186Ser | 7 | Reported (2) | Pathogenic | Pathogenic | | | | | |
| 396 | c.565G>C:p.Gly189Arg | 7 | Reported (1) | Pathogenic | Pathogenic | | | | | |
| 69 | c.583G>A:p.Gly195Arg | 8 | Reported (1) | - | Pathogenic | | | | | |
| 271 | c.659_664del:p.220_221del | 9 | - | - | - | VUS | | | PM2/PM4 | |
| 283X | c.665G>A:p.Gly222Asp | 9 | Reported (4) | Pathogenic | Pathogenic | | | | | |
| 45 | c.724C>T:p.Arg242* | 10 | - | - | - | Pathogenic | PVS1 | PS3 | | PP1 |
| 304 | c.754G>A:p.Gly252Ser | 11 | - | - | - | Likely pathogenic | | | PM1/PM2/PM5 | PP2/PP3 |
| 130 | c.755G>A:p.Gly252Asp | 11 | Reported (1) | Pathogenic | Pathogenic | | | | | |
| 357 | c.763G>T:p.Gly255Trp | 11 | - | - | - | Likely pathogenic | | | PM1/PM2/PM5 | PP2/PP3 |
| 89 | c.848T>A:p.Leu283* | 12 | - | - | - | Pathogenic | PVS1 | PS3 | PM2 | PP1 |
| 291 | c.897+2T>G | 13i | - | - | - | VUS | | | PM2/PM4 | PP1 |
| 349 | c.897+2T>A | 13i | Reported (1) | - | Pathogenic | | | | | |
| 235 | c.951+5G>C | 14i | - | - | - | Likely pathogenic | | PS3 | PM2 | PP1 |
| 266 | c.1194+1G>A | 18i | Reported (3) | Pathogenic | Pathogenic | | | | | |
| 341 | c.1330G>A:p.Gly444Arg | 20 | Reported (7) | Pathogenic /Likely pathogenic | Pathogenic | | | | | |
| 368 | c.1330G>A:p.Gly444Arg | 20 | Reported (7) | Pathogenic /Likely pathogenic | Pathogenic | | | | | |
| M187 | c.[1546G>T;1556G>T] :p.[Gly516Trp;Gly519Val] | 23 | - | - | - | Likely pathogenic | | | PM1/PM2/PM5 | PP1/PP2/ PP3 |
| 67 | c.1662+1G>A | 24i | Reported (47) | Pathogenic | Pathogenic | | | | | |
| 210 | c.1862G>A:p.Gly621Glu | 27 | Reported (1) | Pathogenic | Pathogenic | | | | | |
| 188 | c.1977+5G>C | 29i | - | VUS | - | VUS | | | PM2 | PP1 |
| 252 | c.2134_2160del:p.Pro712_Gly720del | 32 | Reported (2) | Pathogenic | Pathogenic | | | | | |
| 426 | c.2299_2316dup:p.Ile767_Pro772dup | 34 | - | - | - | Likely pathogenic | | PS3 | PM2/PM4 | |
| 214 | c.2356G>A:p.Gly786Arg | 35 | Reported (6) | Pathogenic | Pathogenic | | | | | |
| 173 | c.2357G>A:p.Gly786Glu | 35 | - | - | - | Likely pathogenic | | | PM1/PM2/PM5 | PP2/PP3 |
| 402 | c.2518G>A:p.Gly840Arg | 37 | - | - | - | Pathogenic | | PS2 | PM1/PM2 | PP2/PP3 |
| 193 | c.2815G>A:p.Gly939Ser | 40 | Reported (1) | Pathogenic | Pathogenic | | | | | |
| 82 | c.2869G>A:p.Gly957Ser | 41 | Reported (2) | - | Pathogenic | | | | | |
| 251 | c.3256G>C:p.Gly1086Arg | 46 | - | - | - | Likely pathogenic | | | PM1/PM2/PM5 | PP2/PP3 |
| 83 | c.3338G>A:p.Gly1113Asp | 46 | - | - | - | Likely pathogenic | | | PM1/PM2 | PP2/PP3 |
| 64 | c.3525+1G>A | 48i | Reported (1) | - | Pathogenic | | | | | |
| 81 | ex.24-31 deletion | 24-31 | - | - | - | | | | | |

- Checked whether it was reported in HGMD, ClinVar and EDS Variant Database

**Reported 18/33 (54.5%)**

- Checked whether it was reported in HGMD, ClinVar and EDS Variant Database
- (If not reported) Evaluated it according to the ACMG guidelines 2015

| Patient ID | Variants | Exon | HGMD Pro. (number of cases) | ClinVar | EDS Variant Database | ACMG guidelines 2015 | Criteria for classifying pathogenic | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | PVS | PS | PM | PP |
| | | | Reported (20) | Pathogenic | Pathogenic | - | | | | |
| | | | Reported (20) | Pathogenic | Pathogenic | - | | | | |
| | | | Reported (2) | Pathogenic | Pathogenic | - | | | | |
| | | | Reported (1) | Pathogenic | Pathogenic | - | | | | |
| | | | Reported (1) | - | Pathogenic | - | | | | |
| | | | - | - | - | VUS | | | PM2/PM4 | |
| | | | Reported (4) | Pathogenic | Pathogenic | - | | | | |
| | | | - | - | - | Pathogenic | PVS1 | PS3 | | PP1 |
| | | | - | - | - | Likely pathogenic | | | PM1/PM2/PM5 | PP2/PP3 |
| | | | Reported (1) | Pathogenic | Pathogenic | - | | | | |
| | | | - | - | - | Likely pathogenic | | | PM1/PM2/PM5 | PP2/PP3 |
| 89 | c.848T>A:p.Leu283* | 12 | - | - | - | Pathogenic | PVS1 | PS3 | PM2 | PP1 |
| 291 | c.897+2T>G | 13i | - | - | - | VUS | | | PM2/PM4 | PP1 |
| 349 | c.897+2T>A | 13i | Reported (1) | - | Pathogenic | - | | | | |
| 235 | c.951+5G>C | 14i | - | - | - | Likely pathogenic | | PS3 | PM2 | PP1 |
| 266 | c.1194+1G>A | 18i | Reported (3) | Pathogenic | Pathogenic | - | | | | |
| 341 | c.1330G>A:p.Gly444Arg | 20 | Reported (7) | Pathogenic /Likely pathogenic | Pathogenic | - | | | | |
| 368 | c.1330G>A:p.Gly444Arg | 20 | Reported (7) | Pathogenic /Likely pathogenic | Pathogenic | - | | | | |
| M187 | c.[1546G>T;1556G>T] :p.[Gly516Trp;Gly519Val] | 23 | - | - | - | Likely pathogenic | | | PM1/PM2/PM5 | PP1/PP2/ PP3 |
| 67 | c.1662+1G>A | 24i | Reported (47) | Pathogenic | Pathogenic | - | | | | |
| 210 | c.1862G>A:p.Gly621Glu | 27 | Reported (1) | Pathogenic | Pathogenic | - | | | | |
| 188 | c.1977+5G>C | 29i | - | VUS | - | VUS | | | PM2 | PP1 |
| 252 | c.2134_2160del:p.Pro712_Gly720del | 32 | Reported (2) | Pathogenic | Pathogenic | - | | | | |
| 426 | c.2299_2316dup:p.Ile767_Pro772dup | 34 | - | - | - | Likely pathogenic | | PS3 | PM2/PM4 | |
| 214 | c.2356G>A:p.Gly786Arg | 35 | Reported (6) | Pathogenic | Pathogenic | - | | | | |
| 173 | c.2357G>A:p.Gly786Glu | 35 | - | - | - | Likely pathogenic | | | PM1/PM2/PM5 | PP2/PP3 |
| 402 | c.2518G>A:p.Gly840Arg | 37 | - | - | - | Pathogenic | | PS2 | PM1/PM2 | PP2/PP3 |
| 193 | c.2815G>A:p.Gly939Ser | 40 | Reported (1) | Pathogenic | Pathogenic | - | | | | |
| 82 | c.2869G>A:p.Gly957Ser | 41 | Reported (2) | - | Pathogenic | - | | | | |
| 251 | c.3256G>C:p.Gly1086Arg | 46 | - | - | - | Likely pathogenic | | | PM1/PM2/PM5 | PP2/PP3 |
| 83 | c.3338G>A:p.Gly1113Asp | 46 | - | - | - | Likely pathogenic | | | PM1/PM2 | PP2/PP3 |
| 64 | c.3525+1G>A | 48i | Reported (1) | - | Pathogenic | - | | | | |
| 81 | ex.24-31 deletion | 24-31 | - | - | - | - | | | | |

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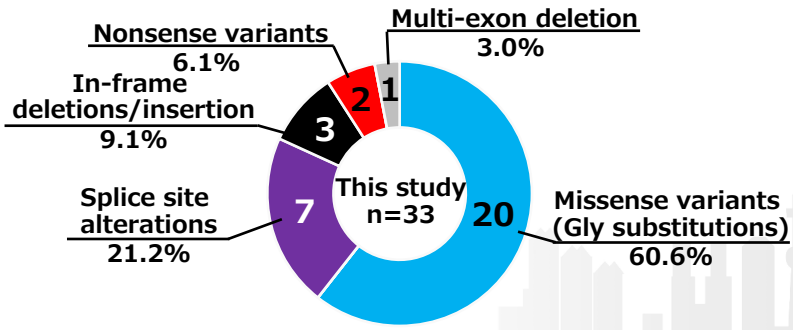
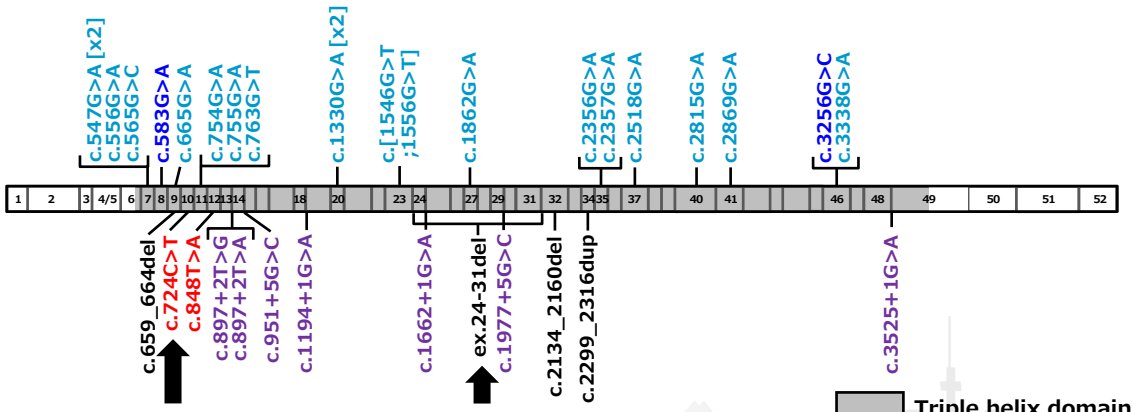
Among the 33 variants,

- **20 missense variants (60.6%)**
  leading to substitutions of glycine (Gly) in the triple helix domain (which included 2 missense variants at the 5' end of exon)
- **7 splice site alterations (21.2%)**
- **3 in-frame deletions or insertion (9.1%)**
- **2 nonsense variants (6.1%)**
- **1 multi-exon deletion (3.0%)**



## The distribution of variants in *COL3A1* detected in this study

- All variants were located in the triple helix domain.
- All missense variants resulted in Gly substitutions.
- All splice site alterations and deletions/insertion were predicted to cause in-frame alterations.

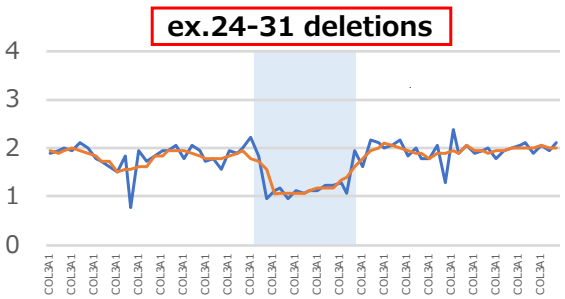



Missense variants that led to substitutions of Gly in the triple helix domain.
Missense variants at the 5' end of exon that led to substitutions of Gly in the triple helix domain.
Splice site alterations.
Nonsense variants.
In-frame deletions or insertion.

## Results

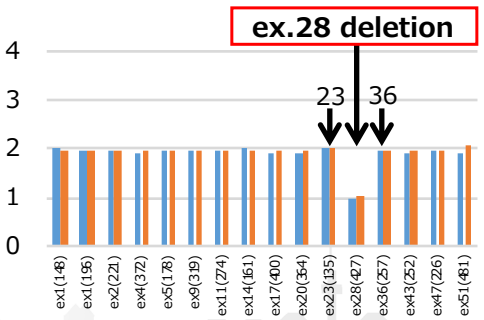
**Case 1 (No.81)** Found to have a multi-exon deletion

CNV visualization method
for Ion AmpliSeq data
(Nishio et al., Mol Genet Genomic Med 6:678-86, 2018)

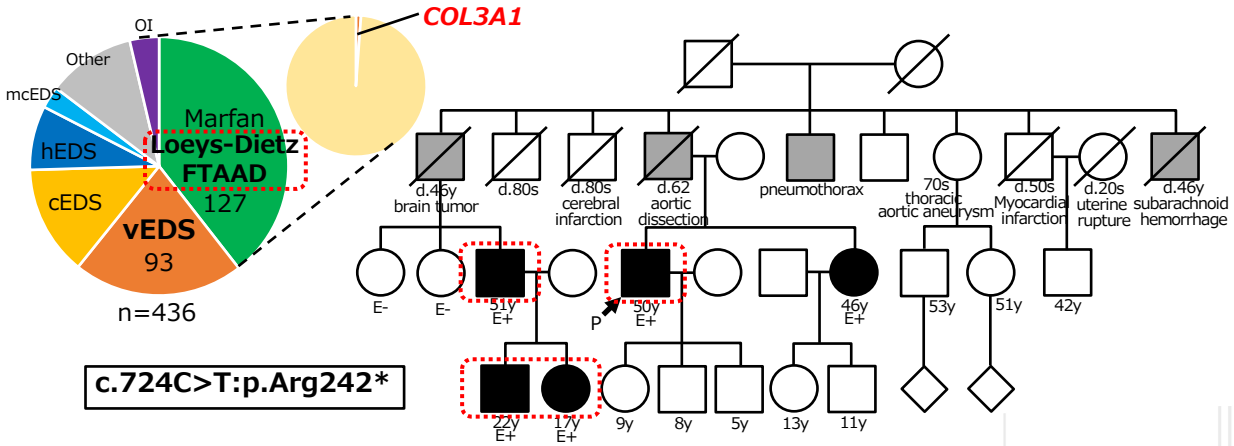



Blue indicates the normalized relative read depth of each amplicon
Red indicates the smoothing value for five relative amplicons

Validation by MLPA
(P155-D2 probemix)

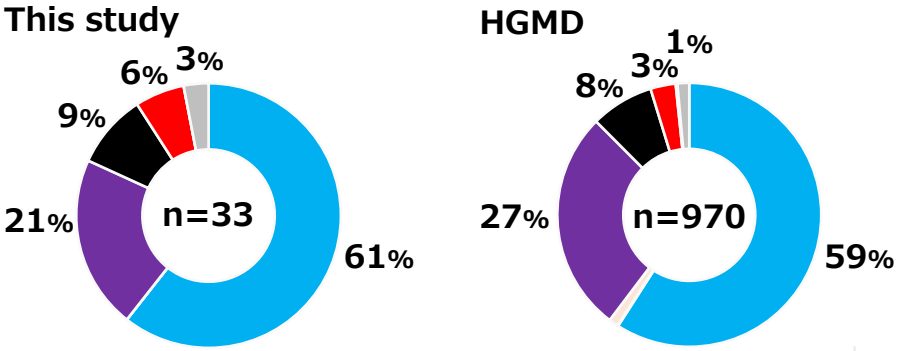


## Case 2 (No.45) Found to have a nonsense variant in *COL3A1*




- The patient was initially suspected as Loeys-Dietz syndrome or FTAAD because of dissection of ascending aorta without skin or skeletal features like Marfan syndrome or vEDS
- The patient as well as his affected cousin underwent valve-replacement surgery safely with no fragility-related complications, and started to have celiprolol after molecular diagnosis
- Two asymptomatic young children of the cousin were also diagnosed molecularly, and started vascular surveillance

## Discussion 1 –variant types–




The frequency of variant types in this study was compatible with those registered in HGMD Professional 2019.1

## Discussion 2 –Clinical Utility–

**Case 1**

This clinical sequencing strategy can detect not only SNV and indel but also CNV

**Case 2**

This clinical sequencing strategy was critically useful in the management of a case clinically indistinguishable from other HCTDs, and of at-risk young relatives enabling early accurate diagnosis

## Conclusions

- This is the first series of NGS panel-based clinical sequencing for vEDS in Japan
- The frequency of variant types in Japanese was compatible with those reported in previous literatures
- This clinical sequencing strategy provide the clinical utility and the cost-effectiveness

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