

[CASE REPORT]

Vascular Ehlers-Danlos Syndrome with a Novel Missense Mutation in *COL3A1*: A Man in His 50s with Aortic Dissection after Interventional Treatment for Hemothorax as the First Manifestation

Kosuke Sakai^{1,2}, Maiko Toda¹, Hiroyuki Kyoyama¹, Hiroaki Nishimura¹, Akitoshi Kojima¹, Yoshiki Kuwabara¹, Yumiko Kobayashi^{1,2}, Satoshi Kikuchi¹, Yusuke Hirata¹, Gaku Moriyama¹, Wataru Watanabe³, Koichi Akutsu⁴, Maki Nakai⁵, Takeshi Yamada⁵, Akihiko Gemma² and Kazutsugu Uematsu¹

Abstract:

Type III collagen is the major protein in the walls of blood vessels and hollow organs; it is decreased in patients with vascular Ehlers-Danlos syndrome (EDS). A 52-year-old man was admitted for severe back pain, and right hemothorax was suspected by chest computed tomography. Immediately after embolization for bleeding bronchial artery, aortic dissection occurred and was treated conservatively in the intensive-care unit. Vascular EDS with a mutation of *COL3A1* cDNA (c.3175G>A) was diagnosed. When vascular EDS is suspected, the patient should be treated prophylactically, and a genetic examination should be performed to confirm the diagnosis.

Key words: vascular Ehlers-Danlos syndrome, *COL3A1*, angiography, aortic dissection, hemothorax

(Intern Med 58: 3441-3447, 2019)

(DOI: 10.2169/internalmedicine.2983-19)

Introduction

Ehlers-Danlos syndrome (EDS) is a rare connective tissue disorder (1, 2). All 13 types of EDS are classified based on characteristic manifestations (3). Vascular EDS, previously known as EDS type IV, is the most severe type, and its symptoms are attributed to a reduction in type III collagen production (4-6). Since type III collagen is a major protein in the walls of blood vessels and hollow organs, the symptoms of vascular EDS include bruising, arterial and bowel fragility, and uterine fragility during pregnancy (6).

Although vascular EDS is due to an autosomal dominant defect in the *COL3A1* gene, half of vascular EDS patients have no family history; initial manifestations like rupture of

blood vessels, intestines, or other organs are diagnostic clues (2, 5-7). These symptoms can also suggest other ailments: Marfan syndrome, Loeys-Dietz syndrome, familial artery aneurysm, arterial tortuosity syndrome, and dissection syndromes (6). To distinguish vascular EDS from these other diseases, it is necessary to identify the *COL3A1* mutation or to demonstrate a reduced production of type III collagen (2).

We herein report a case of vascular EDS presenting initially as hemothorax without any traumatic injury, subsequently manifesting as aortic dissection after embolization of the bronchial artery during angiography and finally being diagnosed by detection of a mutation in the *COL3A1* gene. When hemothorax is seen without any traumatic injury, vascular EDS should be a differential diagnosis, and appropri-

¹Department of Pulmonary Medicine, Saitama Medical Center, Saitama Medical University, Japan, ²Department of Pulmonary Medicine and Oncology, Graduate School of Medicine, Nippon Medical School, Japan, ³Department of Diagnostic Imaging, Saitama Medical Center, Saitama Medical University, Japan, ⁴Department of Cardiovascular Medicine, Nippon Medical School, Japan and ⁵Division of Personalized Genetic Medicine, Nippon Medical School Hospital, Japan

Received: March 7, 2019; Accepted: June 23, 2019; Advance Publication by J-STAGE: August 6, 2019

Correspondence to Dr. Kosuke Sakai, ksakai@nms.ac.jp

Table 1. Laboratory Findings on First Visit.

Hematology		Biochemistry		Serology	
WBCs	9,800 / μ L	AST	29 U/L	C-reactive protein	0.1 mg/dL
Neutrophils	83.3 %	ALT	16 U/L	Anti-nuclear antibody	$\times 80$
Lymphocytes	11.6 %	γ -GTP	39 U/L	Rheumatoid factor	11 U/mL
Eosinophils	0.9 %	ALP	252 U/L	Anti-cardiolipin antibody	8 U/mL
Monocytes	3.9 %	T-Bil	1.2 mg/dL	MPO-ANCA	1 U/mL
Basophils	0.3 %	BUN	15 mg/dL	PR3-ANCA	1 U/mL
Red blood cells	509 $\times 10^4$ / μ L	Cre	0.64 mg/dL	Activated protein C	94 %
Hb	17.0 g/dL	LDH	216 U/L	Cofactor protein S	86 %
Hematocrit	49.7 %	CK	172 U/L	Lupus anticoagulant (dRVVT)	1.1
Platelet	16.0 $\times 10^4$ / μ L	Na	140 mEq/L	Prior to neutralization	43.6 s
Coagulation		K	4.1 mEq/L	After neutralization	39.4 s
APTT	31.2 s	Cl	104 mEq/L	von Willebrand factor	292 %
PT/INR	0.99	Ca	9.4 mg/dL		
D-Dimer	0.66 μ g/mL	Alb	4.5 g/dL		

WBCs: white blood cells, Hb: hemoglobin, APTT: activated partial thromboplastin time, PT: prothrombin time, INR: international normalized ratio, AST: aspartate transaminase, ALT: alanine aminotransferase, γ -GTP: γ -glutamyl transpeptidase, ALP: alkaline phosphatase, T-Bil: total bilirubin, BUN: blood urea nitrogen, Cre: creatinine, LDH: lactate dehydrogenase, CK: creatine kinase, Na: sodium, K: potassium, Cl: chlorine, Ca: calcium, Alb: albumin, MPO-ANCA: myeloperoxidase-antineutrophil cytoplasmic antibody, PR3-ANCA: proteinase3-antineutrophil cytoplasmic antibody, dRVVT: dilute Russell's viper venom time

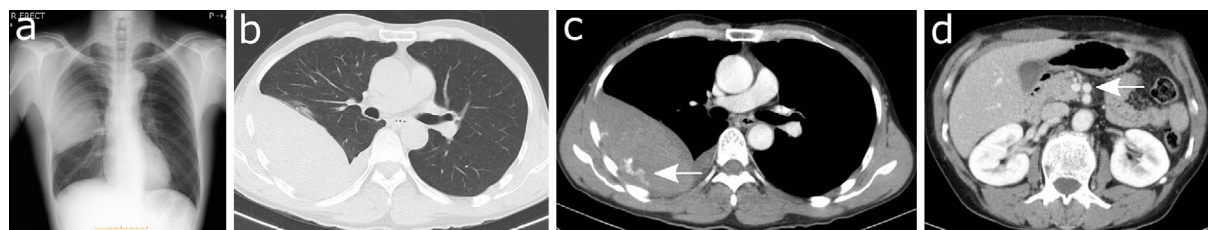


Figure 1. Chest X-ray film and computed tomography (CT) on the first visit. (a) The chest X-ray film shows fan-shaped infiltration spreading from the hilum of the right lung. (b, c) CT reveals a soft tissue mass with uneven density in the right thoracic cavity and contrast medium leak from the intercostal artery into the mass (arrow). (d) There is a 10-mm aneurysm (arrow) in front of the superior mesenteric artery.

ate examinations should be undertaken to make a diagnosis.

Case Report

A 52-year-old man arrived at the emergency room of our hospital on foot complaining of pain under the right scapula and a sudden onset of a cold sweat 7 hours earlier. He had had hyperuricemia since the age of 30 and spondylolisthesis since the age of 40. No abnormal findings had been pointed out by annual health examinations. He was an ex-smoker of 35 pack-years. His father had died from lung cancer, but he had no family history of any connective tissue diseases or sudden death.

He appeared thin with a height of 179.5 cm, weight of 61.0 kg, and body mass index of 18.9 kg/m². He had a blood pressure of 185/100 mmHg and heart rate of 110 beats per minute, without oxygenation failure or a fever. His first physical examination was unremarkable. The laboratory examination showed no anemia with hemoglobin of 17.0 g/dL and hematocrit of 49.7%, no coagulation disorder, and

no antibodies related to collagen diseases (Table 1). The electrocardiogram showed sinus tachycardia.

Chest computed tomography (CT) showed a mixed-density soft tissue mass in the right thoracic cavity and leakage of contrast medium from an intercostal artery into the mass, implying hemothorax in the right thoracic cavity (Fig. 1). After admission to the emergency room, a chest tube was inserted, and blood continued to ooze.

To identify the source of the bleeding, we performed angiography, which revealed irregular leaking of contrast medium to the distal site of the right fifth intercostal artery (Fig. 2a) and an aneurysm at the right sixth intercostal artery (Fig. 2b). The right fourth and sixth intercostal arteries were simultaneously drawn when the right fifth intercostal artery image was constructed, implying that these arteries have a common trunk (Fig. 2c, d). A gelatin sponge was placed at the right fifth intercostal artery for hemostasis.

Immediately afterward, severe back pain developed, and fentanyl to relieve pain was started in the angiography room. Contrast medium was stagnant in a false lumen of the de-

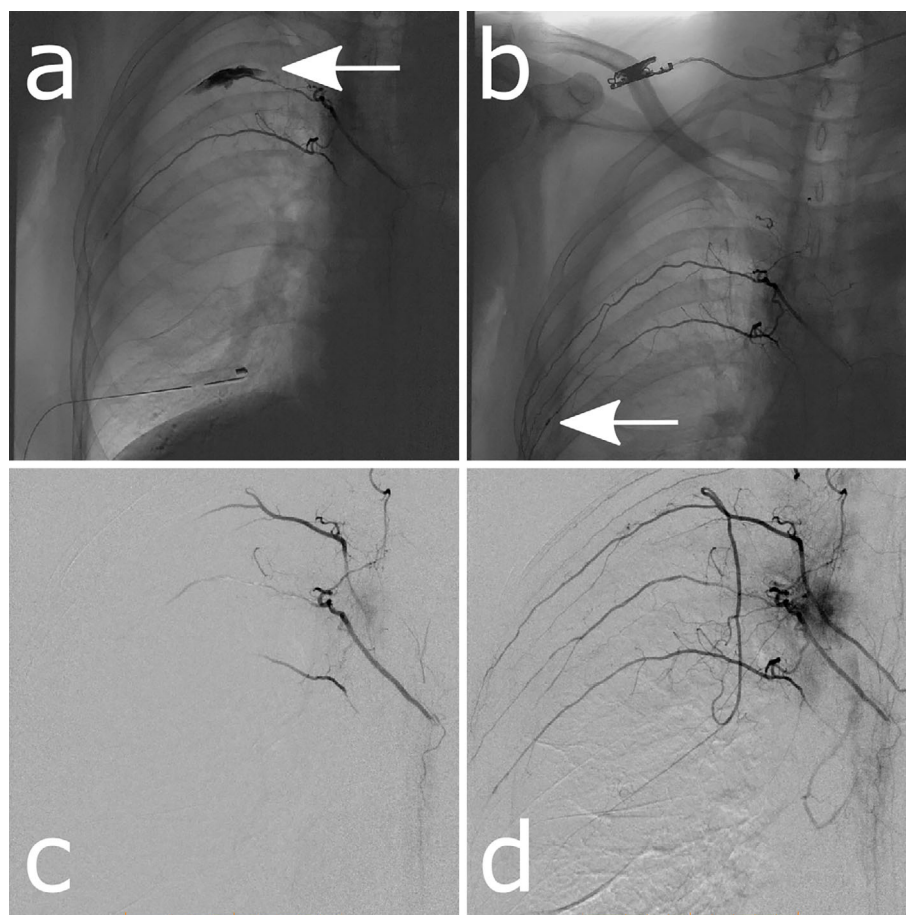


Figure 2. Angiography of bronchial arteries. (a) Contrast medium leaks out irregularly into the distal site of the right fifth intercostal artery (arrow). (b) An aneurysm is apparent at the right sixth intercostal artery (arrow). (c, d) The right fourth and sixth intercostal arteries are revealed by enhancement of the right fifth intercostal artery. These right intercostal arteries are supplied from a common trunk.

scending aorta at the origin of the right fifth intercostal artery (Fig. 3a). CT showed Stanford type A aortic dissection from the thoracic descending aorta to the ascending aorta (Fig. 3b-f). We started to administer nicardipine and continued to observe the patient without surgery in the intensive-care unit (ICU). Five days later, the false lumen had shrunk, and he left the ICU (Fig. 4a-d). He was discharged without recurrence of symptoms 22 days after the aortic dissection was noted and returned to his occupation. The hematoma had also shrunk after his discharge (Fig. 4e, f).

Since connective tissue disorders including vascular EDS were suspected, we referred him to the division of personalized genetic medicine at Nippon Medical School Hospital. To obtain his consent to the examination, a medical geneticist explained about genetic testing based on the following points: maintenance of the confidentiality of the patient's individual information and examination results; possibility of incidental pathogenic findings and the right to refuse to be informed of the results; possibility of familial disease; reconfirmation of his intention regarding genetic testing for his family members, after knowing results of his examination, if a responsible mutation is detected; genetic counsel-

ing; and mental supports by psychiatrists, clinical psychologists, medical geneticists and genetic counselors. After obtaining his written consent, total RNA was extracted from his peripheral blood cells, and a missense mutation (c.3175G>A) of *COL3A1* cDNA (i.e., a glycine-to-arginine substitution at codon 1059) was found (Fig. 5). This result confirmed the diagnosis of vascular Ehlers-Danlos syndrome. To confirm the diagnosis, we tried to check the *COL3A1* gene of his family. Unfortunately, his three children did not consent to this assessment. Celiprolol was therefore started at 50 mg/day and gradually increased to 400 mg/day, while an angiotensin II receptor blocker was added to a calcium channel blocker for stabilization of blood pressure. No vascular complications have recurred thus far.

Discussion

In the present case, vascular EDS was suspected and diagnosed based on the manifestations of vascular vulnerability, including hemothorax without any traumatic injury, aortic dissection immediately after angiography and emboliza-

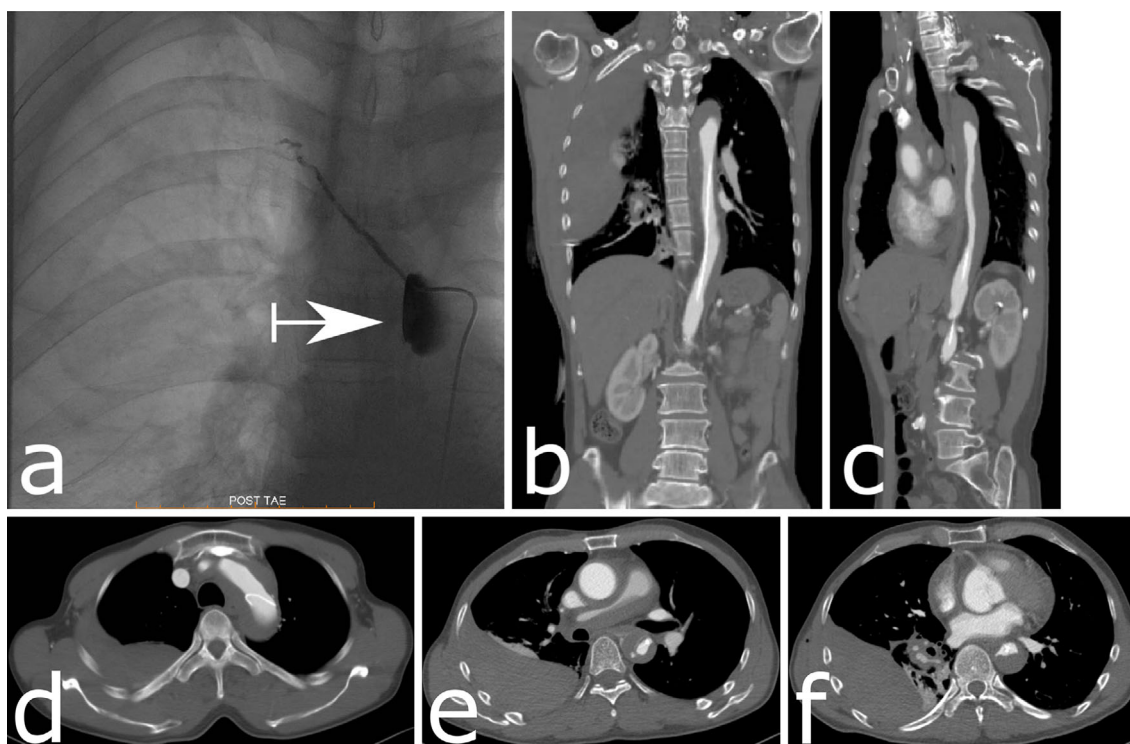


Figure 3. Stanford type A aortic dissection from the thoracic descending aorta to the ascending aorta after embolization of the right fifth intercostal artery. (a) Contrast medium is stagnant at the origin of the right fifth intercostal artery in the descending aorta. (b) A coronal section of the vessels on CT shows dissection in the thoracic descending aorta with a narrow true lumen of the aorta and catheter existing in the true lumen. An uneven density in the right thoracic cavity is revealed. (c) A sagittal section of the vessels on CT also shows dissection in the descending aorta with a narrow true lumen. (d-f) An axial section of the vessels on CT shows dissection at the arch of the aorta, with a catheter and a narrowed true lumen. The right pulmonary trunk and arteries are compressed and narrowed by a mediastinal hematoma. An area of uneven density on the dorsal side in the right thoracic cavity is revealed (d). The dissection extends to the ascending aorta, and there is an area of uneven density on the dorsal side of the right thoracic cavity (e). The dissection with the mediastinal hematoma extends from the descending aorta to the ascending aorta. (f) Contrast-enhanced CT reveals a feature that is similar in appearance to an ulcer-like projection that protrudes into the false lumen at the descending aorta. An area of uneven density stretches to the caudal side in the right thoracic cavity.

tion of the bronchial artery. At the time, it was difficult to diagnose the patient with vascular EDS because he had no family history of any connective tissue diseases or sudden death and had had abnormalities of the joint or skin that were unremarkable. Hyperextensibility of the skin or hypermobility of large joints are often clues to the presence of EDS, but these manifestations are unusual for vascular EDS (1). Symptoms of vascular EDS are related to decreased production of type III collagen, and the diagnostic criteria were revised in 2017 (Table 2) (3). However, these findings are not necessary for the diagnosis of vascular EDS, and there are cases where the disease has had little or no effect on the appearance of the face or skin (8).

Since vascular EDS is likely to be suspected based on the clinical features and family history, and given its rarity (1 in 50,000-200,000 of the population), its diagnosis before the occurrence of serious initial symptoms is difficult and some-

times not confirmed (1, 6). To confirm the diagnosis, a mutational analysis of the *COL3A1* gene or biochemical examination of collagen production is recommended. A biochemical examination to confirm a reduction in type III collagen was not conducted in the present case because recurrent bleeding events due to tissue vulnerability and the detection of a *COL3A1* mutation made us hesitate to conduct a tissue biopsy. Tissues for a biochemical examination may be able to be collected if he suffers from another event, such as intestinal rupture, that requires unavoidable surgical intervention.

Hemothorax was the initial symptom of vascular EDS in this case, and aortic dissection developed immediately after intervention for bronchial arterial bleeding. An aneurysm or dissection or rupture of vessels in the patients of vascular EDS typically involves the renal, iliac, femoral, mesenteric, hepatic, carotid, subclavian, ulnar, popliteal and tibial arter-

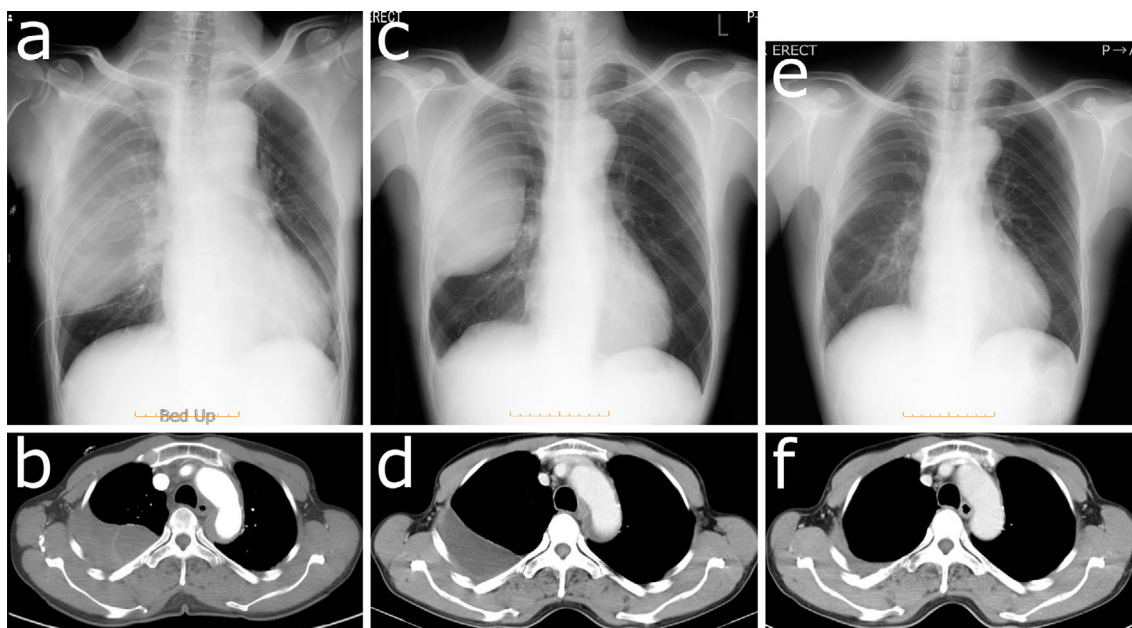


Figure 4. The follow-up chest X-ray film and CT scan. The false lumen of the aorta shrank, and the hemothorax did not expand 1 day (a, b), 15 days (c) and 18 days (d) after the aortic dissection had occurred. The hematoma was almost diminished three months after his discharge (e, f).

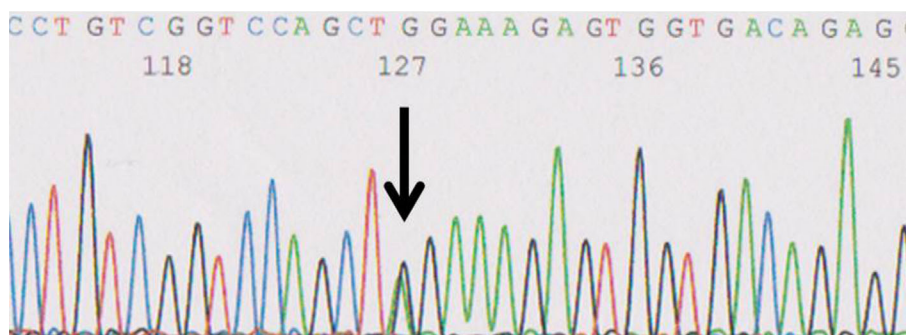


Figure 5. The sequence of the *COL3A1* gene located on 2q31. The cDNA was from the peripheral blood cells. Each nucleotide is indicated by a different color: adenine (A), green; cytosine (C), blue; guanine (G), black; and thymine (T), red. A heterozygous missense mutation (c.3175G>A) was found (arrow).

ies, and coronary rupture is rare (8). Intercostal aneurysm rupture was recently reported in only one case from Japan, and intercostal aneurysm rupture as the onset manifestation is rare, as described in the paper (9). Although pathological evidence of thoracic complications in vascular EDS has already been reported in a few post-mortem cases (10), the occurrence of aortic dissection after intervention has hardly been reported. The complications of angiography in vascular EDS patients are reported to be hemorrhaging from the puncture site, intracranial hemorrhaging, heart rupture, aortic dissection, intraabdominal hemorrhaging, intrathoracic hemorrhaging and others. Such hemorrhaging events occur repeatedly after the first treatment of the complications, and when or where the next complications will occur is unpredictable (11). We should therefore pay close attention when performing guidewire manipulation in order to avoid these complications.

In the present case, hemothorax of uncertain origin was noted and led to the suspicion of blood vessel rupture in the chest. Therefore, angiography had to be performed in order to identify the source of the bleeding. It has been reported that 37% of patients with vascular EDS die of complications from blood vessel injury in the chest, and in all cases of carotid cavernous sinus fistula, coil embolization for the carotid vessels was safely performed (12). When contrast medium was leaking from the right fifth intercostal artery, we realized that the bleeding was continuous and decided to conduct embolization of this artery. Since aortic dissection had occurred after embolization in this case of uncertain pathogenesis, conservative observation might have been an option as long as the blood pressure was stabilized. Aortic dissection can occur in any case; therefore, angiography should be performed cautiously, and alternative candidate causes should continue to be considered. Even though the

Table 2. Diagnostic Criteria of Vascular Ehlers-Danlos Syndrome (3).

Inheritance	Autosomal dominant
Major criteria	Family history of vascular Ehlers-Danlos Syndrome with documented causative variant in <i>COL3A1</i> Arterial rupture at a young age Spontaneous sigmoid colon perforation in the absence of known diverticular disease or other bowel pathology Uterine rupture during the third trimester in the absence of previous C-section and/or severe peripartum perineum tears Carotid-cavernous sinus fistula formation in the absence of trauma
Minor criteria	Bruising unrelated to identified trauma and/or in unusual sites such as cheeks and back Thin, translucent skin with increased venous visibility Characteristic facial appearance Spontaneous pneumothorax Acrogeria Talipes equinovarus Congenital hip dislocation Hypermobility of small joints Tendon and muscle rupture Keratoconus Gingival recession and gingival fragility Early onset varicose veins (under age 30 and nulliparous if female)

cause of aortic dissection in our case was uncertain, an elevated blood pressure during angiography or vascular injury from some type of insult was suspected; the guidewire did not stray into the false lumen. Although aortic dissection can be treated surgically, we chose to treat it conservatively in the ICU to avoid the risk of further serious complications, as these sequential events suggested the presence of a comorbidity, such as a connective tissue disorder.

The lifespan of affected individuals varies widely from 10 to 80 years, and the median age at death is about 51 years (12, 13). The lifespan is influenced by a mutation in *COL3A1*, which encodes the pro- $\alpha 1$ chains of type III collagen; these chains have a (Gly-X-Y)_n repeat structure (14). The exon-skipping mutation of *COL3A1* occurs less frequently than other mutations of *COL3A1*, and patients with exon-skipping mutations have the shortest median survival time. Substitution for triplet glycine residues in the triple helical domain results in a better prognosis than exon-skipping mutations, but substitution by bulky residues results in more severe phenotypes than that by smaller residues. Heterozygosity for *COL3A1*-null alleles delays the onset of complications by 20 years (6, 15). The missense mutation c.3175G>A in *COL3A1* cDNA, which converts glycine to arginine at codon 1059 in the (Gly-X-Y)_n repeat structure in the triple helical domain, was detected in this case and has not been previously reported on the ClinVar website (<https://www.ncbi.nlm.nih.gov/clinvar/>). However, the pathogenic missense mutation c.3176G>T in *COL3A1*, which converts glycine to valine at codon 1059, just as in our case, had been already reported on the ClinVar website. Similar pathogenic mutations leading to substitution for glycine in the (Gly-X-Y)_n repeat structure at other positions in the triple helical domain are the most common mutation

type in the *COL3A1* gene. The (Gly-X-Y)_n repeat structure is necessary for the triple helix formation of the pro- $\alpha 1$ chains of type III collagen encoded by the *COL3A1* gene. In particular, glycine residues are positioned in the center of the triple helix for dense packing along the central axis of chains to form type III collagen and stable collagen fibrils in extracellular spaces. Therefore, substitution of glycine in the (Gly-X-Y)_n repeat structure leads to the production of non-functional type III collagen and vulnerability of collagen tissue (14). We assume that the mutation in our case inhibits the encoding of glycine at the same codon which was previously reported to be pathogenic, and therefore it could also fail to generate the (Gly-X-Y)_n repeat structure.

Blood pressure stabilization is the recommended treatment for patients with vascular EDS. In a randomized trial that was relatively large for a rare disease, celiprolol, a $\beta 1$ -adrenoceptor antagonist with $\beta 2$ -adrenoceptor agonist action, was shown to protect against severe vascular EDS complications, including cardiac and arterial events, by causing vasodilation or by boosting the production of type III collagen (16, 17).

It is difficult to consider vascular EDS as a differential diagnosis before treatment or conducting an examination for complications because it is a rare disease, and its complication is often revealed or assessed under urgent circumstances. In a previous report, vascular EDS was diagnosed before vascular surgery in only 26% of patients (18). Our case report suggests that vascular EDS should be suspected in patients who develop bleeding or blood vessel collapse of uncertain origin, and patients with vascular EDS should be treated prophylactically. For the confirmation of the diagnosis, genetic examinations need to be performed at specialized facilities with specialists in clinical genetics who are

accustomed to handling genetic information, although they are currently approved by the health insurance program in Japan. Medical practitioners must understand the autosomal dominant nature of EDS and carefully consider the results of genetic examinations.

This case was reported at the 45th Annual Meeting of the Japanese Society of Intensive Care Medicine.

The authors state that they have no Conflict of Interest (COI).

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