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SURGERY TODAY

Case Report

Sigmoid Colon Perforation Induced by the Vascular Type of Ehlers–Danlos Syndrome: Report of a Case

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Abstract
The vascular type of Ehlers–Danlos syndrome (vEDS) is a rare inherited disease of the connective tissues, and is caused by abnormal type III collagen resulting from heterogeneous mutations of the type III collagen *COL3A1* gene. We herein report the case of a vEDS patient who developed a sigmoid colon perforation and was given a definitive diagnosis by a genetic and biomolecular assay. The patient demonstrated clinical manifestations caused by tissue weakness such as frequent pneumothorax events and a detached retina. During the operation, we noticed easy bruising and thin skin with visible veins on the patient's abdominal wall. Finally, a diagnosis was confirmed by the reduction of type III collagen synthesis and by the identification of a mutation in the gene for type III collagen. We conclude that it is difficult to diagnose a vEDS patient without clinical experiences and specialized genetic methods. Furthermore, all organs must be treated gently during therapy, because the tissues of vEDS patients are extremely fragile.

Key words Ehlers–Danlos syndrome · Vascular type · Perforation · Type III collagen · *COL3A1*

Introduction

The vascular type of Ehlers–Danlos syndrome (vEDS, Ehlers–Danlos syndrome type IV) is a rare, autosomal dominant disease of the connective tissues caused by abnormal type III collagen resulting from heterogeneous mutations of the type III collagen *COL3A1* gene.[1–4] vEDS is characterized by four clinical criteria: easy bruising, thin skin with visible veins, characteristic facial features, and the rupturing of arteries and organs.[1–4] In addition, classic EDS patients exhibit hypermobility of the large joints and hyperextensibility of the skin.[1,2] Typically, Ehlers–Danlos syndrome (EDS) is divided into six types, and vEDS patients follow a particularly poor clinical course caused by complications from tissue weakness.[1,2] Twenty-five percent of vEDS patients develop one or more complications associated with tissue weakness by 20 years of age, and 80% develop some complications by 40 years. Pepin et al. reported that the calculated median survival time of vEDS patients was 48 years of age.[1] We herein present a case report of a vEDS patient who was clinically and genetically diagnosed following a sigmoid colon perforation, and review the pertinent literature.

Case Report

A 20-year-old male patient was admitted to our hospital with severe abdominal pain. The patient's abdominal wall was very hard, and muscular guarding was palpated. Enhanced computed tomography was performed immediately, and revealed free air and stool containing barium in the abdominal cavity, because the patient's colon had been examined 2 days prior for causal ascites and abdominal pain by a barium enema (Fig. 1A,B). As soon as we diagnosed the patient with generalized peritonitis due to colon perforation, an emergency operation was performed. During the operation, the patient's abdominal skin was observed to be markedly thin, with visible veins. After we decided that the sigmoid colon perforation was the cause of the generalized peritonitis, the lesion was removed and Hartmann's procedure was performed. In addition, it was revealed that the patient suffered from frequent spontaneous pneumothorax events in the past, and that his creatine kinase levels

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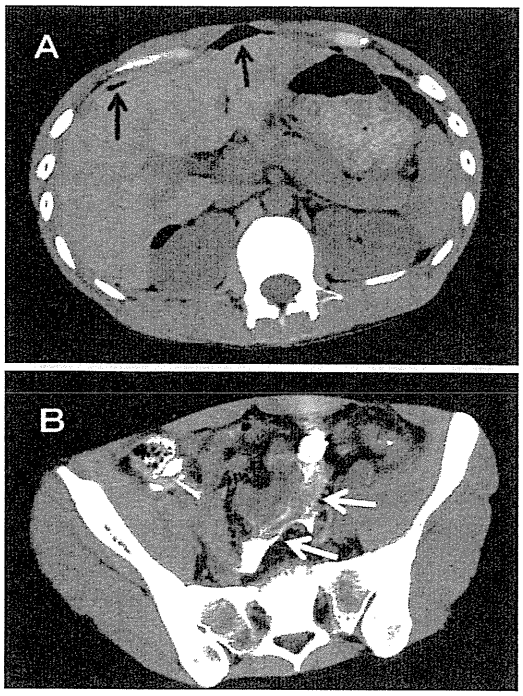

Fig. 1A,B. Enhanced computed tomography at the sigmoid colon perforation. Free air (*black arrows*) and stool containing barium (*white arrows*) were observed in the abdominal cavity

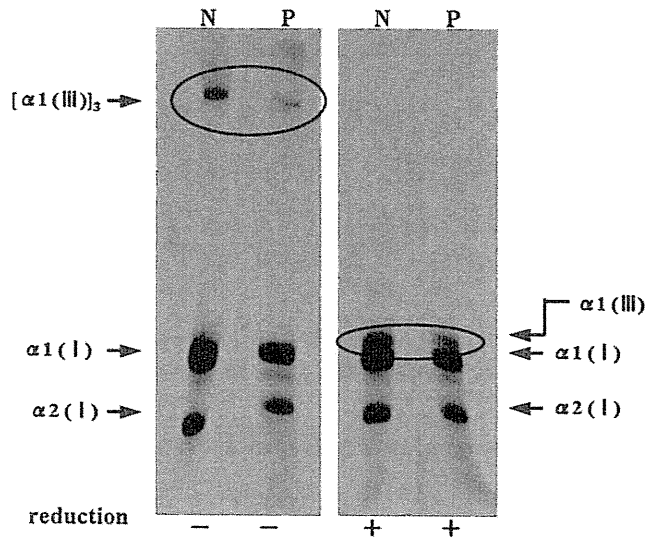

Fig. 2. Production of type I or type III collagen in the patient's cultured fibroblasts. The synthesis of type I collagen in this patient was the same as in controls. However, the synthesis of type III collagen was reduced by approximately 22.7% compared with the normal control values (*inside circle*). *N*, normal control; *P*, patient with vascular type of Ehlers–Danlos syndrome

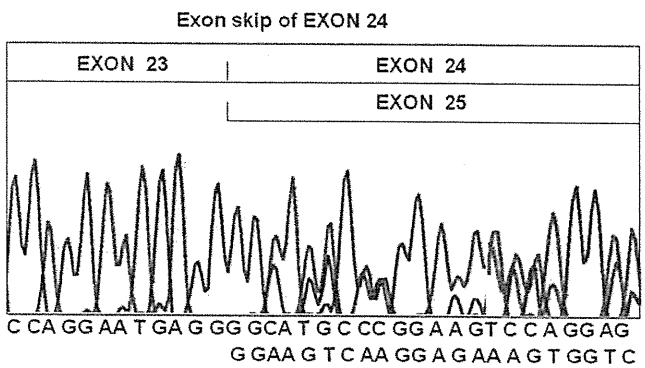

Fig. 3. Genetic analysis of the complementary DNA from the patient's cultured fibroblasts. A skip in exon 24 of *COL3A1*, which encodes collagen type III, was identified by the genetic analyses of the complementary DNA from the cultured fibroblasts

were increased to severalfold higher than in normal subjects. Although a paralytic ileus developed as a complication, the patient was discharged from our hospital 1 month after the operation. However, 3 days after discharge, he was readmitted due to his eighth spontaneous pneumothorax and a detached retina in his left eye. Because many complications caused by tissue weakness had developed over such a short period, very rare vEDS was diagnosed according to the four clinical criteria: easy bruising, thin skin with visible veins, characteristic facial features, and rupture of the arteries and organs.

To confirm this diagnosis, the patient's skin and blood samples were sent to Dokkyo Medical University, and were examined by genetic and molecular biological assays. Accordingly, the diagnosis of vEDS was confirmed by the reduction of type III collagen synthesis in cultured skin fibroblasts and by the identification of a mutation in the gene for type III collagen (*COL3A1*). The synthesis of type I collagen in this patient was the same as in controls. However, the synthesis of type III collagen was reduced by approximately 22.7% compared with normal controls (Fig. 2). A skip in exon 24 of *COL3A1*, which codes for collagen type III, was identified by genetic analysis of the complementary DNA from cultured fibroblasts (Fig. 3). Furthermore, the region near the genomic DNA was amplified by polymerase chain reaction (PCR) for the analyses of genomic DNA; the result revealed a G-to-A transition at the donor splice-site +1 of intron 24 (IVS 24 G+1 to A) of the *COL3A1* gene (Fig. 4). Because the mother of the present patient also demonstrated characteristic facial features and easy bruising of the skin, we genetically examined her blood samples to determine the genetic background of this patient. Consequently, we were able to confirm that the mother had the same mutation in *COL3A1* gene.

Less than 6 months after the sigmoid colon perforation, the patient was admitted with a developing

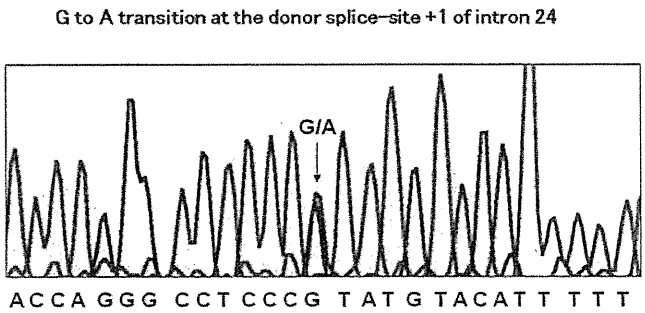

Fig. 4. Sequence analysis of genomic DNA from the patient's blood cells. The region near the genomic DNA was amplified by polymerase chain reaction for the analysis of genomic DNA. The results revealed a G-to-A transition at the donor splice site +1 of intron 24 (IVS 24 G+1 to A) of the *COL3A1* gene

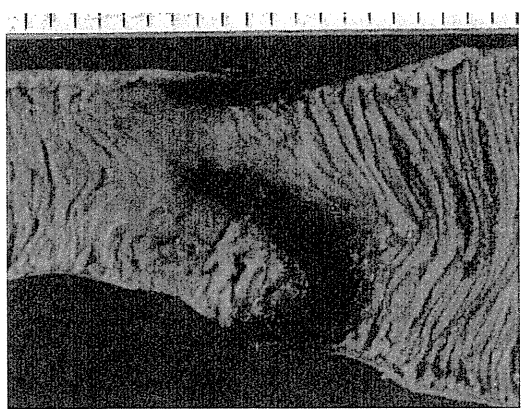

Fig. 5. Resected specimen of the jejunum from the second operation. The seromuscular layer of the patient's jejunum was torn throughout, and the entire layer of the intestine had become partly necrotic

adhesive ileus. Although conservative therapy was appropriated because there was no ischemic change of the intestine at admission, an emergency operation was performed because of the sudden onset of severe abdominal pain, which was not relieved by analgesic drugs. The operative and histopathological findings revealed the seromuscular layer of his jejunum to be torn, thus resulting in partial necrosis of this entire layer of the patient's intestine (Fig. 5). Therefore, we performed a partial resection of the small intestine, and the colostomy was not closed.

Methods of Genetic Examination

Dermal fibroblasts were obtained from the patient's skin and were cultured.[5–7] The protein synthesis of type I and type III collagen were assessed as described previously.[5–8] After RNA was extracted from the cultured fibroblasts, complementary DNA was synthesized by reverse transcription from the RNA as a template. The complementary DNA was amplified by PCR, and analyzed by electrophoresis on polyacrylamide gels to identify the abnormal fragments. Abnormal DNA fragments were directly sequenced by an ABI PRISM 3100 genetic analyzer (ABI Advanced Biotechnologies, Columbia, MD, USA).[2,3,8] Furthermore, genomic DNA was extracted from the blood cells, and all mutations were confirmed in the genomic DNA of *COL3A1* by a sequence analyzer.[2,3,8]

Discussion

Ehlers–Danlos syndrome (EDS) is a rare inherited disease of the connective tissue.[1,2] Most surgeons generally consider EDS to be a dermatologic disease.[1–4] However, patients who are affected by EDS, particularly the vascular EDS type (vEDS), develop complications associated with tissue weakness, and surgical or interventional therapy is often required.[1–4,9–11] Until genetic and biochemical testing was sufficiently developed, a considerable number of patients who died unexpectedly could not be diagnosed as having vEDS. In the present case, the reason for the colonic perforation was unclear after a histopathological examination, and 6 months passed until the diagnosis of vEDS could be made by genetic and biomolecular assays. Even if we suspected the possibility of vEDS based on the patient's clinical symptoms, the genetic and biomolecular assays could not be easily performed in most hospitals. Fortunately, we obtained advice from an authority in genetics and had technical support with the genetic and biomolecular assays. If this patient had not been definitively diagnosed, it is likely that the patient and his family might have lost any hope. Our belief is that a system for diagnosing rare inherited diseases, such as vEDS, should therefore be established as expeditiously as possible in Japan.

In general, most surgeons encounter vEDS patients who are affected by perforative peritonitis and perform surgery by creating an intestinal stoma, because the abdominal cavity is polluted with stool and the patient's tissues are very fragile. Furthermore, the intestinal stoma helps in the management of constipation, which these patients often experience to a severe extent.[1] The existence of an intestinal stoma is also preferable in order to prevent high intestinal pressures. However, patients who receive a colostomy creation are typically frustrated by the limited lifestyle. Therefore, while we understand why a patient may prefer bowel reconstruction, it is difficult to proceed down this path. It is necessary to consider the future of the vEDS patients, as it may be safer not to remove the intestinal stoma to

prevent high intrabowel pressure that causes constipation and adhesive ileus. It is an important to note that complications and tissue weakness increase in vEDS patients after the age of 20 years.[1,2] Several authors have recommended that the perforative lesion and its distal colon should be removed at the same time to prevent reperforation in the sigmoid colon and rectum.[10] Other authors have also recommended a subtotal colectomy as a reasonable treatment because of the high rate of reperforation in vEDS patients.[12] Although these suggestions have validity and are based on a safety-first concept, we were unable to perform a subtotal colectomy for the present vEDS patient at the time of the first operation, when a definitive diagnosis had not yet been determined. Moreover, it is difficult for us to perform both a partial resection of the small intestine and a subtotal colectomy, even at a second operation, because of the risk of short bowel syndrome and anastomotic leakage. It appears that a unique procedure for perforation of the colon in vEDS patients cannot be standardized, because individual patients have widely divergent background factors, such as age, performance status, accuracy of the diagnosis, frequency of perforation, and medical expertise in their country.

We recommend a therapeutic approach for the ileus in vEDS patients based on the clinical course of the present vEDS patient. vEDS patients who are affected by ileus must be surgically treated before too many fissures develop in the intestine, regardless of the presence of ischemic changes. In general, patients who are diagnosed with a paralytic ileus or adhesive ileus after prior operations are conservatively treated by decompression with a nasogastric tube or a Miller–Abbott tube. However, we were unable to treat our vEDS patient conservatively, because the wall of his small intestine was easily torn and became necrotic under high pressure. The timing for a surgical operation must be carefully considered, and a massive bowel resection should always be prevented if at all possible.

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RESEARCH ARTICLE

AMERICAN JOURNAL OF medical genetics PART A

Delineation of Dermatan 4-*O*-Sulfotransferase 1 Deficient Ehlers–Danlos Syndrome: Observation of Two Additional Patients and Comprehensive Review of 20 Reported Patients

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Loss-of-function mutations in *CHST14*, dermatan 4-*O*-sulfotransferase 1 (D4ST1) deficiency, have recently been found to cause adducted thumb-clubfoot syndrome (ATCS; OMIM-#601776) and a new type of Ehlers–Danlos syndrome (EDS) coined as EDS Kosho Type (EDSKT) [Miyake et al., 2010], as well as a subset of kyphoscoliosis type EDS without lysyl hydroxylase deficiency (EDS VIB) coined as musculocontractural EDS (MCEDS) [Malfait et al., 2010]. Lack of detailed clinical information from later childhood to adulthood in ATCS and lack of detailed clinical information from birth to early childhood in EDSKT and MCEDS have made it difficult to determine whether these disorders would be distinct clinical entities or a single clinical entity with variable expressions and with different presentations depending on the patients' ages at diagnosis. We present detailed clinical findings and courses of two additional unrelated patients, aged 2 years and 6 years, with EDSKT with a comprehensive review of 20 reported patients with D4ST1 deficiency, which supports the notion that these disorders constitute a clinically recognizable form of EDS. The disorder, preferably termed D4ST1-deficient EDS, is characterized by progressive multisystem fragility-related manifestations (joint dislocations and deformities, skin hyperextensibility, bruisability, and fragility; recurrent large subcutaneous hematomas, and other cardiac valvular, respiratory, gastrointestinal, and ophthalmological complications) resulting from impaired assembly of collagen fibrils, as well as various malformations (distinct craniofacial features, multiple congenital contractures, and congenital defects in cardiovascular, gastrointestinal, renal, ocular, and central nervous systems) resulting from inborn errors of development.

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Am J Med Genet Part A 155:1949–1958.

Key words: dermatan 4-*O*-sulfotransferase 1 deficiency; adducted thumb-clubfoot syndrome; Ehlers–Danlos syndrome Kosho type; musculocontractural Ehlers–Danlos syndrome; congenital contractures; progressive multisystem fragility-related manifestations; malformations

Additional supporting information may be found in the online version of this article.

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INTRODUCTION

Dermatan 4-*O*-sulfotransferase 1 (D4ST1) is a regulatory enzyme in the glycosaminoglycan biosynthesis that transfers active sulfate to position 4 of the *N*-acetyl-D-galactosamine residues of dermatan sulfate [Evers et al., 2001; Mikami et al., 2003]. Dermatan sulfate, as well as chondroitin sulfate and heparan sulfate, constitutes glycosaminoglycan sidechains of proteoglycans; and has been implicated in cardiovascular disease, tumorigenesis, infection, wound repair, and fibrosis via dermatan sulfate-containing proteoglycans such as decorin and biglycan [Trowbridge and Gallo, 2002]. Carbohydrate sulfotransferase 14 (*CHST14*), localized on 15q12, is the gene encoding D4ST1. Recently, loss-of-function mutations in *CHST14* (D4ST1 deficiency) have been found to cause adducted thumb-clubfoot syndrome (ATCS; OMIM#601776) in 11 patients from four families [Dündar et al., 2009] and a variant of Ehlers–Danlos syndrome (EDS) in six patients from six families [Miyake et al., 2010], tentatively coined as EDS Kosho Type (EDSKT) in the London Dysmorphology Database (http://www.lmdatabases.com/index.html) and POSSUM (http://www.possum.net.au/). ATCS was originally recognized as a new type of arthrogryposis, focused on characteristic clinical pictures from birth to early childhood, including adducted thumbs and talipes equinovarus as well as facial dysmorphisms (prominent forehead, large fontanelle, hypertelorism, down-slanting palpebral fissures, low-set ears), and arachnodactyly [Dündar et al., 1997; Sonoda and Kouno, 2000; Dündar et al., 2001; Janecke et al., 2001]. In a recent study by Dündar et al. [2009], ATCS has been categorized again as a connective tissue disorder, based on additional clinical pictures from childhood to adolescence, including skin fragility and bruisability, joint laxity, and osteopenia. EDSKT comprises a pattern of distinct craniofacial features, multiple congenital contractures, progressive joint and skin laxity, and progressive multisystem fragility-related manifestations, including recurrent large subcutaneous hematomas and other cardiac, respiratory, gastrointestinal, ophthalmological complications [Yasui et al., 2003; Kosho et al., 2005, 2010].

Very recently, Malfait et al. [2010] have independently found mutations in *CHST14* in three patients from two families, who were diagnosed with kyphoscoliosis type EDS without lysyl hydroxylase deficiency (EDS VIB). They concluded that their series and ATCS, as well as EDSKT, formed a phenotypic continuum based on their clinical observations and identification of an identical mutation in both conditions, and proposed to coin the disorder as "musculocontractural EDS" (MCEDS) [Malfait et al., 2010]. However, it is still an unsolved problem whether ATCS, EDSKT, and MCEDS would be distinct clinical entities or a single clinical entity with variable inter- and intrafamilial expressions and with different presentations depending on the patients' ages at diagnosis [Miyake et al., 2010], because detailed clinical information are lacking in ATCS from later childhood to adulthood and in EDSKT and MCEDS from birth to early childhood.

Here, we present detailed clinical findings and courses of two additional unrelated patients, aged 2 years and 6 years, with EDSKT, which would contribute to delineate comprehensive phenotypic spectrum of D4ST1 deficiency.

CLINICAL REPORTS

Patient 1

The patient, a Japanese boy, was the second child of a healthy 31-year-old mother and a healthy 33-year-old nonconsanguineous father. He was born by cesarean for breech presentation at 38 weeks and 3 days of gestation. His birth weight was 3,092 g (+0.2 SD), length 46 cm (−1.3 SD), and OFC 34 cm (+0.4 SD). At age 15 days, he was referred to our hospital for the treatment of bilateral talipes equinovarus. He had a round face with a large fontanelle, hypertelorism, short palpebral fissures, blue sclerae, strabismus, a short nose with a hypoplastic columella, low-set and rotated ears, a high palate, a long philtrum, a thin upper lip vermillion, a small mouth, and microretrognathia (Fig. 1A, B). He had arachnodactyly, flexion-adduction contractures of bilateral thumbs, flexion contractures of the interphalangeal (IP) joints in the other fingers, flexion contractures of bilateral elbows and knees, and rigidity of bilateral hip joints (Fig. 1C). He also had widely spaced nipples, a redundant and translucent skin, an umbilical hernia, and bilateral cryptorchidism (Fig. 1C). Talipes equinovarus was treated with incision of bilateral Achilles' tendons at age 2 months, followed by serial plaster casts and braces. Skin fragility was observed at the procedure. It was surgically corrected at age 1 year and 11 months. Gross motor development was delayed: He raised his head at 6 months, sat without support at age 1 year, stood up assisted at age 1 year and 6 months, and walked assisted after surgical correction of talipes equinovarus. He had bruises easily on the occiput

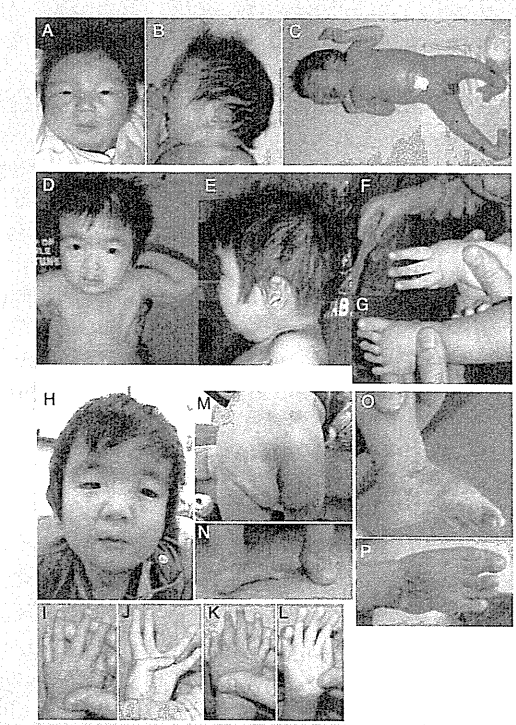

FIG. 1. Clinical photographs of Patient 1 at age 15 days (A–C), at age 1 year and 3 months (D–G), and at age 2 years and 10 months (H–P).

and buttocks after falling, which were absorbed spontaneously. Bleeding time was 1.3 min (normal values, 1–5 min), prothrombin time-international normalized ratio (PT-INR) 1.00 (normal values, 0.81–1.38 sec), and activated partial thromboplastin time (APTT) 27.9 sec (normal values, 23–36 sec).

When seen by us at age 1 year and 3 months, his craniofacial shape became square with a broad, bossed forehead, and hypertelorism with downslanting palpebral fissures became evident (Fig. 1D, E). Skin redundancy and tapering fingers and toes were noted (Fig. 1D, F, G). Ear rotation and flexion contractures of fingers improved (Fig. 1E, F).

When last seen by us at age 2 years and 10 months, he weighed 9.86 kg (−2.4 SD), height 84.9 cm (−2.1 SD), and OFC 45.5 cm (−2.4 SD). His face was slender, and was characterized by an unclosed fontanelle, hypertelorism, short and downslanting palpebral fissures, blue sclerae, strabismus, a short nose with a hypoplastic columella, low-set ears, a high palate, a long philtrum, a thin upper lip vermillion, a small mouth, and microretrognathia (Fig. 1H). He had a Marfanoid habitus, generalized joint laxity, a flat and thin thorax, and distinctive fingers (tapering with enlargement of distal phalanges) (Fig. 1I–L), and talipes valgus and planus with extremely soft subcutaneous tissues at the heels (Fig. 1N–P). The distal IP joints in bilateral index to little fingers and the IP/metacarpophalangeal (MP) joints in bilateral thumbs could hardly be flexed or extended. The MP joints in bilateral index to little fingers could be moved with poor flexion and hyperextension (see Supplementary Video 1 online). He had hyperextensible to redundant skin with bruisability and fine palmer creases (Fig. 1J, L, M). He suffered from constipation (defecation twice a week), treated with oral magnesium oxide. Ophthalmological examinations showed mild esotropia, and amblyopia due to severe hyperopic astigmatism. A cardiac ultrasonography showed no defects or valve abnormalities but mild dilation of the ascending aorta at the sinus of Valsalva. A brain CT showed no ventricular enlargement (Fig. 3O, P). G-banded chromosomes were normal. The Kinder Infant Developmental Scale [Cheng et al., 2010] showed mild developmental delay with the overall developmental quotient as 65 (physical/motor, 35; manipulation, 58; receptive language, 77; expressive language, 103; conceptual thinking, 77; social relationships with children, 68; social relationships with adults, 116; home training, 68; feeding, 42). He had orchiopexy and a surgical correction of an umbilical hernia at age 2 years and 7 months.

Patient 2

The patient, a Japanese boy, was the first child of a healthy 25-year-old mother and a healthy 28-year-old nonconsanguineous father. He was born by normal vaginal delivery at 38 weeks of gestation. His birth weight was 2940 g (+0.3 SD), length 49.1 cm (+0.3 SD), and OFC 32 cm (−0.5 SD). He was admitted for the treatment of bilateral adducted thumbs and talipes equinovarus. His craniofacial features included a large fontanelle, a high forehead, hypertelorism, short and downslanting palpebral fissures, blue sclerae, strabismus, a short nose with a hypoplastic columella, low-set and rotated ears, a high palate, a long philtrum, a thin upper lip vermillion, a small mouth, and microretrognathia (Fig. 2A). He had arachnodactyly, flexion-adduction contractures of bilateral thumbs, flexion contractures of the IP joints in the other fingers, rigidity of bilateral hip joints, and mild pectus excavatum (Fig. 2A, B). He also had widely spaced nipples, a redundant skin, and bilateral cryptorchidism (Fig. 2A). He suckled poorly and hated to be hugged tightly, suggesting hyperalgesia to pressure. Talipes equinovarus was treated with serial plaster casts. Gross motor development was delayed: He raised his head at 7 months, sat without support at age 1 year and 2 months, crawled at age 1 year and 6 months, pulled himself up by holding to something at age 1 year and 6 months, and walked unassisted at age 2 years and 6 months. His fontanelle was closed at age 3 years.

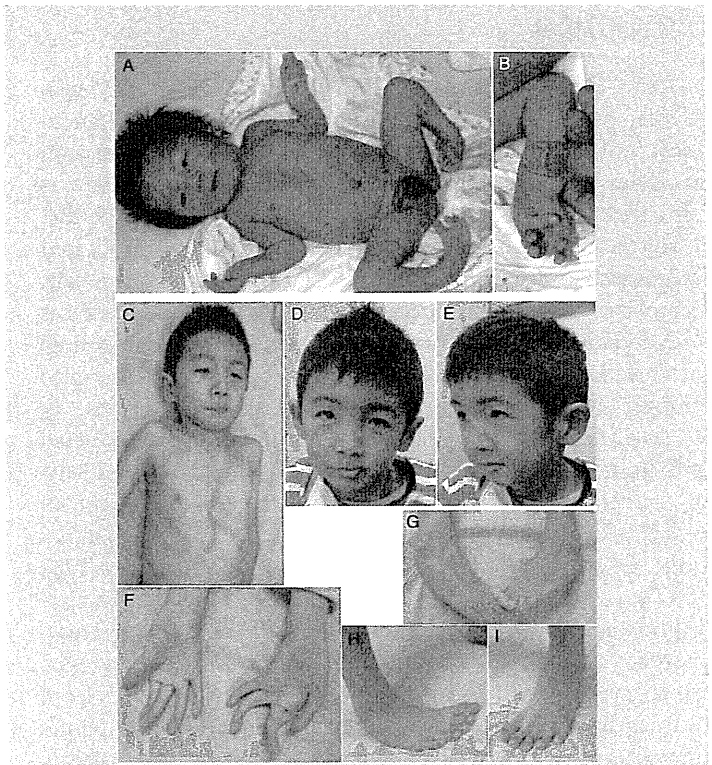

FIG. 2. Clinical photographs of Patient 2 at birth (A, B) and at age 6 years and 6 months (C–I).

At age 3 years, he developed a large subcutaneous hematoma over the skull after falling. Hematomas on the lower legs frequently occurred. He had recurrent dislocations of bilateral shoulders.

When last seen by us at age 6 years and 6 months, he weighed 16.4 kg (−1.4 SD), height 112 cm (−1.0 SD), and OFC 51.5 cm (−0.2 SD). He could jump unassisted. His craniofacial features included hypertelorism, short and downslanting palpebral fissures, blue sclerae, strabismus, a short nose with a hypoplastic columella, low-set ears, a high palate, a long philtrum, a thin upper lip vermillion, a small mouth, and microretrognathia (Fig. 2D, E). He had a Marfanoid habitus, generalized joint laxity, and pectus excavatum (Fig. 2C). His fingers were cylindrical and slender (Fig. 2F). He showed talipes equinovarus when lying down (Fig. 2G) and talipes planus when standing (Fig. 2H, I). The subcutaneous tissues at the heels were extremely soft. The distal IP joints in bilateral index to little fingers and the IP joints in bilateral thumbs

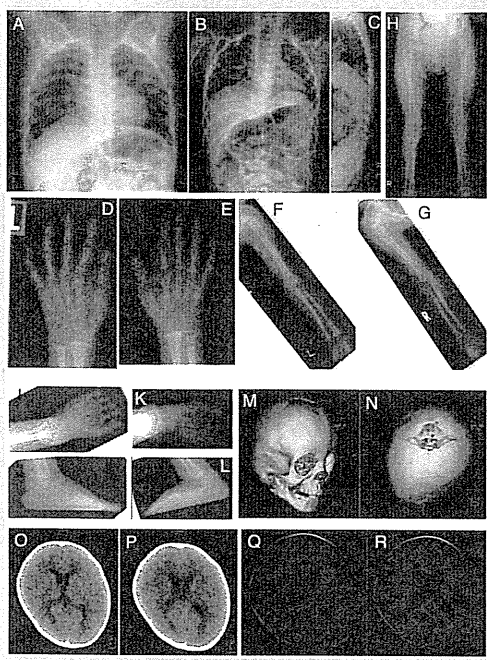

FIG. 3. Radiographs of Patient 1 at age 1 year and 10 months (A) and 2 years and 6 months (B–L). Cranial and brain CT of Patient 1 at age 2 years and 6 months (M–P). Brain MRI of Patient 2 at age 1 year and 10 months (Q, R).

could hardly be flexed or extended. The proximal IP joints in bilateral index to little fingers and the MP joints in all fingers could be flexed and extended, but could not be moved separately and smoothly. His skin was hyperextensible and bruisable. Fine palmar creases were also noted (Fig. 2F). He occasionally had constipation and abdominal pain. A cardiac ultrasonography showed trivial mitral valve prolapse, patent ductus arteriosus, and dextrocardia. A brain MRI showed bilateral ventricular enlargement (Fig. 3Q, R). G-banded chromosomes were normal. His intelligence was normal.

SKELETAL INVESTIGATIONS

Radiographs of Patient 1 were reviewed. At age 2 years and 6 months, he had mild scoliosis (Fig. 3B), which was not noted at age 1 year and 10 months (Fig. 3A). Physiological lumbar lordosis was not present (Fig. 3C). The left hip joint was dislocated (Fig. 3H). Long bones of the legs and arms showed over modeling, with narrowing diaphysis and widening metaphysis (Fig. 3F–H). Bilateral tibiae and fibulae were medially curved (Fig. 3H). Short bones of the hands (Fig. 3D, E) and feet (Fig. 3I–L) also showed over modeling, as well as osteoporotic changes in the feet (Fig. 3I–L).

MUTATION ANALYSIS

Genomic DNA was extracted from peripheral blood leukocytes of the patients and their parents, and was amplified with PCR using four primer sets for *CHST14* (sequences available on request). Through direct sequencing of the PCR products, compound heterozygous mutations were detected in both patients: c.842 C > T causing p. Pro281Leu (p.P281L) and c.878 A > G causing p.Tyr293Cys (p.Y293C) in Patient 1; c.626 T > C causing p.Phe209Ser (p.F209S) and c.842 C > T causing p.Pro281Leu (p.Y293C) in Patient 2 (data not shown). The parents had one of the two heterozygous mutations observed in their children.

DISCUSSION

We have presented detailed clinical characteristics and courses of two new unrelated pediatric patients with compound heterozygous *CHST14* mutations. The features showed striking resemblance to those of patients with EDSKT in their infancy to early childhood [Kosho et al., 2005, 2010]. *CHST14* mutations (P281L/Y293C) in Patient 1 were identical to those found in two patients with EDSKT [Miyake et al., 2010]. F209S found in Patient 2, which was not listed on a database of common gene variations in the Japanese population (JSNP) [Haga et al., 2002], was the mutation that has never found in previous patients with ATCS, EDSKT, or MCEDS.

To date, 22 patients (12 males, 10 females) from 14 families, including present patients, have been reported to have homozygous or compound heterozygous mutations in *CHST14* (Tables I and II) [Dündar et al., 1997; Sonoda and Kouno, 2000; Dündar et al., 2001; Janecke et al., 2001; Yasui et al., 2003; Kosho et al., 2005; Dündar et al., 2009; Kosho et al., 2010; Malfait et al., 2010; Miyake et al., 2010]. Eight families were of Japanese origin, three of Turkish origin, one of Austrian origin, and one of Indian origin. The median patients' age at their initial publication was 4 years and 1 month (range, 0 day–32 years): 7 months (range, 0 day–6 years) in ATCS, 12.5 years in EDSKT (range, 2 years–32 years), and 21 years (range, 12 years–22 years) in MCEDS.

CHST14 mutations included V49X in two families (ATCS, MCEDS), K69X in one (EDSKT), R135G in one (ATCS), L137Q in one (ATCS), F209S in one (EDSKT), R213P in one (ATCS), P281L in eight (EDSKT), C289S in one (EDSKT), Y293C in four (one ATCS, three EDSKT), and E334GfsX107 in one (MCEDS). Sulfotransferase activity of COS-7 cells transfected with *CHST14* containing K69X, P281L, C289S, or Y293C mutation was decreased at almost the same level, suggesting that loss-of-function mutations in *CHST14*, that is to say D4ST1 deficiency, would cause these disorders [Miyake et al., 2010].

Characteristic craniofacial features at birth to early infancy (large fontanelle, hypertelorism, short and downslanting palpebral fissures, blue sclerae, short nose with hypoplastic columella, low-set and rotated ears, high palate, long philtrum, thin upper lip vermilion, small mouth, and micro-retrognathia) were noted in most patients with ATCS, EDSKT, and MCEDS. Slender and asymmetrical facial shapes with protruding jaws from school age, commonly observed in patients with EDSKT, were also described in ATCS2 at age 15 years, ATCS3 at age 6 years, ATCS7 at age 8 years [Dündar et al., 2009], and in MCEDS1 at age 21 years [Malfait et al., 2010]. A pair of ATCS siblings had palatal defects: ATCS4 with cleft lip and palate, which was surgically repaired, and ATCS5 with cleft soft palate [Sonoda and Kouno, 2000].

Congenital multiple contractures, most specifically adduction–flexion contractures of thumbs and talipes equinovarus,

TABLE I. Clinical Characteristics of Reported Patients With D4ST1 Deficiency (Continued)

								Craniofacial															Skeletal									Cutaneous						Cardiovascular				Gastrointestinal		
Family	Patient	Origin	*CHST14* Mutations	Age at initial publication (d, days; m, months; y, years)	Sex	Large fontanelle (early childhood)	Hypertelorism	Down-slanting palpebral fissures	Blue sclerae	Short nose with hypoplastic columella	Ear deformities	Palatal abnormalities	Long philtrum and thin upper lip	Small mouth/micro-retrognathia in infancy	Short neck	Slender face/protruding jaw from school age	Facial asymmetricity from school age	Malfanoid habitus/slender build	Congenital multiple contractions	Recurrent/chronic joint dislocations	Pectus deformities	Spinal deformities	Peculiar fingers (tapering ~ slender and cylindrical)	Progressive talipes deformities	Hyperextensibility/ redundancy	Bruisability	Fragility/atophic scars	Fine/Acrogeria-like palmar creases	Hyperalgesia to pressure	Recurrent subcutaneous infections/fistula	Congenital heart defects	Valve abnormalities	Large subcutaneous hematomas	(Hemo) pneumathorax	Constipation	Others
ATCS																																										
1	1	Turkish	V49X homo	3.5y	F	+	+	+	+		P, PR	H	+		+				+				+								–									
	2			1.5y	M	+	+	+	+	+	P, PR		+		+	+a		+a	+	+a	Exa	KSa	+	Planus, valgus#			+a				–									
	3			6y	F		+	+		+	P		+			+	+	+	+		F–T	S	+	Varus			+													
2	4	Japanese	Y293C homo	4y	M	+	+	+		+	LS	CL/CP	+						+	+	Ca		+	Planus, valgus							ASD									
	5			7m	M	+	+	+		+	LS	CSP	+						+	+			+																	
3	6	Austrian	R213P homo	0d†	M	+	+	+			PR, LS				+				+			S	+								ASD, CoA									Common mesentery
	7			12m	M	+	+	+	+		D, PR				+	+a		+a	+		F–Ta		+	Planus, valgus#			+a				–									Absence of gastrocolic omentum, spontaneous volvulus of small intestine
4	8	Turkish	R135G / L137Q	1–4m†	F														+																					
	9			1–4m†	M														+																					
	10			1–4m†	M														+																					
	11			3m	M	+	+	+	+		D, LS	H	+		+				+				+								–									
MCEDS																																										
1	1	Turkish	V49X homo	22y	F	+		+		+	PR, LS	H	+			+	+	+	+		F–T	KS	+	Planus, valgus	+	+	+	+		+	–	–								Hiatal hernia, gastroesphageal reflux, abdominal pain
	2			21y	F	+		+	+			H	+						+	+	F–T	S	+	Small, broad	+	+	+	+												
2	3	Indian	E334GfsX107 homo	12y	F				+		LS		–					+	+	+		KS	+	Planus, valgus	+	+	+	+		+										Duodenum obstruction due to malrotation
EDSKT																																										
1	1	Japanese	P281L / Y293C	11y	F	+	+	+	+	+	LS	H	+	+		+	+	+	+	+	Ex	KS	+	Planus, valgus	+	+	+	+	+	+	ASD	TR, TVP, MVP	+	–	+	Gastric ulcer§		
2	2	Japanese	P281L homo	14y	F	+	+	+	+	+	LS	H	+	+		+	+	+	+	+	F–T	S	+	Cavus, valgus	+	+	+	+	+	+	–		+	+§	+	Diverticular perforation§		
3	3	Japanese	P281L homo	32y	M	+	+	+	+	+	LS	H	+	+		+	+	+	+	+	F–T	KS	+	Planus, valgus	+	+	+	+	+	+		AR, ARD, MR	+	+	+	Diverticular perforation		
4	4	Japanese	K69X / P281L	32y	M		+		+			H				+	+	+	+		Ex	KS	+	Planus, valgus	+	+	–	+	–	–			+	+	–			
5	5	Japanese	P281L / C289S	20y	F	+	+	+	+	+	LS	H	+	+		+	+	+	+	+	Ex	S	+	Planus, valgus	+	+	+	+	+	+		AR, MR, IE	+	–	+			
6	6	Japanese	P281L / Y293C	4y	F	+	+	+	+	+	LS	H	+						+		Ex	S	+	Planus	+	+	+	+	+	+			+	–	+			
Present report (EDSKT)																																										
7	7	Japanese	P281L / Y293C	2y	M	+	+	+	+	+	PR, LS	H	+	+					+	+		F–T	S	+	Planus, valgus	+	+	+	+	–	+	–	ARD	–	–	+			
8	8	Japanese	F209S / P281L	6y	M	+	+	+	+	+	PR, LS	H	+	+					+	+	+	Ex		+	Planus	+	+	–	+	+	–	PDA	MVP	+	–	+	Abdominal pain		

ATCS, adducted thumb-clubfoot syndrome; EDSKT, Ehlers–Danlos Syndrome, Kosho Type; MCEDS, Musculocontractural Ehlers–Danlos Syndrome
+, present; Blank, information not available; †, died; F, female; M, male; P, prominent; PR, posteriorly rotated; LS, low set; D, dysplastic; H, high; CL, cleft lip; CP, cleft palate; CSP, cleft soft palate;
Ex, excavatum; F–T, flat and thin; Ca, carinatum; KS, kyphoscoliosis; S, scoliosis; ASD, atrial septal defect; CoA, coarctation of aorta; PDA, patent ductus arteriosus;
TR, tricuspid valve regurgitation; TVP, tricuspid valve prolapse; MVP, mitral valve prolapse; AR, aortic valve regurgitation; ARD, aortic root dilation; MR, mitral valve regurgitation; IE, infectious endocarditis
ATCS Patient 1 died at age 6 years [Dündar et al., 2009].
a, Information from reassessment by Dündar et al. [2009], describing ATCS P2 at age 15 years and ATCS P7 at age 8 years
b, Information from reassessment by Kosho et al. [2010], describing EDSKT1 at age 16 years and EDSKT2 at age 32 years.

TABLE II. Clinical Characteristics of Reported Patients With D4ST1 Deficiency

		Urogenital			Ophthalmological						Development			Growth (prenatal)				Growth (postnatal)				
Family	Patient	Nephro (Cysto) lithiasis	Cryptorchidism	Others	Breast development	Strabismus	Refractive errors	Glaucoma/elevated intraocular pressure	Others	Hearing impairment	Ventricular abnormalities (brain)	Gross motor delay	Age of unassisted walk (y, years; m, months)	Mental delay	Gestational weeks	Birth length (centile or SD)	Birth weight (centile or SD)	Birth OFC (centile or SD)	Age (y, years; m, months)	Height (centile or SD)	Weight (centile or SD)	OFC (centile or SD)	References
ATCS																							
1	1					+					Enl	+	No	+	Term				3.5y	25-50th	50th	50th	Dündar et al., 1997
	2					+		+			Asym	+		+c	Term				1.5y	50th	10th	10–25th	Dündar et al., 1997
																			15ya	25–50th	<3rd		Dündar et al., 2009
	3					+													6y	10–25th	<3rd		Dündar et al., 2009
2	4		+	Hydronephrosis										–	39wk	–0.6	–0.9	–0.8	4y2m	–3.9	–1.8	–0.9	Sonoda and Kouno, 2000
	5		+	Hydronephrosis, inguinal hernia								–		–	38wk	–1.6	–1.3	–0.5	7m	–2.1	–1.4	–1.1	Sonoda and Kouno, 2000
3	6		+	Horseshoe kidney							Enl				32wk	25th	10th	25th					Janecke et al., 2001
	7		+			+					Asym	–		–	38wk	50th	25th	50th	8ya	10-25th	<3rd		Janecke et al., 2001
																							Dündar et al., 2009
4	8																						Dündar et al., 2001
	9																						Dündar et al., 2001
	10																						Dündar et al., 2001
	11	+	+	Inguinal hernia							Enl, Asym								3m	<3rd	3–10th	10th	Dündar et al., 2001
MCEDS																							
1	1	+			–		My	+	Retinal detachment, phthysis bulbi	+		+	4y		42wk	±0	–0.67		22y	–2.0		±0	Malfait et al., 2010
	2	+		Hydronephrosis, renal ptosis, ureteral stenosis	–		My	+	Retinal detachment	+		+	2y		42wk				14y	+0.68		>2.0	
2	3						My		Microcornea			+			Term		–0.88						Malfait et al., 2010
EDSKT																							
1	1			Bladder dilation, recurrent UTI, involuntary contractionb	–b	+	Hy	–	Microcornea	+	Enl	+	2y	+d	42wk	–0.1	–1.3	–1.0	7y	+0.8	–1.0	±0	Kosho et al., 2005
																			11y	+0.2	–2	+0.2	Kosho et al., 2010
																			16y	+0.3	–0.4	+0.2	
2	2	+		Atonic bladder, recurrent UTI	–b	+	My, As	+b		+		+	No	–	Term		–2.0		15y	–3.2			Kosho et al., 2005
																							Kosho et al., 2010
3	3		+	Hypogonadism		+	My, As	–	Microphthalmia	+		+		–	40wk	+1.3	+0.5	+0.8	30y	+1.2	–1.7		Kosho et al., 2010
4	4					–	–	–	Retinal detachment	–				–					23y	–0.4	–2.4		Yasui et al., 2003
																							Kosho et al., 2010
5	5	+		Delayed menarche, irregular menstruation	–	+	My, As	+		–		+	2y2m	–	39wk	–1.1	–0.4	+1.0	19y	–0.1	–0.8		Kosho et al., 2010
6	6					+	My, As	+		+		+	1y5m	–	41wk	–1.2	–0.5	–0.6	4y	–1.2	–1.3	–0.9	Kosho et al., 2010
Present report																							
7	7		+			+	Hy, As, Am	–				+	No	+	38wk3d	–1.3	+0.2	+0.4	2y	–2.2	–2.4	–1.2	Patient 1
8	8		+			+				–	Enl	+	2y6m	–	38wk	+0.3	+0.3	–0.5	6y	–0.7	–1.4	+0.1	Patient 2

ATCS, adducted thumb-clubfoot syndrome; EDSKT, Ehlers-Danlos Syndrome, Kosho Type; MCEDS, Musculocontractural Ehlers-Danlos Syndome
+, present; Blank, information not available; UTI, urinary tract infection; Hy, hyperopia; My, myopia; As, astigmatism; Am, amblyopia; Enl, enlargement; Asym, asymmetry; No, not ambulant
a, Information from reassessment by Dündar et al. [2009], describing ATCS P2 at age 15 years and ATCS P7 at age 8 years.
b, Information from reassessment by Kosho et al. [2010], describing EDSKT1 at age 16 years and EDSKT at age 32 years.
c, IQ was 91 at Porteus test and 86 at Goodenough test at age 7 years and 2 months [Janecke et al., 2001];
d, mild learning dissability

were cardinal features in patients with ATCS, EDSKT, and MCEDS. Peculiar fingers described as "tapering," "slender," and "cylindrical" were also common features. Aberrant finger movement was described in EDSKT1 [Kosho et al., 2010], EDSKT7, and EDSKT8. EDSKT1, EDSKT2, EDSKT3, and EDSKT5 were found to have tendon abnormalities such as anomalous insertions of flexor muscles, which might result in contractures [Kosho et al., 2005, 2010]. In childhood, spinal deformities (scoliosis, kyphoscoliosis) and talipes deformities (planus, valgus) occurred and progressed. Malfanoid habitus, recurrent joint dislocations, and pectus deformities (flat and thin, excavatum, carinatum) were also evident. Talipes equinovarus in seven EDSKT patients and MCEDS3 was surgically repaired [Kosho et al., 2005, 2010; Malfait et al., 2010]. EDSKT3 received tendon transplantations for defects of tendons to bilateral thumbs, and EDSKT4 received surgical fixation of bilateral ankle joints as well as surgery for carpal tunnel syndrome [Kosho et al., 2010]. MCEDS1 underwent surgery for rapidly worsened kyphoscoliosis at age 14 years [Malfait et al., 2010].

Bone mineral density (BMD) was decreased in ATCS2 at age 15 years (Z score −1.6), ATCS3 at age 6 years (Z score −4.6) [Dündar et al., 2009], EDSKT2 at age 29 years (Z score −2.4 for the lumbar spine 1–4, −2.3 for the 33% radius), and EDSKT3 at age 31 years (Z score −3.7 for the lumbar spine 1–4, −3.6 for the femoral neck) [unpublished data], whereas BMD was normal in ATCS7 at age 8 years [Dündar et al., 2009] and EDSKT1 at age 15 years (Z score +1.6 for the lumbar spine 1–4, −0.9 for the femoral neck) [unpublished data]. Urine N-telopeptide of collagen type I (NTX), an osteoclast marker, was increased at 92.8 nmol BCE/mmol Cr in EDSKT1 at age 16 years, 70.3 nmol BCE/mmol Cr in EDSKT2 at age 28 years, and 238.4 nmol BCE/mmol Cr in EDSKT3 at age 31 years [unpublished data] (normal values for premenopausal females, 9.3–54.3; males, 13.0–66.2), whereas serum bone specific alkaline phosphatase (BAP), an osteoblast marker, was normal at 12.5 U/L in EDSKT1 at age 16 years, 25.6 U/L in EDSKT2 at age 28 years, and 15.1 U/L in EDSKT3 at age 31 years (normal values, 9.6–35.4) [unpublished data]. These results in biochemical markers of bone turnover suggested an increase in osteoclast activity with normal osteoblast activity, which could cause oeteopenia or osteoporosis.

Radiologically, diaphysial narrowing of phalanges and metacarpals was noted in EDSKT1 at age 11 and 16 years, EDSKT2 at age 10 and 28 years, EDSKT3 at age 31 years, EDSKT5 at age 19 years [Kosho et al., 2005, 2010], and EDSKT7 at age 2 years and 6 months. Talipes valgus and planus or cavum, with diaphysial narrowing of phalanges and metatarsals, were noted in ATCS7 at age 8 years [Dündar et al., 2009], EDSKT1 at age 11 and 16 years, EDSKT2 at age 14 and 28 years, EDSKT6 at age 4 years [Kosho et al., 2005, 2010], and EDSKT7 at age 2 years and 6 months. Tall vertebral bodies were noted in EDSKT1 at age 11 and 16 years, EDSKT2 at age 14 and 28 years, EDSKT3 at age 31 years, EDSKT4 at age 31 years, EDSKT5 at age 19 years [Kosho et al., 2005, 2010], and MCEDS2 at age 21 years [Malfait et al., 2010], whereas they were not noted in EDSKT6 at age 2 years [Kosho et al., 2010] and EDSKT7 at age 2 years and 6 months.

Cutaneous features were common in most patients with EDSKT and MCEDS, including hyperextensibility to redundancy, bruisability, fragility leading to atrophic scars, acrogeria-like fine palmar creases or wrinkles, hyperalgesia to pressure, and recurrent subcutaneous infections with fistula formation, which lead to skin defects including decubitus necessitating plastic surgery in EDSKT2 [Kosho et al., 2005, 2010]. Excessive palmar creases were observed in ATCS2, ATCS3, and ATCS7, and delayed wound healing and ecchymoses were also recorded ATCS patients [Dündar et al., 2009]. Palmar creases increased and became deeper according to the ages, as compared among photographs of EDSKT1 at age 11 and 16 years, EDSKT2 at age 5 years and 32 years, EDSKT3 at age 32 years, EDSKT5 at age 19years, and EDSKT6 at age 4 years [Kosho et al., 2005, 2010].

Seven patients with EDSKT suffered from large subcutaneous hematomas, which sometimes progressed acutely and massively to be treated intensively (admission, blood transfusion, surgical drainage). These lesions were supposed to be caused by rupture of subcutaneous arteries or veins. Hematoma formation was mentioned in a follow-up observation of ATCS patients [Dündar et al., 2009]. Bleeding time was prolonged in ATCS7 (9 min) [Dündar et al., 2009] and EDSKT4 (11 min) [Yasui et al., 2003; Kosho et al., 2010], whereas it was normal in EDSKT1 (3 min) [Kosho et al., 2005], EDSKT3 (1 min) [unpublished data], and EDSKT7 (1.3 min). EDSKT1 had, to prevent large subcutaneous hematomas, intranasal administration of 1-desamino-8-D-arginine vasopressin (DDAVP) after injuries [Kosho et al., 2005, 2010]. A large hematoma over the buttocks in EDSKT4 was treated with intranasal DDAVP and intramuscular conjugated estrogen [Yasui et al., 2003; Kosho et al., 2010].

Two ATCS and two EDSKT patients had congenital heart defects (atrial septal defect was the most common, observed in three), and five EDSKT patients had cardiac valve abnormalities. EDSKT5 suffered from infectious endocarditis probably resulting from aortic valve or mitral valve regurgitation, and underwent surgery. Three adult patients with EDSKT developed pneumothorax or hemopneumothorax, treated with chest tube drainage; and two of them suffered from diverticular perforation, treated surgically. Various gastrointestinal abnormalities were observed: Constipation in seven EDSKT patients and abdominal pain in one EDSKT and one MCEDS patients, as well as common mesentery in ATCS6, absent gastrocolic omentum and spontaneous volvulus of small intestine in ATCS7, gastric ulcer necessitating partial gastrectomy in EDSKT1, and duodenum obstruction due to malrotation treated surgically in MCEDS3 [Janecke et al., 2001; Dündar et al., 2009; Kosho et al., 2010; Malfait et al., 2010].

Urological complications included nephrolithiasis or cystolithiasis in one ATCS, two EDSKT, and two MCEDS patients; hydronephrosis in two ATCS and one MCEDS patients, dilated or atonic bladder with recurrent urinary tract infection in two EDSKT patients, and horseshoe kidney in one ATCS patient. Hydronephrosis in MCEDS2 was caused by renal ptosis and ureteral stenosis, for which a ureteral stent was placed with a laparoscopic procedure, complicated by severe hemorrhage due to excessive tissue fragility [Malfait et al., 2010].

Cryptorchidism was observed in five ATCS and three EDSKT male patients. EDSKT3, who received orchiopexy, showed hypogonadism in adulthood. In female patients older than adolescence, poor breast development was noted in three EDSKT (EDSKT1 and

EDSKT2 showed normal menstruation cycles; EDSKT5 showed delayed menarche and irregular menstruation cycles) and two MCEDS patients. No female patients have been reported to be pregnant.

Various ophthalmological complications were observed: Strabismus in four ATCS and seven EDSKT patients, refractive errors in six EDSKT and three MCEDS patients, glaucoma or elevated intraocular pressure in one ATCS, three EDSKT, and two MCEDS patients; microcornea or microphthalmia in two EDSKT and one MCEDS patients, and retinal detachment in one EDSKT and two MCEDS. Retinal detachment in EDSKT4 [Kosho et al., 2010] and glaucoma in MCEDS1 [Malfait et al., 2010] required surgery. Hearing impairment was noted in four EDSKT patients (predominantly for high-pitched sound in EDSKT1, EDSKT2, and EDSKT6) and two MCEDS.

Gross motor developmental delay was observed in two ATCS, seven EDSKT, and three MCEDS patients; and ages of unassisted walk in patients who accomplished it ranged from 1 year and 5 months to 4 years (median, 2 years and 1 month). EDSKT2, at age 32 years, could not walk unassisted because of severe foot deformities and muscle weakness of the legs [Kosho et al., 2010]. An underlying myopathic process was suggested in ATCS2 because of reduced amplitude muscle action potentials with normal distal latency time and nerve conduction velocity, whereas muscle biopsy did not reveal any histological abnormality [Dündar et al., 1997]. Mild mental delay was suggested in two ATCS and two EDSKT patients. ATCS2 was reported to have global psychomotor delay at the initial publication [Dündar et al., 1997], whereas his IQ was around 90 at age 7 years and 2 months [Janecke et al., 2001]. Five ATCS and two EDSKT patients showed ventricular enlargement and/or asymmetry on brain ultrasonography, CT or MRI. ATCS7 also showed absence of the left septum pellucidum [Janecke et al., 2001]. EDSKT6 had tethering of a spinal cord, and underwent duraplasty [Kosho et al., 2010].

Growth assessment was performed using data described with SD scores, excluding data described with centile scores. Patients with *CHST14* mutations showed mild prenatal growth retardation: The mean birth length −0.5 SD and the median −0.6 SD (n = 9; range, −1.6 SD to +1.3 SD); the mean birth weight −0.6 SD and the median −0.67 SD (n = 11; range, −2.0 SD to +0.5 SD); and the mean birth OFC −0.2 SD and the median −0.5 SD (n = 8; range, −1.0 SD to +1.0 SD). Postnatal growth was also mildly impaired with slenderness and relative macrocephaly: The mean height −0.9 SD and the median −0.6 SD (14 data from 12 patients; range, −3.9 SD to +1.2 SD); the mean weight −1.5 SD and the median −1.4 SD (11 data from 9 patients; range, −2.4 SD to −0.4 SD); the mean OFC −0.2 SD and the median ±0 SD (10 data from 8 patients; range, −1.2 SD to >2.0 SD).

Light microscopic investigations on skin specimens from EDSKT5 and EDSKT6 showed that fine collagen fibers were predominant in the reticular to papillary dermis and normally thick collagen bundles were markedly reduced [Miyake et al., 2010]. Electron microscopic investigations of the specimens showed that collagen fibrils were dispersed in the reticular dermis, compared with regularly and tightly assembled ones observed in the control, whereas each collagen fibril was smooth and round, not varying in size and shape, similar to each fibril of the control [Miyake et al., 2010]. These findings suggested that the main pathological basis of this disorder would be insufficient assembly of collagen fibrils, compatible with the evidence that dermatan sulfate of decorin proteoglycan, a key regulator of collagen fibril assembly that contains both chondroitin sulfate and dermatan sulfate in its glycosaminoglycan chains and controls the distance between collagen fibrils, was found to be completely lost and replaced by chondroitin sulfate in patients' fibroblasts [Miyake et al., 2010]. However, both light microscopic and electron microscopic findings of skin were assessed as normal in ATCS7 [Dündar et al., 2009]. In MCEDS2, most collagen bundles were small-sized, some of which were composed of variable diameter collagen fibrils separated by irregular interfibrillar spaces [Malfait et al., 2010].

This comprehensive review of the patients with loss-of-function mutations in *CHST14* (D4ST1 deficiency) supports the notion that ATCS, EDSKT, and MCEDS would be a single clinical entity with variable inter- and intra-familial expressions and with different presentations depending on the patients' ages at diagnosis or at publication. The disorder, we preferably would like to coin simply as EDS due to D4ST1 deficiency or D4ST1 deficient EDS (DD-EDS), is a clinically recognizable syndrome, characterized by progressive multisystem fragility-related manifestations including joint dislocations and deformities, skin hyperextensibility, bruisability, and fragility; recurrent large subcutaneous hematomas, and other cardiac valvular, respiratory, gastrointestinal, and ocular complications, which are considered to result from connective tissue weakness and be consequences of insufficient decorin-mediated assembly of collagen fibrils caused by D4ST1 deficiency. The disorder also shows various malformations including distinct craniofacial features, multiple congenital contractures, and congenital defects in cardiovascular, gastrointestinal, renal, ocular, and central nervous systems, which might not simply be accountable for connective tissue weakness but could be considered as inborn errors of development. In a recent review focusing on ATCS, Zhang et al. [2010] state that D4ST1 deficiency is the only recognized condition resulting from a defect specific to DS biosynthesis, and that the disorder emphasizes the roles D4ST1 play in human development and extracellular matrix maintenance.

DD-EDS could be detected at birth from characteristic craniofacial and skeletal features and molecular genetic testing gives definitive diagnosis. Initial screening for congenital cardiac, ocular, and renal abnormalities and hearing loss would be necessary. In infancy, orthopedic intervention for talipes equinovarus (serial plaster casts, surgery) as well as physical therapy for motor developmental delay would be the center of management. Laxatives and/or enema are considered in patients with constipation. Surgical fixation is considered for cryptorchidism in males. Regular follow-up for ophthalmological (strabismus, refractive errors, glaucoma), otological (otitis media with effusion, hearing loss), urological (urination, bladder enlargement), and cardiovascular (valve abnormalities, aortic root dilation) problems should be continued. After walking independently, attention should be paid to progressive foot deformities and trauma that could cause skin lacerations, joint dislocations, and massive subcutaneous hematomas. Intranasal DDAVP after injuries is considered to prevent large subcutaneous hematomas. From adolescence, assessment of spinal deformities (scoliosis, kyphoscoliosis) and secondary sex

characteristics (breast development in females and gonadal function in males) would be necessary. In adulthood, appropriate treatments should be performed on occasional emergency complications ([hemo]peumothorax, diverticular perforation). Wrist-type sphygmomanometer would be suitable for patients with hyperalgesia to pressure [unpublished observation].

Very recently, Janecke et al. [2011] have claimed that it would lead to confusion for clinicians and researchers to categorize the D4ST1 deficiency into a type of EDS and that an appropriate term should be "Dermatan sulfate-deficient adducted thumb-clubfoot syndrome." The reasons were described as follows: Clinically, "adducted thumb" and "clubfoot" would be the most distinguishable features at birth; etiologically, the molecular basis would differ substantially from EDS. In reply to the article, we have presented sufficient evidences for categorizing the disorder into a type of EDS: Clinically, the disorder would satisfy all the hallmarks of EDS (skin hyperextensibility, joint hypermobility, and tissue fragility affecting the skin, ligaments, joints, blood vessels, and internal organs), and the patients should be treated as having generalized connective tissue fragility in the lifelong management; etiologically, multisystem fragility in the disorder was found to be caused by impaired assembly of collagen fibrils caused by dermatan sulfate loss in the decorin glycosaminoglycan chain [Kosho et al., submitted].

In conclusion, ATCS, EDSKT, and MCEDS; which were found independently to be caused by D4ST1 deficiency, would be a single clinical entity with variable expressions and with different presentations depending on the patients' ages. The syndrome is characterized by a unique set of clinical features including progressive multisystem fragility-related manifestations (joint dislocations and deformities, skin hyperextensibility, bruisability, and fragility; recurrent large subcutaneous hematomas, and other cardiac valvular, respiratory, gastrointestinal, and ocular complications) resulting from impaired assembly of collagen fibrils, as well as various malformations (craniofacial features, multiple congenital contractures, and congenital defects in cardiovascular, gastrointestinal, renal, ocular, and central nervous systems) resulting from inborn errors of development.

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LETTER TO THE EDITORS

Human Mutation

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A Response to: Loss of Dermatan-4-sulfotransferase 1 (D4ST1/CHST14) Function Represents the First Dermatan Sulfate Biosynthesis Defect, "Dermatan Sulfate-Deficient Adducted Thumb–Clubfoot Syndrome". Which Name is Appropriate, "Adducted Thumb–Clubfoot Syndrome" or "Ehlers–Danlos Syndrome"?

We thank Janecke et al. [2011] for their letter about a recently recognized dermatan 4-O-sulfotransferase 1 (D4ST1) deficiency caused by loss-of-function *CHST14* (MIM# 608429) mutations, independently found in an arthrogryposis syndrome "Adducted Thumb–Clubfoot Syndrome" (ATCS) [Dündar et al., 2009], a specific form of Ehlers-Danlos syndrome (EDS) as we have proposed (EDS, Kosho Type; EDSKT) [Miyake et al., 2010], and a subset of kyphoscoliosis type EDS without evidence of lysyl hydroxylase deficiency (EDS type VIB) coined as "Musculocontractural EDS" (MCEDS) [Malfait et al., 2010]. Janecke et al. [2011] proposed that these three conditions constitute a clinically recognizable and genetically identical type of connective tissue disorder and that the disorders should not be categorized into a form of EDS, but be termed collectively "Dermatan Sulfate-Deficient Adducted Thumb–Clubfoot Syndrome" to avoid possible confusion for both clinicians and researchers. The proposal is based on their clinical and molecular recognition of the disorder. First, the presence of multiple congenital malformations such as facial dysmorphism, cleft lip/palate, intestinal abnormalities, renal abnormalities, and features such as nephrolithiasis and muscle hypotonia in these patients are not typical in EDS, though features such as joint laxity, skin hyperextensibility/fragility, and bleeding diathesis are typical in EDS. Second, the molecular basis in the disorder is different from that in EDS.

EDS comprises a heterogeneous group of heritable connective tissue disorders, with the hallmarks being skin hyperextensibility, joint hypermobility, and tissue fragility affecting the skin, ligaments, joints, blood vessels, and internal organs [Steinmann et al., 2002]. Dominant-negative effects or haploinsufficiency of mutant procollagen α-chain genes or deficiency of collagen-processing enzymes have been found to cause EDS [Mao and Bristow, 2001]. In a revised nosology, EDS was classified into six major types [Beighton et al., 1998] and several other forms have also been identified based on the molecular and biochemical abnormalities [Abu et al., 2008; Giunta et al., 2008; Kresse et al., 1987; Schalkwijk et al., 2001; Schwarze et al., 2004].

Homozygous or compound heterozygous *CHST14* mutations have been found in 11 patients aged 0 day to 6 years at the initial publication (from four families) with ATCS [Dündar et al., 1997, 2001, 2009; Janecke et al., 2001; Sonoda and Kouno, 2000], in six patients aged 2–32 years (from six families) with EDSKT [Kosho et al., 2005, 2010; Miyake et al., 2010; Yasui et al., 2003], and in three patients aged 12–22 years (from two families) with MCEDS [Malfait et al., 2010]. Lack of detailed clinical information from later childhood to adulthood in ATCS and lack of detailed clinical information from birth to early childhood in EDSKT and MCEDS have made it difficult to determine whether the three conditions would be distinct clinical entities or a single clinical entity with variable expressions and with different presentations depending on the patients' ages at diagnosis [Miyake et al., 2010], though the latter notion was suspected to be appropriate [Janecke et al., 2011; Malfait et al., 2010]. We, therefore, have just published an article in *American Journal of Medical Genetics Part A*, describing detailed clinical findings and courses of two additional unrelated EDSKT patients, aged 2 and 6 years, which could definitely unite the three conditions [Shimizu et al., 2011]. Furthermore, we have presented a comprehensive review of all reported patients with D4ST1 deficiency, which concludes that the three conditions constitute a clinically recognizable disorder, characterized by progressive multisystem fragility-related manifestations and various malformations and allows us to term the disorder "D4ST1-deficient EDS" [Shimizu et al., 2011]. The clinical manifestations are summarized in Table 1.

We have categorized D4ST1 deficiency into a form of EDS for substantial reasons. Clinically, the disorder satisfies all the hallmarks of EDS [Steinmann et al., 2002]. All patients we have encountered were diagnosed with EDS and have been managed as having generalized connective tissue fragility, such as preventing skin wounds, hematomas, joint dislocations, and progressive talipes and spinal deformities. Careful surgical suturing of torn skin and regular evaluations of internal organs (e.g., cardiac valve abnormalities, aortic root dilation, and bladder enlargement) and ocular abnormalities are also conducted. ATCS is surely a helpful term to detect and diagnose patients at birth, but it is indeed questionable whether the term would be appropriate for the lifelong management of patients with the disorder. Furthermore, clinical manifestations extending beyond the core features of EDS are considered not as excluding information from EDS as Janecke et al. [2011] have claimed, but as wide clinical variability in EDS such as muscle hypotonia and chronic pain in most of the types, talipes equinovarus and facial characteristics in vascular type, and congenital hip dislocation in arthrochalasia type [Beighton et al., 1998; Voermans et al., 2009].

Etiologically, multisystem fragility in D4ST1 deficiency was illustrated to be caused by impaired assembly of collagen fibrils resulting from loss of dermatan sulfate (DS) in the decorin glycosaminoglycan side chain [Miyake et al., 2010], which justifies terming the

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Table 1. Clinical Manifestations in D4ST1 Deficiency

Craniofacial	*Cardiovascular*
Large fontanelle (early childhood)	Congenital heart defects (ASD)
Hypertelorism	Valve abnormalities (MVP, MR, AR, ARD)
Short and downslanting palpebral fissures	Large subcutaneous hematomas
Blue sclerae	*Gastrointestinal*
Short nose with hypoplastic columella	Constipation
Ear deformities (prominent, posteriorly rotated, low set)	Diverticula perforation
Palatal abnormalities (high, cleft)	*Respiratory*
Long philtrum and thin upper lip	(Hemo) pneumothorax
Small mouth/microretrognathia (infancy)	*Urogenital*
Slender face with protruding jaw (from school age)	Nephrolithiasis/cystolithiasis
Asymmetric face (from school age)	Hydronephrosis
Skeletal	Dilated/atonic bladder
Marfanoid habitus/slender build	Inguinal hernia
Congenital multiple contractures (fingers, wrists, hips, feet)	Cryptorchidism
Recurrent/chronic joint dislocations	Poor breast development
Pectus deformities (flat, excavated)	*Ocular*
Spinal deformities (scoliosis, kyphoscoliosis)	Strabismus
Peculiar fingers (tapering, slender, cylindrical)	Refractive errors (myopia, astigmatism)
Progressive talipes deformilies (valgus, planus, cavum)	Glaucoma/elevated intraocular pressure
Cutaneous	Microcornea/microphthalmia
Hyperextensibility/redundancy	Retinal detachment
Bruisability	*Hearing*
Fragility/atrophic scars	Hearing impairment
Fine/acrogeria-like palmar creases	*Neurological*
Hyperalgesia to pressure	Ventricular enlargement/asymmetry
Recurrent subcutaneous infections/fistula	*Development*
	Hypotonia/gross motor delay

ASD: atrial septal defect; MVP: mitral valve prolapse; MR: mitral valve regurgitation; AR: aortic valve regurgitation; ARD: aortic rot dilation.

disorder a form of EDS. However, ultrastructural findings in the skin from patients with ATCS and MCEDS were not consistent with those in patients with EDSKT, characterized by intact collagen fibrils not assembled regularly or tightly [Miyake et al., 2010]. For patients with ATCS, the skin was assessed as normal [Dündar et al., 2009]. For those with MCEDS, most collagen bundles were found to be small sized, some of which were composed of variable diameter collagen fibrils separated by irregular interfibrillar spaces [Malfait et al., 2010]. Ultrastructural and glycobiological studies on the skin from other patients as well as those on other affected tissues such as bone, muscle, and intestine would be necessary to delineate the wide spectrum of pathophysiology. Involvement of other DS-containing proteoglycans such as biglycan should also be investigated. Various malformations observed in the disorder might not simply be explained by connective tissue fragility, as they are considered to be inborn errors of development [Dündar et al., 2009; Zhang et al., 2010].

Based on the clinical, molecular, ultrastructural, and glycobiological data to date, D4ST1 deficiency is characterized by a unique set of clinical features consisting of progressive multisystem fragility-related manifestations and various malformations (Table 1). Further clinical and etiological evidences would solve the problem regarding which name should be the most appropriate: "Dermatan Sulfate-Deficient Adducted Thumb-Clubfoot Syndrome" or "D4ST1-Deficient EDS." Until then, we propose that the name "D4ST1-Deficient EDS (Adducted Thumb-Clubfoot Syndrome)" would be preferable.

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綜　説

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Discovery and Delineation of a New Type of Ehlers-Danlos Syndrome Caused by Dermatan 4-O-sulfotransferase Deficiency

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Key words: Ehlers-Danlos syndrome (Kosho Type), dermatan 4-O-sulfotransferase deficiency, decorin, arthrogryposis, connective tissue fragility

エーラスダンロス症候群（古庄型），デルマタン硫酸4-O-硫酸基転移酵素欠損症，デコリン，先天性多発関節拘縮，結合組織脆弱性

I　エーラスダンロス症候群とは

エーラスダンロス症候群（Ehlers-Danlos症候群；EDS）は，皮膚・関節の過伸展性，各種組織の脆弱性を特徴とする先天性疾患の総称であり[1]，古典型（Classical type），関節型（Hypermobility type），血管型（Vascular type），後側彎型（Kyphoscoliosis type），多発関節弛緩型（Arthrochalasia type），皮膚脆弱型（Dermatosparaxis type）の6つの主病型に分類されている。いずれもコラーゲン分子そのもの，または修飾酵素の遺伝子変異により生じる[2][3]。近年，主病型に属さない新たな病型が，その生化学的，遺伝学的基盤とともに相次いで発見されている（表1）[4]-[12]。最近我々は，顔貌上の特徴，先天性多発関節拘縮，進行性の結合組織脆弱性（皮膚弛緩，関節弛緩・変形，巨大皮下血腫など）を呈する全く新しいタイプのEDS（EDS, Kosho Type；EDSKT）を見出した。本稿では，患者さんとの出会い，原因遺伝子単離，病態解明，そして疾患概念確立に至る経緯を紹介する。

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II　患者さんとの出会いと原因遺伝子探索の道のり

1人目の患者さん（表2，3におけるEDSKT1）に出会ったのは，筆者が埼玉県立小児医療センター遺伝科に勤務していた2000年である。それまでに，同センターで古典型患者さんを診察する機会があり，皮膚が容易に裂け，関節脱臼を繰り返す，といった症状に悩まされているにも関わらず，医療者を含めた周囲の理解が得られず，また日本においてはほとんど専門家がいない「日の当たらない」疾患であることを感じていた。当時7歳の女児は，顔貌上の特徴，内反足を含む先天性多発関節拘縮，くも状指を有し，年齢とともに皮膚関節の過伸展性・脆弱性が目立ってきていた（図1）。初診時の担当医は，多発関節弛緩型との診断をしていた。筆者が再診で偶然担当し，詳しく経過を聞くと，転倒など軽微な外力で巨大な皮下血腫を生じる重症例であることが明らかになった。診察上も，独特な手足の変形や手掌の皺といったきわだった特徴を呈していた。以後1年弱の外来診療のなかで，原因検索として，典型的ではないが出血症状から血管脆弱性の可能性を考え，まずは血管型EDSの可能性を考えた。皮膚生検を行い，培養皮膚線維芽細胞を用いたIII型コラーゲン蛋白分析を千葉大学皮膚科簇持 淳助教授（現獨協医科大学皮膚科教授）に依頼した。結果，

表1　Ehlers-Danlos 症候群の分類

	頻度/患者数	遺伝形式	原因遺伝子
大病型			
古典型（Classical type）	1/20,000	AD	*COL5A1*, *COL5A2*
関節型（Hypermobility type）	1/5,000～20,000	AD	大多数は不明 少数例で TNXB
血管型（Vascular type）	1/50,000～250,000	AD	*COL3A1*
後側彎型（Kyphoscoliosis type）	1/100,000	AR	*PLOD*
多発関節弛緩型（Arthrochalasia type）	約30人	AD	*COL1A1**, *COL1A2**
皮膚脆弱型（Dermatosparaxis type）	8人	AR	*ADAMTS-2*
その他の病型			
Brittle cornea syndrome	11人	AR	*ZNF469*
EDS-like syndrome due to tenascin-XB deficiency	10人	AR	*TNXB*
Progeroid form	3人	AR	*B4GALT7*
Cardiac valvular form	4人	AR	*COL1A2*
EDS-like spondylocheirodysplasia	8人	AR	*SLC39A13*
D4ST-1-deficient EDS	22人	AR	*CHST14*

AD：常染色体優性遺伝，AR：常染色体劣性遺伝
COL5A1：V型プロコラーゲン α1鎖遺伝子，*COL5A2*：V型プロコラーゲン α2鎖遺伝子，*TNXB*：テナシンX遺伝子
PLOD：リジルヒドロキシラーゼ遺伝子，＊：スプライス異常によるエクソン6のスキップ
ADAMTS-2：プロコラーゲンI N-プロテイナーゼ遺伝子
ZNF469：コラーゲン生合成・組織化に関わる転写因子の遺伝子
B4GALT7：β4ガラクトース転移酵素-7（GalT-I）遺伝子
SLC39A13：亜鉛トランスポーター機能を持つタンパクの遺伝子
CHST14：デルマタン4-O-硫酸基転移酵素（D4ST-1）遺伝子

III型コラーゲンの産生は正常であり，血管型EDSは否定され，病型を確定することはできなかった。

2人目の患者さん（EDSKT2）とは，筆者が2003年に当院勤務となってからの出会いである。取り組むべきテーマを見つけられずにいたある日，福嶋義光部長（医学部遺伝医学・予防医学講座教授）に興味のある疾患について聞かれた。「EDSです」と答えると，「外勤先の療育センターに1人いる」とのことであった。26歳になっているはずの女性は，同センターへの通院を終了していた。カルテを見ると，出生直後から当院整形外科で入院・通院加療をしていることがわかった。古いカルテ庫から当院のカルテを取り出すと，EDSKT1と全く同様に出生時には内反足，手指関節拘縮，顔貌上の特徴を認め，その後，皮膚裂傷，関節脱臼，巨大皮下血腫など進行性の結合組織脆弱性を呈していた（図1）。

2人は，これまでの分類には当てはまらない新しい型のEDSであると直感し，論文を作成することにした。文献検索をすると，後側彎型（旧分類ではVI型）に類似した脊椎変形，皮膚や動脈の脆弱性を呈しているものの，後側彎型の病因であるlysyl hydroxylaseの欠損を認めないEDS VIB型（lysyl hydroxylaseの欠損を伴う通常の後側彎型をEDS VI Aと分類する場合）に分類されていたPakistan姉弟例[1)13)]に酷似した臨床像であることを見出した（図2）。Lysyl hydroxylaseの欠損の有無をスクリーニング（尿デオキシピリジノリン／ピリジノリン比）したが，予想どおり正常パターンであり欠損はなかった。そこで，暫定分類をEDS VI B型としてAmerican Journal of Medical Genetics誌に報告した[14)]。EDSKT2およびPakistan姉弟例の両親が血族結婚であったことから，常染色体劣性遺伝の可能性が推測された。病態解明へ向け，2005年度信州医学振興会医学研究助成および2007年度信州若手研究者萌芽研究支援事業の支援を得て，関連遺伝子解析を行ったが，原因遺伝子を同定することはできなかった。

新規疾患として確立するためにも，また，原因遺伝子を同定するためにも，新たな患者さんの収集が必要であった。そんな折，遠方に在住のEDS患者さんから「自分の病状は他の病型のだれとも違うと思うので，相談にのってほしい」と話を持ちかけられた。この患者さんがEDSKT3であり，自身の病型診断のために