

The Vascular Type of Ehlers-Danlos Syndrome

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Abstract

Vascular type of Ehlers-Danlos syndrome (EDS), also known as EDS type IV (NIM#130050) is a life-threatening autosomal dominant inherited disorder of connective tissue, caused by mutations of the *COL3A1* gene. Vascular EDS causes severe fragility of connective tissues with arterial and intestinal ruptures and complications of surgical and radiological treatment, and is of particular importance to medical professionals of many specialties: surgeons, internists, radiologists, and obstetricians. An accurate diagnosis may help in the management of visceral complications. In addition, when a family is faced with new information concerning a positive genetic test for vascular EDS, it is crucial that follow-up care for the family include comprehensive genetic counseling. After the genetic diagnosis of a *COL3A1* mutation, various medical specialists, including the clinical geneticists must cooperate to perform genetic counseling and to provide a system of long-term follow up for individuals with vascular EDS.

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Key words: Ehlers-Danlos syndrome (EDS), vascular type of Ehlers-Danlos syndrome (vascular EDS, EDS type IV), *COL3A1*, genetic counseling

Introduction

Vascular type of Ehlers-Danlos syndrome (EDS), also known as EDS type IV (MIM#130050) has the most severe clinical findings of all known EDSs. In Japan, most medical professionals are unfamiliar with this disease.

EDSs^{1,2}

The EDSs are characterized by abnormalities of the molecules that configure the extracellular matrix, such as collagen, or its modifying enzymes.

The EDSs occur as heritable diseases that cause hyperextensibility of the skin, hypermobility of the large joints and easy bruising. Although the previous EDS type classification system consisted of numbers assigned to as many as 11 disorders, the new classification consists of 6 types which are given a simple name associated with the main symptoms. The new classification system was developed during a conference at Villefranche in 1997 and drew upon the accumulated clinical experience and advances in molecular genetics to define a classic type, a hypermobility type, a vascular type, a kyphoscoliosis type, an arthrochalasia type, and a dermatosporaxis type of

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Table 1 Classification of Ehlers-Danlos syndromes^{1,2,3}

New classification Villefranche (1997)	Former classification Berlin (1988)	MIM #	Inheritance	Biochemical defects
Classic	I	130000	AD	COL5A1,
	II	130010		COL5A2
Hypermobility	III	130020	AD	Not known
Vascular	IV	130050	AD	COL3A1
Kyphoscoliosis	VI	225400	AR	Lysyl hydroxylase
Arthrochaliasia	VIIA, VIIB	130060	AD	COL1A1, COL1A2
Dermatosporaxis	VIIC	225410	AR	Type I collagen N-peptidase
others	V	305200	XR	
	VIII	130080	AD	
	X	225310	AR	
	XI	147900	AD	

MIM #, the number in the catalog of Mendelian Inheritance in Man
(<http://www.ncbi.nlm.nih.gov/>)

AD, autosomal dominant; AR, autosomal recessive; XR, X-linked recessive

EDS (**Table 1**)³. Each type of EDS differs in regard to the main symptoms, the causative gene and the inheritance pattern. As a result, the genetic heterogeneity of EDS is very strong.

In the diagnosis of EDS, it is important to clarify which type of EDS is present on the basis of the clinical symptoms and family history. When the causative gene has been determined, the protein analysis by cultured skin fibroblasts and gene mutation analysis are helpful for establishing a definitive diagnosis. It is important for each clinical entity of EDS to be considered as a different disease that results from a different causative gene.

Vascular Type of EDS (EDS Type IV)

Clinical Symptoms of Vascular EDS

Vascular EDS is recognized on the basis of 4 main clinical findings, which include the rupture of blood vessels, or of internal organs such as the uterus and intestines, a striking facial appearance, easy bruising, and translucent skin with visible veins^{1,2,4} (**Table 2**). The systemic arteries, which are rich in type III collagen, may undergo dissection, aneurysm, or rupture. An arterial rupture may be preceded by either an aneurysm, arteriovenous fistula, or a dissection but may also occur spontaneously^{4,5}. About half of the arterial complications involve the thoracic

or abdominal arteries, and the rest are divided between those in the head and neck and those in the limbs. Sudden death may sometimes occur. Furthermore, in addition to the formation of vascular lesions, ruptures of hollow organs (intestines, pregnant uterus) that are rich in type III collagen, as well as recurrent pneumothorax are characteristic of vascular EDS. Although frequent easy bruising is seen, as in the other EDSs, the hyperextensibility of the skin and the hypermobility of the large joints are unusual, unlike other EDSs. Pepin *et al.*⁴ and Oderich *et al.*⁵ have examined the natural history of vascular EDS in detail. Complications are rare during childhood, but 25% of vascular EDS individuals have one or more complications by 20 years of age, and 80% or more have at least one complication by 40 years of age. The most common cause of death is arterial rupture. Pregnant women with vascular EDS have a mortality rate of 15% due to vascular EDS-related complications during pregnancy.

Mechanisms of Vascular EDS

Vascular EDS is an autosomal dominantly inherited disease caused by a one allele mutation of the *COL3A1* gene coding for type III procollagen, resulting in qualitative or quantitative abnormalities of mature type III collagen protein. Type III

Table 2 Diagnostic criteria for vascular EDS³

The combination of any two of the major diagnostic criteria should have a high specificity for vascular EDS; further testing is strongly recommended to confirm the diagnosis. The presence of one or more minor criteria supports the diagnosis of vascular EDS but is not sufficient to establish the diagnosis.

Major diagnostic criteria

- Arterial rupture
- Intestinal rupture
- Uterine rupture during pregnancy
- Family history of the vascular type of EDS

Minor diagnostic criteria

- Thin, translucent skin (especially noticeable on the chest/abdomen)
- Easy bruising (spontaneous or with minimal trauma)
- Characteristic facial appearance (thin lips and philtrum, small chin, thin nose, large eyes)
- Acrogeria (an aged appearance to the extremities, particularly the hands)
- Hypermobility of small joints
- Tendon/muscle rupture
- Early-onset varicose veins
- Arteriovenous carotid-cavernous sinus fistula
- Pneumothorax/pneumohemothorax
- Chronic joint subluxations/dislocations
- Congenital dislocation of the hips
- Talipes equinovarus (clubfoot)
- Gingival recession

procollagen protein consists of 343 repetitions of glycine (Gly)-X-Y (X and Y are other amino acids) in each of 3 amino acids which is a feature of the collagen specificity. The mature type III collagen fibers consist of a helix with 3 alpha type III collagen chains. In vascular EDS, with most of the gene mutations being of a one allele of *COL3A1*, the dominant negative effect is produced; therefore the mature normal type III collagen proteins which are not produced in half amounts, but decrease to one-eighth in their usual number (**Fig. 1**)^{2,4,6}. In vascular EDS, the symptoms also develop as a result of the normal *COL3A1* protein being reduced by half (haploinsufficiency), as has become clear from a study⁷ on some mutations of *COL3A1*. Although vascular EDS is inherited in an autosomal dominant manner, half of the affected individuals have no family members affected with vascular EDS⁴⁻⁶.

Recently, mutations of the genes encoding type I or II transforming growth factor beta receptor (*TGFBR1/2*), which are two of the causative genes of Marfan syndrome⁸, were found in the individuals who had clinical features of vascular EDS, but lacked the *COL3A1* mutation^{9,10} (sometimes termed

the Loey-Dietz syndrome type II). At present, the involvement of these genes in the familial aortic aneurysm syndromes, in which clinical findings are very similar, have not been clarified, although the involvement of multiple genes is considered to be the cause.

Diagnosis and Gene Testing for Vascular EDS

Vascular EDS often shows aortic dissection or aneurysms^{11,12}. The clinical entities caused by autosomal dominant forms can also reveal the mode of inheritance from family histories and pedigrees. When family histories are taken, consideration must be given to whether any family members who have died suddenly. Marfan syndrome should be considered if the presenting vascular complication is an aortic aneurysm or dissection. Vascular EDS and Marfan syndrome can be distinguished relatively easily on the basis of the findings of a physical examination. Individuals with Marfan syndrome typically have arachnodactyly, lens dislocation, and dilatation or aneurysm of only the aorta¹². A clinical diagnosis of Marfan syndrome is based on the family history and the observation of the characteristic

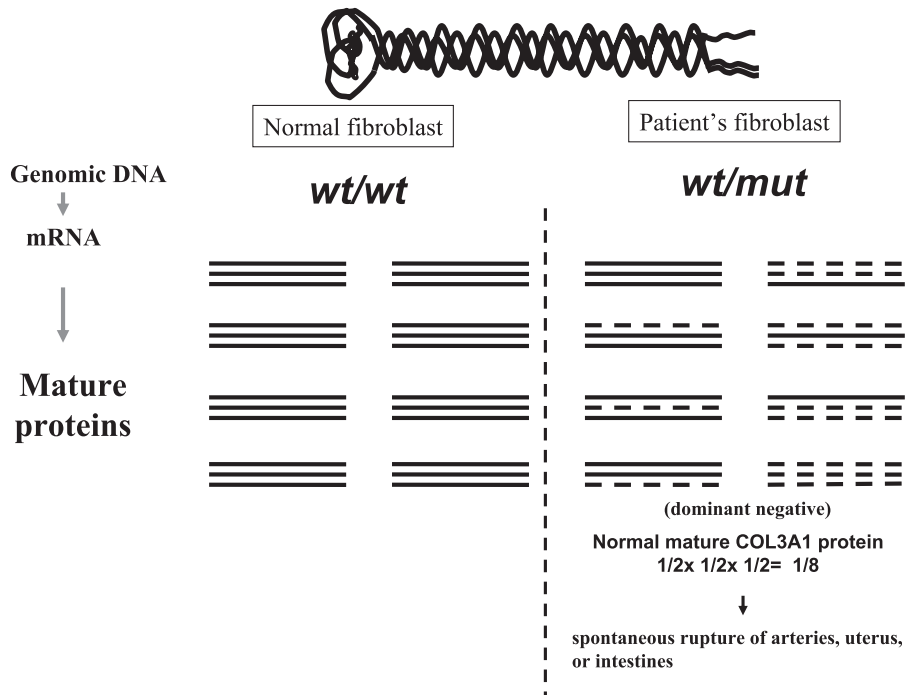


Fig. 1 Mechanisms of vascular EDS. The mature type III collagen fibers consist of a helix with three alpha type III collagen chains. In vascular EDS, with most of the gene mutations of a one allele of *COL3A1*, the dominant negative effect is produced, so the mature normal type III collagen proteins decrease to one-eighth of their usual number.

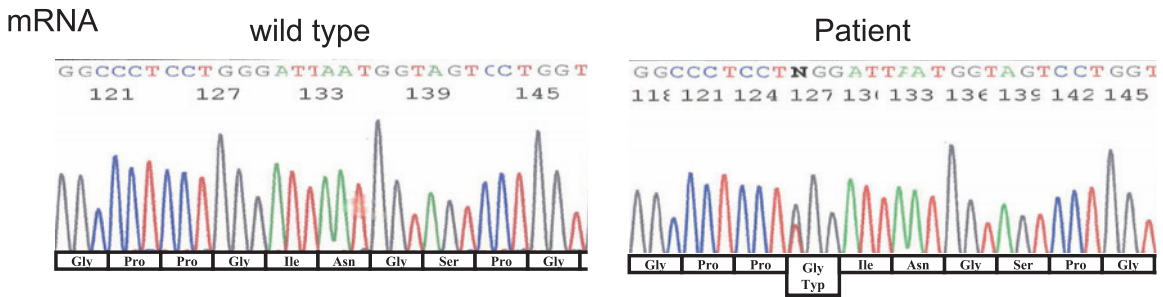
findings in multiple organ systems. Furthermore, in Japan, because most health professionals are not familiar with vascular EDS, confusion has occurred in some cases, for example, it is considered to be as the classic type of EDS.

To confirm the diagnosis of vascular EDS, a reduction in the amount of type III collagen protein or the identification of a *COL3A1* gene mutation is needed. However, the collagen reduction in a protein level determination is sometimes difficult to evaluate^{6,13}. Pepin *et al.*⁴ have demonstrated that the early and definitive diagnosis of the disease should also be considered to facilitate the early recognition and treatment of complications. More than 100 mutations of the *COL3A1* gene have been reported, and the mutation positions of the *COL3A1* gene are assumed to be various and without a "hot-spot" of mutation⁴. There are two types of mutation of the *COL3A1* gene. About two-thirds of the identified mutations of the *COL3A1* gene result in the substitution of other amino acids for glycine residues in the [Gly-X-Y]₃₄₃ triplets of the triple helical domain of the gene. The remaining mutations affect splice

sites in the *COL3A1* gene with a marked preference for the 5' (donor) splice-site¹⁴ in an intron. The different types of clinical complications have not been found to be associated with specific mutations in the *COL3A1* gene² (Fig. 2).

We previously reported the first genetically confirmed case of vascular EDS in Japan¹⁵. We have established a *COL3A1* gene diagnosis technique to identify the mutations of the *COL3A1* gene in Japan⁶. Because the *COL3A1* gene consists of 52 exons, total RNA is extracted from the skin fibroblasts of the affected individuals. Reverse transcriptase polymerase chain reaction (RT-PCR) amplification for the triple-helical domain of the *COL3A1* gene covering 3.8 kb is performed and direct sequencing is done, allowing the identification of gene mutations. Concerning a correlation with the specific mutation position of the *COL3A1* gene, and the severity of the vascular EDS, our results in Japan⁶ were not different from the previous studies^{4,5} (Table 3). However, unlike previous reports^{4,5}, our study described many individuals with a *COL3A1* mutation had pneumothorax⁶. We have considered adding

1) Substitutional mutation in Gly residues



2) Splicing mutation

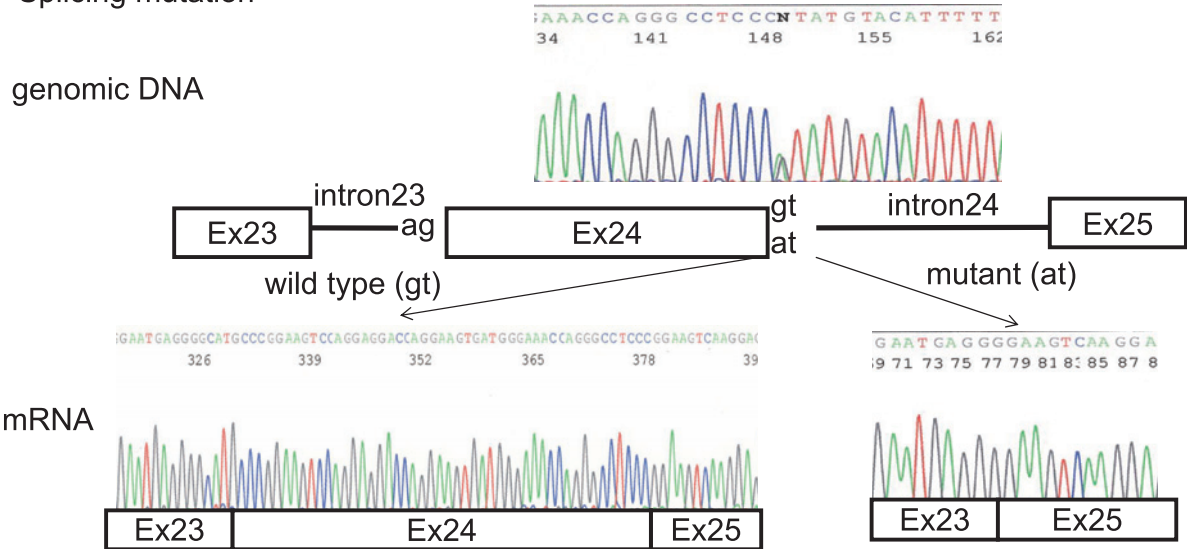


Fig. 2 Schematic representation of the two types of COL3A1 gene mutation

- (A) A one base substitution in an exon of the triple helical domain of the COL3A1 gene causes other amino acids to replace glycine residues in the [Gly-X-Y]₃₄₃ triplets.
- (B) Mutations in splice sites of the COL3A1 gene cause in the 5' (donor) splice-site. The exon-intron structure of COL3A1 with acceptor (ag) and donor (gt) splice sites in introns adjacent to exons. When there is a one base substitution in splice donor site (gt → at) of intron 24 in genomic DNA, skipping of an exon (exon24) consequently occurs and leads to fusion of the former exon (exon23) and the next exon (exon25) in mutant mRNA.

vascular EDS to the differential diagnosis of patients with a history of pneumothorax in adolescence, or those with the repeated pneumothorax with a family history of vascular EDS. Recently, vascular EDS has been diagnosed at the stage when pneumothorax has occurred¹⁶; the importance of which is evident in allowing the careful surveillance for, and perhaps the prevention of, unrevealed vascular complications.

Management and Genetic Medicine for Vascular EDS

At present, the treatment of individuals with vascular EDS and a COL3A1 mutation consists only of symptomatic therapy and the early detection of complications. Furthermore, in vascular EDS, as the

blood vessels are vulnerable because of the effects of the abnormal type III collagen, a corresponding prudence in angiographic or surgical technique is called for⁴. Surgical intervention for bowel rupture is necessary and is usually lifesaving. The complications during and after surgery are related to tissue and vessel friability, which results in recurrent arterial or bowel tears, fistulae, poor wound healing, and suture dehiscence. Individuals with vascular EDS and a TGFBR1/2 mutation have a much lower incidence (5%) of fatal complications from vascular surgery, despite the similarity of these findings to those of individuals with COL3A1 mutations^{9,17}.

Pregnant women with vascular EDS require

Table 3 Summary of clinical features of the vascular EDS individuals in Japan

	Age at first complication	thin skin	Complications			Family History of sudden death	Defects in COL3A1	
			Arterial dissection or rupture	Bowel rupture	Pneumothorax		Base substitution	Splicing mutation
Our cases ⁶ 9 cases	26.3	87%	87%	44%	77%	44%	67% Gly → Trp Gly → Glu Gly → Asp (3) Gly → Ser	33%
Washington Univ ⁴ 220 cases	23.5	ND	79%	8%	ND	48%		
Mayo Clinic ⁵ 31 cases	28.5	77%		84%	16%	58%		

All cases of vascular EDS in our study⁶ were diagnosed with mutation analysis of the *COL3A1* gene. The study in Washington Univ⁴ is evaluated by protein level determination of *COL3A1*. The study in Mayo Clinic⁵ is evaluated from clinical diagnostic criteria; the diagnosis in 77% of individuals vascular EDS was confirmed with protein level determination.

careful monitoring during the antenatal period, with particular attention to the possibility of arterial ruptures or rupture of the uterus. When the first complication of vascular EDS is treated, rupture may occur again⁴. The timing and site of a repeat rupture cannot be predicted by the first event. The surveillance may include periodic arterial screening through venous subtraction angiography and computed tomography¹⁸, magnetic resonance¹⁹, or multi-detector computed tomography (MDCT)²⁰. However, arteriography is not recommended because of the risk of vascular injury⁴. The EDS Foundation in the United States, which is an association of individuals with EDS created an informative data source, the Emergency Physician's Reference of Vascular EDS, intended for physicians and based on the findings of the pertinent literature²¹. Attention has been directed to the emergency response to this disease, as promoted by these educational campaigns, and the importance of an organized correspondence effort, has also been established. The symptoms of vascular EDS develop suddenly in many cases, and the establishment of emergency medical systems that recognize this possibility is of the utmost importance.

When the diagnosis of vascular EDS has been established, the development of an effective medical follow-up system with an awareness of the potential

complications is essential. The effective monitoring of the complications of vascular EDS, including the prevention of sudden death, is of primary importance both for those individuals affected with the disease, and for their family members. Unfortunately, in vascular EDS the symptoms may appear without warning and sudden death may occur. There is no effective treatment, at present, for these catastrophic events. Another factor making appropriate care difficult is that individuals with vascular EDS are treated in various departments of a hospital and some caregivers are not familiar with this disease. In Europe and the United States, an organized effort is underway to provide education about vascular EDS to improve the early detection of complications following diagnosis, and to develop an effective network for communicating new information.

However, in Japan, many health professionals are not familiar with vascular EDS, which is sometimes mistaken for the classic type of EDS⁶. Delays in the diagnosis of vascular EDS remain common, even when the clinical features are typical, which may lead to inadequate or inappropriate treatment and management, as occurs with many rare diseases. Moreover, 50% of the children of the person with vascular EDS will have a mutant gene due to the dominant mode of inheritance even if there is no

family history of vascular EDS. Because the predicted penetration rate of the disease is high, the siblings of an individual with vascular EDS as well as his/her children have a 50% risk of vascular EDS and may show the symptoms of the disease because it tends to occur in adulthood. When a family is faced with new information concerning a positive genetic test for vascular EDS, it is crucial that follow-up care include comprehensive genetic counseling⁴. As mentioned above, the genetic testing of the persons suspected of having vascular EDS should be performed not only for the medical reasons, but also for genetic counseling^{22,23}. When genetic testing for vascular EDS is performed, the purpose should be understood as should the ethical considerations relating to presymptomatic diagnosis and the implications for the children or of the individuals with vascular EDS. The situations will differ in each case. When the diagnosis of vascular EDS is under investigation, we find it best to provide genetic counseling and psychological support for the individuals with vascular EDS and their families before genetic testing. In our hospital, the genetic testing of *COL3A1* is performed following a clinical genetic conference in the Division of Clinical Genetics²². Furthermore, as in the example in which the clinical findings and the responsible genes overlap Marfan syndrome^{6,7}, vascular EDS is gaining in recognition in recent years. In the case of familial aortic aneurysms, it is necessary to systematically examine the genes of not only *COL3A1*, but also of *TGFBR1* and 2; hence, a diagnostic paradigm is needed.

Research into Potential Treatments for Vascular EDS

There is still no effective treatment to prevent the complications associated with vascular EDS. Although gene therapy is an important option for the treatment of genetic disorders, defective gene cannot be replaced in dominantly inherited diseases such as vascular EDS. The recently developed RNA interference (RNAi) technology, which enables gene posttranscriptional expression silencing, may be applicable. Research into the treatment approach for the dominantly inherited diseases, for which cures

have been not yet been developed is advancing²⁴. We are now examining the possibility of gene therapy for vascular EDS through research on using previously untried techniques. Using *in vitro* research, the discovery of a control effect was parsed onto a unique target with RNAi to the *COL3A1* mutation allele using the vascular EDS model fibroblasts. The normal mRNA was not affected within vascular EDS fibroblasts, but it was found that the mutant mRNA could be controlled specifically. It is anticipated that this technique will provide a future cure for individuals with vascular EDS, and may enable research into the treatment of other autosomal dominantly inherited diseases for which do not have an effective treatment. Further study of these clinical applications is thus required.

The Future Medical Approaches to Vascular EDS

The clinical awareness and timely diagnosis of vascular EDS remains inadequate, as the disease is often diagnosed only after life-threatening complications or death. Although vascular EDS is a rare disease, physicians should be aware of the existence of vascular EDS to improve the prognosis and the quality of life for individuals for vascular EDS⁴⁶. The increased appreciation of the risks and complications of vascular EDS necessitates consistent follow-up. The complications of vascular EDS require hospitalization and consultation with medical professionals in various fields: surgeons, internists, radiologists, obstetricians, and clinical geneticists. While vascular EDS is of particular importance to these specialists, there is currently no specific treatment for the underlying condition; however, knowledge of the diagnosis may help in the management of visceral complications, pregnancy, and genetic counseling^{6,22}. Therefore, a network of the medical specialists, including clinical geneticists needs to be established after the genetic diagnosis of the *COL3A1* mutation to perform genetic counseling and to provide a system of long-term follow-up for individuals with vascular EDS.

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