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SHORT COMMUNICATION

A novel point mutation in type III collagen gene resulting in exon 24 skipping in a case of vascular type Ehlers-Danlos syndrome

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Abstract A novel point mutation in a patient with vascular type Ehlers-Danlos syndrome is herein reported. The patient experienced pneumothorax and multiple arterial troubles during the past 10 years. The patient's hand skin was atrophic, and a paper-like atrophic scar was seen. The collagen microfibrils from the patient's skin demonstrated irregular contour, electron density variegation, and smaller, irregular fibril diameters. The patient's fibroblasts produced apparently much less type III collagen comparing with control. A skipping of exon 24-originated sequences of the patient's mRNA of type III collagen was proved. A single base pair mutation of thymine to guanine at the second position of the intron 24 of *COL3A1* was finally found. This is the first report of the mutation of the second position of exon 24 of *COL3A1*.

Keywords Ehlers-Danlos syndrome · Type III collagen · Exon skipping

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Introduction

Ehlers-Danlos (EDS) syndrome is a group of diseases demonstrating the abnormalities in the connective tissue, its central symptoms being hyperelasticity, fragility of the skin, and joint hypermobility [3]. Vascular EDS [2, 16], a disease characterized by hollow organ troubles, i.e., arterial aneurysm, bowel rupture, and pneumothorax, in addition to the skin symptoms, is one among EDS. The cause of vascular EDS is attributed to the abnormality in the type III collagen [4], which leads to impaired extracellular matrix (ECM) structure formation, and finally results in labile hollow organs. The major genetic abnormality of type III collagen is a point mutation and an exon skipping, which contribute to most of the cases [10]. To date, a number of molecular abnormalities of type III collagen is summarized in the literature, but the information is somewhat limited [9, 12]. Thus, among all population of vascular EDS, it is supposed that the genetic and molecular abnormalities are determined in only a small population of the patients. Therefore, the accumulation of information about molecular abnormalities would be still important.

In this report, a case of vascular EDS patient is presented. In this patient, the abnormality was found to be a skipping of exon 24, which is originated from a one base pair mutation of intron 24.

Case report

A 38-year-old male experienced pneumothorax in 1990, left renal infarction due to dissecting aneurysm of left renal artery in 1999, right renal infarction due to dissecting aneurysm of right renal artery in 2005 [1], and then pseudoaneurysm of splenic artery, left gastric artery, and intramuscular

hemorrhage of the left thigh in 2006. The patient did not have any family history of the arterial trouble. Because of the repetitive arterial trouble, the patient was transferred to our clinic for examination of vascular EDS.

Upon consultation, the patient's hand skin was atrophic, and cutaneous veins were easily visible (Fig. 1a, b). The patient had hypermobilities in his wrist and finger joints (Fig. 1c, d). In the left axillary area, atrophic scars were present (Fig. 1e). An angiogram of renal infarction and a dissecting aneurysm of right renal artery is shown in Fig. 1f. These clinical features fulfilled three major and minor criteria [2], and the patient was thus clinically diagnosed to have vascular EDS. A skin biopsy was done from the patient's forearm. Upon Elastica van Gieson stain, elastic fibers of the patient did not show significant abnormality compared with normal ones (Fig. 1g, h).

The patient's collagen shows abnormal morphology and composition

An electron microscopic observation of the patient's skin demonstrated collagen microfibrils with smaller diameters, irregular contours, and uneven electron densities, compared with those of control (Fig. 2a, b). The collagen microfibril diameter of control was 91.89 ± 9.44 nm (mean ± SD); in contrast, in the patient, the distribution of the diameters was biphasic with the mean ± SD of 76.33 ± 12.77 nm (Fig. 2c).

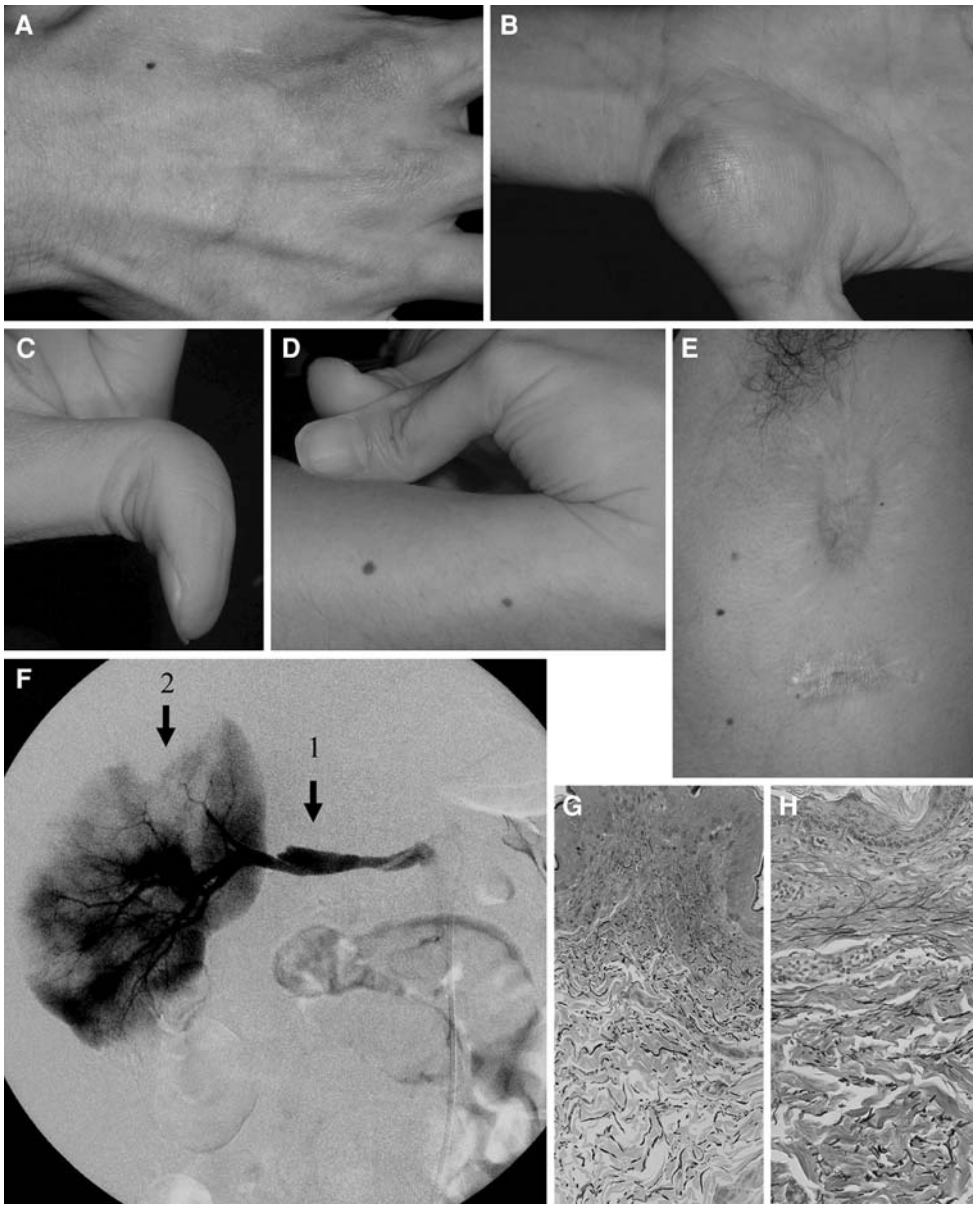

Fig. 1 Clinical features of the patient. **a** and **b** Appearance of the patient's skin. **a** Dorsal aspect; **b** Ventral aspect. Note that the patient's skin is apparently thin and small cutaneous veins are clearly visible. **c**, **d** Hypermobilities of the patient's joints. **c** Interpharangeal joint of the patient's thumb can be bent back by nearly 90°. **d** Thumb sign. The patient can touch his forearm with his thumb of the same side. **e** Paper-like atrophic scar on the left axillary area. These scars were formed at the insertion points of tubes for treatment of pneumothorax in 1990. **f** Arterial phase of renoangiogram representing a dissecting aneurysm in the right renal artery (*arrow 1*) and a wedge-shaped renal infarction on the renal apex (*arrow 2*). **g** Elastica van Gieson stain of the skin specimen of the patient. In the patient, elastic fibers were stained equally dark as the control. **h** The same stain of control specimen

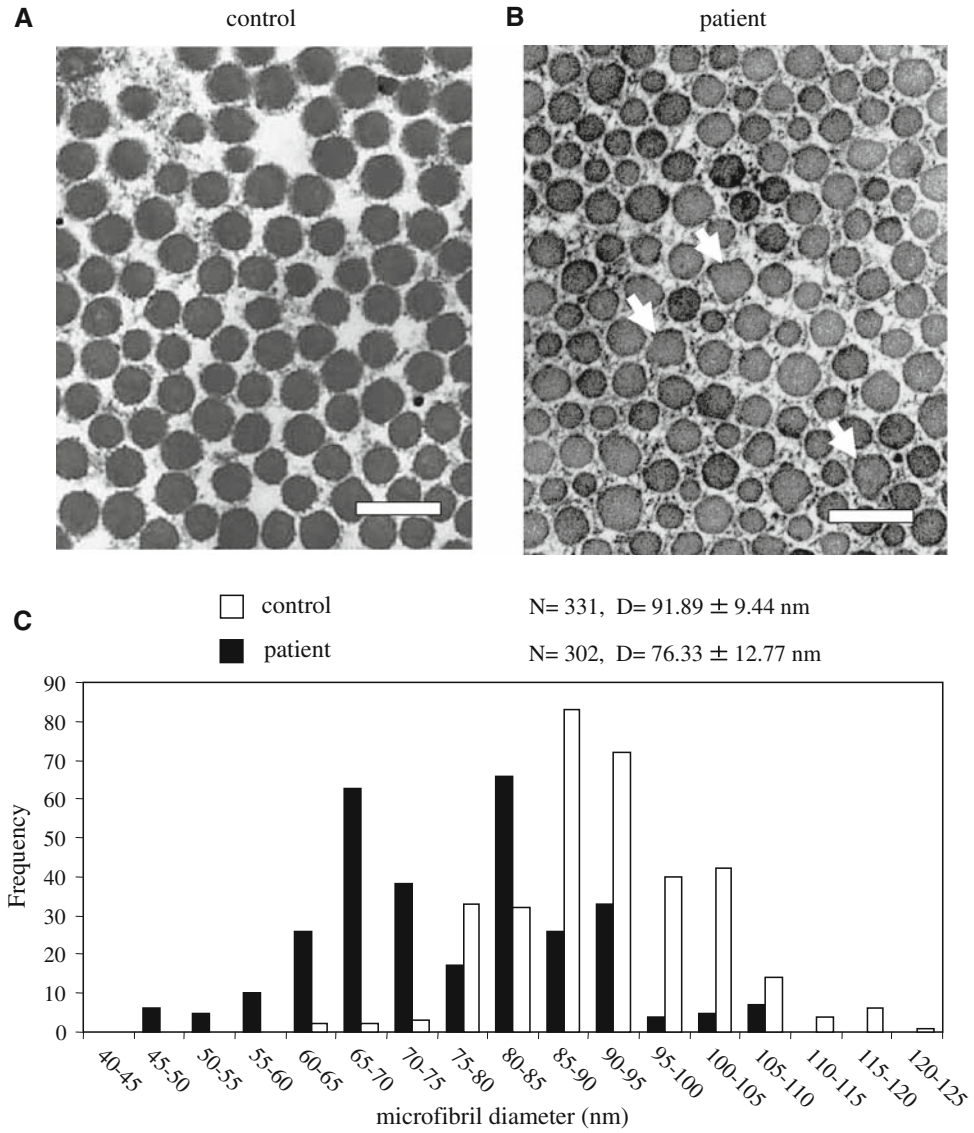

Fig. 2 Electron microscopic view of collagen microfibrils in the dermis of control individual (**a**) and from patient of our case (**b**). *White arrows* indicate the collagen microfibrils with irregular contours. *Bars* indicate 200 nm. **c** Histograms of collagen microfibril diameter. *Empty bars* represent that of normal individual; *filled bar*: that of the patient of our case. Average microfibril diameters ± SD are shown on the top of the panel

Analysis using ^{3}H-proline-labeled collagens from fibroblasts revealed that the position and the amount of α1 (I) and α2 (I) chains from both control and the patient did not demonstrate any evident difference; however, the amount of both trimeric and monomeric α1 (III) chains of the patient were greatly decreased, compared with that of the control (Fig. 3a).

The patient's *COL3A1* showed a novel mutation

Total RNA from cultured fibroblasts was subjected to reverse-transcriptase PCR (RT-PCR) with primers which correspond to 5′ untranslated region and 3′ terminal of *COL3A1* mRNA, respectively. The RT-PCR product was either directly sequenced, or cloned into a vector, and then the sequencing was done [13, 18]. Using the direct sequencing method, the sequence could not be determined in progress around the exon 24 of type III collagen. However, it could be finally read when the RT-PCR products were cloned into a vector, namely sequencing based on PCR cloning of the RT-PCR product [13]. Then a genomic PCR using a panel of primers around exon 24 of type III collagen revealed a single base mutation from T to G at the second position in the intron 24 (Fig. 3b). The mutation in the second position of exon 24 was the first case ever reported, thus, we identified this mutation as a novel one.

Our case fulfilled enough diagnostic criteria for vascular EDS proposed in 1997 [2] and the diagnosis was confirmed by the molecular and genomic analysis. Cutis laxa would be listed as a possible differential diagnosis. Our case lacked flabby, rubber-like skin and no significant morphological abnormality of elastic fiber was observed. From these findings, the diagnosis of cutis laxa would be negative in our case.

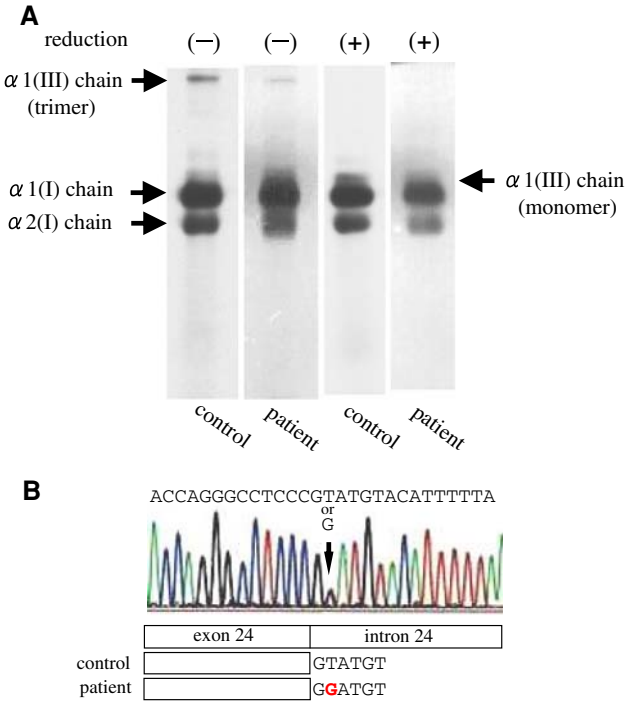

Fig. 3 **a** Analysis of ^{3}H proline-labeled collagens on SDS-PAGE [17]. Images of pepsin-treated samples are shown. The presence and absence of reduction by 2-mercaptoethanol is indicated on the top of the panel. The trimeric α1 (III) chain disappears after reduction and it shifts to the position just above the monomeric α1 (I) chain. **b** Sequence analysis of PCR product of exon 24-intron 24 junction of the patient's genome. Mutated nucleotide is indicated by an *arrow* in the upper panel, exon 24-intron 24 junction is expressed in the upper bar, and difference of genomic sequences at the beginning of intron 24 in the control and patient's genomes are contrasted in the middle and bottom bar. The first six sequences of the intron 24 are shown

A mutation in a single allele of *COL3A1* can reduce the production of normal molecules to one eighth [8]. The final amount of type III collagen in the ECM decreases because of the decay of unstable abnormal mRNA, malsecretion or poor incorporation of remaining seven eighths of abnormal molecule into the ECM [5–7, 14]. Even if there is no abnormality in the type III collagen molecule, homozygous as well as heterozygous defect of the collagen leads vascular EDS phenotypes [11, 14], indicating that merely a decrease of the amount of type III collagen leads to vascular EDS. Our case lacked exon 24 (54 bp) of *COL3A1*, which corresponds to a deletion of 18 amino acids in the second quarter of type III collagen molecule. It is feasible that the final amount of type III collagen incorporated in the collagen microfibrils in our case was also less, or varied. Irregular contours, variable diameter distribution, and variegation of electron densities of collagen microfibrils of our case might reflect this speculation.

As the cause of vascular EDS, point mutations of type III collagen comprise two-thirds of reported cases, and exon skipping follows, which accounts for the rest about one third of the cases [10]. As the cause of exon skipping, Schwartze et al. [13] reported that within 42 analyzed cases of EDS with exon skipping, 37 cases had one base mutation within intron. At the beginning of one intron in pre-mRNA, acceptor sequences for splicing exist. Mutations within this region disrupt the acceptor site, and therefore, splicing in the exon- intron junction does not occur. Instead, an acceptor site of the preceding intron is spliced [13]; the mutation in our case fits this model. The mutation of the first base of the intron sequence is the most common, and then the mutations in the second or the fifth base of the intron are frequent [13]. Until now, mutation in the fourth base of the intron has not been reported. Concerning exon 24 skipping, all the known cases had single base mutation of G to A in the first position of intron and therefore, the mutation from T to G in the second position of intron 24 in type III collagen is the first case ever reported.

Concerning the formation of impaired ECM in vascular EDS, information of the relationship between the location of mutation within type III collagen molecule and the dermal collagen morphology is still limited [15]. Precise analysis based on the accumulation of biochemical as well as morphological information is awaited.

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References

1. Ando T, Ohno H, Hirata Y et al (2005) Spontaneous recovery from renal infarction resulting from renal artery dissection. Int J Urol 12:405–408
2. Beighton P, De Paepe A, Steinmann B, Tsipouras P, Wenstrup RJ (1998) Ehlers-Danlos syndromes: revised nosology, Villefranche, 1997. Ehlers-Danlos National Foundation (USA) and Ehlers-Danlos Support Group (UK). Am J Med Genet 77:31–37
3. Burton JL (1992) Disorders of connective tissue. In: Champion RH, Burton JL, Ebling FJG (eds) Textbook of dermatology. Blackwell, Oxford, pp 1791–1797
4. Byers PH (1995) Ehlers-Danlos syndrome type IV: a genetic disorder in many guises. J Invest Dermatol 105:311–313
5. Byers PH, Holbrook KA, Barsh GS, Smith LT, Bornstein P (1981) Altered secretion of type III procollagen in a form of type IV Ehlers-Danlos syndrome. Biochemical studies in cultured fibroblasts. Lab Invest 44:336–341
6. Chiodo AA, Sillence DO, Cole WG, Bateman JF (1995) Abnormal type III collagen produced by an exon-17-skipping mutation of the *COL3A1* gene in Ehlers-Danlos syndrome type IV is not incorporated into the extracellular matrix. Biochem J 311:939–943
7. Cole WG, Chiodo AA, Lamande SR et al (1990) A base substitution at a splice site in the *COL3A1* gene causes exon skipping and generates abnormal type III procollagen in a patient with Ehlers-Danlos syndrome type IV. J Biol Chem 265:17070–17077
8. Dalgleish R (1998) The human collagen mutation database. Nucleic Acids Res 26:253–255
9. Kuivaniemi H, Tromp G, Bergfeld WF, Kay M, Helm TN (1995) Ehlers-Danlos syndrome type IV: a single base substitution of the

last nucleotide of exon 34 in *COL3A1* leads to exon skipping. J Invest Dermatol 105:352–356
10. Kuivaniemi H, Tromp G, Prockop DJ (1997) Mutations in fibrillar collagens (types I, II, III, and XI), fibril-associated collagen (type IX), and network-forming collagen (type X) cause a spectrum of diseases of bone, cartilage, and blood vessels. Hum Mutat 9:300–315
11. Liu X, Wu H, Byrne M et al (1997) Type III collagen is crucial for collagen I fibrillogenesis and for normal cardiovascular development. Proc Natl Acad Sci USA 94:1852–1856
12. Pepin M, Schwarze U, Superti-Furga A, Byers PH (2000) Clinical and genetic features of Ehlers-Danlos syndrome type IV, the vascular type. N Engl J Med 342:673–680
13. Schwarze U, Goldstein JA, Byers PH (1997) Splicing defects in the *COL3A1* gene: marked preference for 5′ (donor) spice-site mutations in patients with exon-skipping mutations and Ehlers-Danlos syndrome type IV. Am J Hum Genet 61:1276–1286
14. Schwarze U, Schievink WI, Petty E et al (2001) Haploinsufficiency for one *COL3A1* allele of type III procollagen results in a phenotype similar to the vascular form of Ehlers-Danlos syndrome, Ehlers-Danlos syndrome type IV. Am J Hum Genet 69:989–1001
15. Smith LT, Schwarze U, Goldstein J, Byers PH (1997) Mutations in the *COL3A1* gene result in the Ehlers-Danlos syndrome type IV and alterations in the size and distribution of the major collagen fibrils of the dermis. J Invest Dermatol 108:241–247
16. Steinmann B, Superti-Furga A, Royce PM (1993) The Ehlers-Danlos syndrome. In: Royce PM, Steinmann B (eds) Connective tissue and its heritable disorders: molecular, genetic and medical aspects. Wiley-Liss, New York, pp 351–407
17. Tanaka S (1989) SDS-polyacrylamide gel electrophoresis (SDS-PAGE). In: Nagai Y, Fujimoto D (eds) Experimental method for collagens. Kodansha, Tokyo, pp 63–77
18. Watanabe A, Kosho T, Wada T et al (2007) Genetic aspects of the vascular type of Ehlers-Danlos syndrome (vEDS, EDS IV) in Japan. Circul J 71:261–265