


# The Ehlers–Danlos Syndromes, Rare Types

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Caroline van Mourik, B.Ed., M.Sc., Ph.D. (Biology and Geology), has been active in the Swedish patient-organization, previously as Chair but later mainly as the scientific editor. She even presented a paper at the first international symposium on EDS in Ghent, 2012 and frequently lectures about EDS, both for the medical field as well as laymen.

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The Ehlers–Danlos syndromes comprise a clinically and genetically heterogeneous group of heritable connective tissue disorders, which are characterized by joint hypermobility, skin hyperextensibility, and tissue friability. In the Villefranche Nosology, six subtypes were recognized: The classical, hypermobile, vascular, kyphoscoliotic, arthrochalasia, and dermatosparaxis subtypes of EDS. Except for the hypermobile subtype, defects had been identified in fibrillar collagens or in collagen-modifying enzymes. Since 1997, a whole spectrum of novel, clinically overlapping, rare EDS-variants have been delineated and genetic defects have been identified in an array of other extracellular matrix genes. Advances in molecular testing have made it possible to now identify the causative mutation for many patients presenting these phenotypes. The aim of this literature review is to summarize the current knowledge on the rare EDS subtypes and highlight areas for future research. © 2017 Wiley Periodicals, Inc.

**KEY WORDS:** Ehlers–Danlos syndromes; heritable connective tissue disorders; collagen

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## INTRODUCTORY STATEMENT

For each genetic EDS subtype, a subcommittee of authors performed a comprehensive literature search. All articles were reviewed for relevance and additional articles were identified from the literature. The articles were summarized and divided into themes: (1) the history of the EDS subtype, (2) mechanisms of disease, (3) allelic heterogeneity, (4) clinical description, (5) genotype–phenotype correlations and penetrance, (6) management, and (7) differential diagnosis. The summary of these themes was critically reviewed by all authors.

Subcommittees:

- Classical EDS due to *COL1A1* p.(Arg312Cys) (cEDS-*COL1A1*): Fransiska Malfait
- Classical-like EDS due to Tenascin-X deficiency (clEDS): Serwet Demirdas, Nicol Voermans
- Cardiac-valvular EDS (cvEDS): Fransiska Malfait
- Arthrochalasia EDS (aEDS): Tomoki Kosho, Cecilia Giunta, Marianne Rohrbach, Fransiska Malfait
- Dermatosparaxis EDS (dEDS): Tim Van Damme, Fransiska Malfait
- Kyphoscoliotic EDS (kEDS-*PLOD1*): Angela Brady, Neeti Ghali, Cecilia Giunta, Marianne Rohrbach, Tim Van Damme, Anthony Vandersteen
- Kyphoscoliotic EDS (kEDS-*FKBP14*): Cecilia Giunta, Marianne Rohrbach, Tim Van Damme, Fransiska Malfait

- Brittle cornea syndrome (BCS): Marianne Rohrbach, Tim Van Damme
- Spondylodysplastic EDS (spEDS-*B4GALT7* and spEDS-*B3GALT6*): Sylvie Fournel-Gigleux, Tim Van Damme, Fransiska Malfait
- Spondylodysplastic EDS (spEDS-*SLC39A13*): Cecilia Giunta
- Musculocontractural (mcEDS): Tomoki Kosho, Fransiska Malfait
- Myopathic EDS (mEDS): Roberto Mendoza-Londono, Fransiska Malfait
- Periodontal EDS (pEDS): Ines Kapferer-Seebacher, Michael Pope, Anthony Vandersteen, Johannes Zschocke

## CLASSICAL EDS DUE TO *COL1A1* p.(Arg312Cys) (*COL1A1*-cEDS)

Synonyms: Classic-like Ehlers–Danlos syndrome with propensity to arterial rupture, Vascular-like EDS

### The History of Classical EDS due to *COL1A1* p.(Arg312Cys) (*COL1A1*-cEDS)

Nuytinck et al. [2000] reported two children with a classical EDS phenotype, including skin hyperextensibility, easy bruising, atrophic scarring, and joint hypermobility, with a c.934C>T, p.(Arg312Cys) mutation. Malfait et al. [2007] identified the same mutation in an adult who suffered from a rupture of medium-sized arteries, reminiscent of vascular EDS. In addition, two other arginine-to-cysteine (Arg-to-Cys)

substitutions in the pro $\alpha$ 1(I) chain of type I collagen, c.1720C>T, p.(Arg574Cys) and c.3277C>T, p.(Arg1093Cys<sup>o</sup>), were identified in two other adults with vascular rupture, but without EDS-signs [Malfait et al., 2007]. The p.(Arg312Cys) mutation has subsequently been identified in two other individuals with EDS and complications of vascular fragility [Ritelli et al., 2013; Gaines et al., 2015]. In view of the major clinical overlap of the p.(Arg312Cys)-associated phenotype with classical EDS due to *COL5A1* or *COL5A2* mutations, both conditions are grouped within the same clinical entity (“Classical EDS”) in the new EDS classification. Patients with the p.(Arg312Cys) mutation are particularly at risk for vascular events, highlighting the benefit of molecular confirmation in classical EDS patients for management purposes.

*In view of the major clinical overlap of the p.(Arg312Cys)-associated phenotype with classical EDS due to *COL5A1* or *COL5A2* mutations, both conditions are grouped within the same clinical entity (“Classical EDS”) in the new EDS classification.*

The prevalence of this condition is unknown.

### Mechanism of Disease

The pathogenetic basis for the phenotype resulting from these specific Arg-to-Cys substitutions is currently not well understood. Ultrastructural studies of dermal collagen fibrils have shown fibrils with variable diameters, and slightly irregular contour, and, in case of the p.(Arg312Cys), flower-like abnormalities [Malfait et al., 2007].

Several mechanisms have been suggested to be involved in the pathogenesis [Malfait et al., 2007], including local destabilization of the triple helix due to loss of the stabilizing arginine residue; introduction of a cysteine residue, which can lead to disulfide-bonding with other collagenous or non-collagenous proteins, either intracellularly or in the extracellular matrix (ECM), thereby disturbing normal physiological interactions; interference with pericellular processing of the amino-propeptide of procollagen type I, and/or local unwinding of the region surrounding the mutations, thereby disturbing specific interactions with type I collagen ligands [Malfait et al., 2007].

### Allelic Heterogeneity

Three different heterozygous *COL1A1* mutations, leading to a Arg-to-Cys substitution have been reported in association with vascular fragility: c.934C>T, p.(Arg312Cys); c.1720C>T, p.(Arg574Cys); and c.3277C>T, p.(Arg1093Cys).

### Clinical Description

To date, six patients from five families have been reported with the p.(Arg312Cys) (Table S1). These include two females (respectively 5 and 43 years old) and five males (respectively 7, 16, 39, and 53 years old). [Nuytinck et al., 2000; Malfait et al., 2007; Ritelli et al., 2013; Gaines et al., 2015].

The hallmark of this condition is the “vascular fragility,” leading to spontaneous dissection or rupture of medium-sized arteries, in combination

with other EDS signs. It is a rare, but important differential diagnosis of vascular EDS.

#### *Reported clinical features for the p.(Arg312Cys) substitution*

- Reproductive, including pregnancy  
Premature preterm rupture of the fetal membranes (PPROM) was reported in one patient. One patient was reported to have neonatal hypotonia. No pregnancy-related complications were reported (one known pregnancy in an affected female).
- Craniofacial features  
None of the patients had characteristic features of vascular EDS. One patient was reported to have redundant skin folds on the eyelids and soft earlobes, reminiscent of classical EDS. One patient had blue sclerae, high palate, and hypoplastic uvula.
- Musculoskeletal system  
Generalized joint hypermobility was reported in four patients. One patient had congenital bilateral hip dislocation and a traumatic shoulder dislocation. Another patient was also reported to have sporadic joint dislocations. Pectus excavatum was reported twice. Two patients complained from chronic joint pain. None of the patients had a history of fractures; DEXA Z-score was normal in two patients.
- Skin and integument  
Skin involvement included skin hyperextensibility (n = 4); soft, doughy skin (n = 2); thin or translucent skin (n = 3); friable skin/skin splitting (n = 2); atrophic scars (n = 4); delayed wound healing (n = 1); unusual tenderness upon touch (hyperalgesia) (n = 1); piezogenic papules (n = 1); molluscoid pseudotumors (n = 1); varicose veins (n = 1). Easy bruising was reported in all patients (n = 6). Finally one patient had hiatal and abdominal and inguinal hernias.
- Ocular involvement  
Blue sclerae were observed on one patient. One patient was operated for strabismus.
- Cardiovascular system  
All three adults had severe cardiovascular complications. The patient reported by Malfait et al. [2007] had a

spontaneous dissection of the right iliac artery at 43 years. Her affected 16-year-old son did not have complications of vascular fragility, except for unusual bruising. The patient, reported by Gaines et al. [2015] suffered from a spontaneous rupture of the common iliac artery at 39 years. The patient, reported by Ritelli et al. [2013] had mild mitral and aortic valve regurgitation, left ventricular wall thickening, aortic root dilatation, vertebral artery tortuosity, and a hepatic hemangioma at 53 years. The two children reported by Nuytinck et al. [2000] did not show signs of vascular fragility at the time of report. Clinical follow-up is not available for these patients.

#### *Other Arg-to-Cys substitutions that lead to vascular fragility*

EDS-like signs have only been observed in association with the p.(Arg312Cys) mutation. One patient harboring a p.(Arg574Cys) (male, 42 years) suffered from a dissection of the left femoral artery and an aortic aneurysm. One patient harboring a p.(Arg1093Cys) (male, 40 years) had a left kidney infarction at age 34 years, and a dissection of the infrarenal aorta and left iliac artery, with aneurysm of the left renal artery at age 39 years. He also had mitral valve bulging [Malfait et al., 2007].

### Genotype–Phenotype Correlation and Penetrance

The Arg-to-Cys substitutions in the  $\alpha 1$  (I) collagen chain seem to be associated with specific phenotypes: Classical EDS with vascular fragility for p.(Arg312Cys) and isolated vascular fragility for p.(Arg574Cys) and p.(Arg1093Cys). In addition, three other Arg-to-Cys substitutions have been reported for this gene: c.3040C>T, p.(Arg1014Cys) is associated with autosomal dominant Caffey disease [Gensure et al., 2005], whereas c.3106C>T, p.(Arg1036Cys) and c.3196C>T, p.(Arg1066Cys) are reported in patients with an OI/EDS overlap phenotype without signs of vascular fragility [Cabral et al., 2007;

Lund et al., 2008]. The pathogenic basis for these specific clinical consequences is unknown.

Penetrance is unknown.

### Management

Key management guidelines focus on the cardiovascular system.

Specific management guidelines include:

- Measurement of aortic root size and assessment of heart valves by echocardiogram at the time of diagnosis or by age 5 years
- Echocardiogram at 5-year intervals, even if the initial echocardiogram is normal
- Vigilant observation and control of blood pressure can reduce the risk of arterial rupture
- Further vascular surveillance ought to be considered
- Consider bone densitometry evaluation

Guidelines for management of musculoskeletal problems, skin involvement, ophthalmologic and dental follow-up, and pregnancy should follow those formulated for other forms of EDS (for reference: See “management guidelines for the classical Ehlers–Danlos syndrome,” by Bowen et al., this issue).

### Differential Diagnosis

- Vascular EDS
- Classical EDS due to *COL5A1* or *COL5A2* mutations
- OI

### CLASSICAL-LIKE EDS DUE TO TENASCIN-X DEFICIENCY (cIEDS)

#### The History of the Classical-Like EDS

This type of EDS was first described by Burch et al. [1997]. The authors described a 26-year-old male patient with congenital adrenal hyperplasia

(CAH) due to 21-hydroxylase deficiency as well as hyperextensible skin, hypermobile joints, easy bruising, and poor wound healing. Skin biopsy of this patient showed small collagen fibers of normal shape and a complete absence of tenascin XB (TNX). Cultured dermal fibroblasts also lacked TNX. PCR of the patients DNA revealed a 30 kb deletion in chromosome 6 overlapping both the *CYP21B* gene and the *TNXB* gene. Subsequently, Schalkwijk et al. [2001] measured TNX in the serum of 151 EDS patients (classical and vascular type), 168 diseased controls (psoriasis and rheumatoid arthritis), and 21 healthy controls. They detected a complete TNX deficiency in five EDS patients and three affected siblings (one of which with the contiguous gene syndrome). Mutation analyses revealed mostly homozygous mutations in the *TNXB* gene for these patients. The authors concluded that the TNX-deficient type is very similar to the classical type of EDS with two major differences: (1) No atrophic scarring was apparent, and (2) the inheritance pattern was autosomal recessive.

The prevalence of this condition is unknown.

#### Mechanism of Disease

TNX is one of the three known large matricellular proteins of the tenascin family [Mao and Bristow, 2001; Valcourt et al., 2015]. Although the precise function of TNX is unknown, it is known to play a role in the ECM as it is highly expressed in connective tissue of muscle, around tendons, ligaments, and in skin [Mao and Bristow, 2001]. The glycoprotein is encoded by the *TNXB* gene in humans. Classical-like EDS is caused by a complete lack of TNX due to homozygous or compound heterozygous *TNXB* mutations, that lead to nonsense-mediated mRNA decay (NMD), or biallelic deletion of *TNXB*. As a result, the TNX protein is completely absent [Mao and Bristow, 2001].

### Allelic Heterogeneity

A total of 24 patients with a complete TNX-deficiency without involvement of the *CYP21B* gene have been reported. In 19 of these patients (15 families), the molecular diagnosis is known. Homozygous and compound heterozygous mutations have been identified. Mutations have been identified throughout the *TNXB* gene, and include missense, frameshift, and nonsense mutations.

There is a registry of reported *TNXB* gene variants [Dalglish, 1998].

In five out of the 24 reported patients, no genetic confirmation of the clinical diagnosis after TNX serum measurement was performed.

### Clinical Description

The clinical phenotype of the 24 patients with a complete TNX-deficiency without involvement of the *CYP21B* gene was reviewed (Table S1) [Schalkwijk et al., 2001; Peeters et al., 2004; Lindor and Bristow, 2005; Voermans et al., 2007, 2009b; O’Connell et al., 2010; Hendriks et al., 2012; Péniisson-Besnier et al., 2013; Sakiyama et al., 2015; Demirdas et al., 2016].

The absence of TNX throughout the body leads to a phenotype resembling the classical type of EDS. The hallmarks of the disorders are GJH, hyperextensible, soft and/or velvety skin, without the typical atrophic scarring seen in classical EDS, easy bruising, and an autosomal recessive inheritance pattern.

- Reproductive, including pregnancy  
Egging et al. [2008] retrospectively investigated genitourinary and obstetric complications in seven women with classical-like EDS, aged 38–57 years (six from the cohort of Schalkwijk et al. [2001] and Patient 1 from the series of Lindor and Bristow [2005]). These women have had a total of 13 pregnancies and 12 deliveries. Two women (one with CAH due to 21-hydroxylase deficiency as a contiguous gene syndrome; one with additional spina bifida who decided to have no biological

children) did not have any pregnancies. One out of 13 pregnancies ended in intrauterine death of the fetus, and one out of 12 deliveries was complicated with post-partum hemorrhage. No premature births or neonatal complications were reported. No urinary incontinence was seen. Vaginal ( $n=1$ ), uterine ( $n=2$ ), and rectal ( $n=1$ ) prolapse were present. One undefined prolapse was mentioned. Three out of five women had partus-related complications (vaginal uterine extirpation after uterine prolapse, post-partum hemorrhage, intra-uterine death at 24 weeks with post-partum hemorrhage, and precipitous second stage during at term deliveries). The authors concluded that pregnancy is without major complications in TNX-deficient patients, apart from one incident of postpartum hemorrhage. However, uterine and vaginal prolapse regularly occurs in TNX-deficient women, even at a young age, suggesting laxity of the genitourinary tissues. Furthermore, no premature births have been observed in the offspring; however, some patients had been born prematurely themselves.

Demirdas et al. [2016] described the gynecologic and obstetric history of seven women in their cohort. Three of these women had previously been included in the study by Egging et al. [2008]. The other four women reported a total of 10 pregnancies, two of which ended in intrauterine demise of the fetus. Furthermore, four out of 10 deliveries were complicated with post-partum hemorrhage, two women had perineal rupture, and one pregnancy was complicated by PPRM. In this cohort, another woman was reported to have pelvic instability without ever having been pregnant [Egging et al., 2008].

Obviously, some caution must be taken in making conclusions and extrapolating data from such a small group of TNX-deficient patients ( $n=11$ ) [Egging et al., 2008].

- Age of onset

Symptoms of patients with the TNX-deficiency have been described as starting as early as 5 years of age in a girl by Hendriks et al. [2012] and 7 years

of age by O'Connell et al. [2010]. Furthermore, in some adult cases, a childhood onset of hypermobility and dermatological symptoms was reported. Demirdas et al. [2016] reported that all patients ( $n=17$ ) had a clinical onset in childhood, ranging from the neonatal age to puberty. The most encountered initial symptoms in this group were (sub)luxations, hypermobility, and velvety/hyperextensible skin. Based on the data we found, we conclude that patients already experience symptoms such as skin hyperextensibility, a velvety skin, easy bruising/spontaneous ecchymosis, subluxations, and joint hypermobility in childhood. Piezogenic papules of the feet and pes planus are also apparent at the pediatric age.

- Craniofacial features

Craniofacial features reported in the patients included a slight asymmetry of the face ( $n=1$ ), lax skin of the cheeks ( $n=1$ ), and a narrow and/or high arched palate ( $n=4$ ).

- Musculoskeletal system

Frequently described musculoskeletal features included joint and/or muscular pains. Furthermore, one paper reported frequently observed deformities of the hands and feet. Twelve out of 17 included patients had pes planus and four patients had short/broad feet with brachydactyly of the toes. Four of the patients had deformities of the fingers and acrogeric hands [Demirdas et al., 2016].

- Skin and integument

It is noted that all reported patients had hyperextensible skin, frequently described to be soft and velvety of structure. The atrophic scars typical for classical EDS were not observed. Bilateral inguinal hernia was described in a male patient and unilaterally in a female patient. An umbilical hernia was described in a 6-year-old male.

- Ocular involvement

Ocular involvement was infrequently reported in patients with TNX-deficient classical-like EDS. One patient was described to have esotropia/amblyopia [Lindor and Bristow, 2005], another to have astigmatism, and a third patient was described to have bilateral conjunctivochalasis. Five out of 17

patients from the cohort of Demirdas et al. [2016] were described to have frequent subconjunctival hemorrhage.

- Dental involvement

Recurrent periodontitis was reported in one, and another patient was described to have dental crowding due to a narrow palate.

- Cardiovascular system

Peeters et al. [2004] investigated the cardiac features in seven TNX-deficient patients (all from the cohort of Schalkwijk et al. [2001]). They found a systolic murmur at the apex in one patient. Three of the seven patients were found to have mitral valve abnormalities (billowing of the mitral valve in two patients of the same family and a severe mitral valve prolapse (MVP) in another patient). Although the number of patients was small and such abnormalities are not infrequent in the general population, the authors recommended echocardiography at baseline and if a cardiac murmur appears [Peeters et al., 2004]. Subsequently, Lindor and Bristow [2005] also described a patient who had mitral valve surgery due to a MVP. Demirdas et al. [2016] also described cardiologic features of their patients. Four out of 17 of the patients had hypertension and two patients (4/17 patients were previously included by Peeters et al. [2004]) had mitral valve abnormalities. One patient developed a post-partum cardiomyopathy [Demirdas et al., 2016].

- Gastrointestinal system

Two patients reported by Lindor and Bristow [2005] suffered from bleeding of gastrointestinal structures, namely the sigmoid and duodenum, secondary to diverticulitis and as complications of spontaneous ileus. Hendriks et al. [2012] reported a gastric hemorrhage due to ulcers in a male patient initially reported by Schalkwijk et al. [2001]. He died at the age of 57 years due to a septic shock following elective mitral valve replacement surgery, which was complicated by a sinuspiriformis perforation by a transesophageal ultrasound probe [Hendriks et al., 2012; Knuijt et al., 2014]. Sakiyama et al. [2015] also presented a patient who had recurrent gastrointestinal perforation due to tissue fragility (diverticulitis, spontaneous ileus

and a subsequent perforation of the duodenum). Demirdas et al. [2016] did not observe severe gastrointestinal problems in their patients. However, one patient had a gastric ulcer at age 16 and a bowel perforation due to diverticulitis at age 48 years [Demirdas et al., 2016].

- Neuromuscular features and motor development

A total of six articles describe research concerning muscular function in patients with TNX-deficiency or their muscle tissue [Voermans et al., 2007, 2009b; Ottenheijm et al., 2012; Gerrits et al., 2013; Pénişon-Besnier et al., 2013; Sakiyama et al., 2015]. All papers conclude that there is some degree of muscle weakness in patients with TNX-deficiency. Voermans et al. [2007] studied a cohort of 40 EDS patients, among which 10 with TNX-deficiency (six of the cohort of Schalkwijk et al. [2001], and four additional patients). Muscle weakness, myalgia, and easy fatigability were reported by the majority of patients, whereas all patients were able to walk independently without aids. Mild-to-moderate muscle weakness (80%) and reduction of vibration sense (60%) were common. Other findings were axonal polyneuropathy (40%) on nerve conduction studies and mild myopathic features on muscle biopsy (20%). Patients with hypermobile EDS (hEDS) caused by TNXB haploinsufficiency were less affected [Voermans et al., 2009b]. This was confirmed in a quantitative study on isometric function of the thigh muscles in seven patients (four of the cohort of Schalkwijk et al. [2001] and three of the other patients in the study of Voermans et al. [2009b] and Gerrits et al. [2013]). The results showed that muscle weakness in this type of EDS is most likely due to increased compliance of the series-elastic component of muscle tissue and failure of maximal voluntary muscle activation. Further proof of this concept was obtained in single fiber study of muscle tissue of four of these patients

(one of the cohort of Schalkwijk et al. [2001] and three of the other patients in the study of Voermans et al. [2009b] showing that in response to the increased compliance of the extracellular matrix in muscle of TNX-deficient EDS patients, a marked intracellular stiffening of the sarcomere protein titin occurs. The stiffening of titin most likely compensates for the muscle weakness [Ottenheijm et al., 2012]. The report by Pénişon-Besnier et al. [2013] presented a 42-year-old male patient with proximal limb muscle weakness, subclinical heart involvement, minimal skin hyperextensibility, no joint abnormalities, and a history of easy bruising. He had been asymptomatic until age 30 but mentioned low performances at upper bodybuilding exercises. Since then, he experienced gradually worsening lower limb weakness, leading to inability to run from age 40, frequent falls, and the recent need of a banister for stairs. He was initially diagnosed as having a primary myopathy and only later diagnosed with EDS [Pénişon-Besnier et al., 2013].

Motor development was not studied in the included papers. We detected no comments on delayed motor development other than mild to moderate muscle weakness.

- Neurological features and neuromotor development

Cognitive development was not studied in the included papers. Intellectual disability was not reported.

- Other

Severe edema of the ankles and/or feet was described in four patients.

- Family history

Demirdas et al. [2016] asked their patients about family history and found that 4/11 mothers and 5/11 fathers complained of (sub)luxations (n = 4), pes planus (n = 2), easy bruising (n = 1), arthralgia (n = 4), hyperextensible skin (n = 3), hypermobility (n = 1), and inguinal hernia (n = 1). Of the 17 patient's siblings, 11 out of 26 were tested and proven heterozygous carriers. Three of these 11 carrier siblings

reported hypermobile joints [Demirdas et al., 2016].

## Genotype–Phenotype Correlations and Penetrance

No genotype–phenotype correlations are reported within the group. We assume that penetrance is high, but the research to support this assumption is lacking. Family members with a haploinsufficient mutation in the *TNXB* gene have been described to have symptoms of joint hypermobility in 60% of the cases, but more research is needed to confirm this [Zweers et al., 2003].

## Management

No specific guidelines for management of patients with classical-like EDS are available. Guidelines for management of musculoskeletal problems, skin involvement, cardiovascular involvement, and pregnancy should follow those formulated for other forms of EDS (for reference: See “management guidelines for the classical Ehlers–Danlos syndrome,” by Bowen et al., this issue).

Specific management guidelines may include:

- Musculoskeletal

In case of operations, special attention for the effect of general anesthesia and of adequate positioning and support is important to prevent pressure or stretch neuropathies [Voermans et al., 2006]. Furthermore, intubation and endoscopic studies should be performed carefully in order to prevent rupture of trachea or esophagus [Besselink-Lobanova et al., 2010; Hendriks et al., 2012]

- Pregnancy

Gynecological follow-up throughout pregnancy is not warranted based on the retrospective study in five patients [Egging et al., 2008]. However, the severe complications that have been reported during insertion of a trachea tube and a transesophageal ultrasound probe call for a very careful handling of patients, especially in emergency

situations. Therefore, we advise a clinical delivery for all patients

### Differential Diagnosis

- Classical EDS
- Congenital myopathies, including collagen VI- and collagen XII-related disorders

## THE CONTIGUOUS GENE SYNDROME WITH CONGENITAL ADRENAL HYPERPLASIA AND TNX-DEFICIENCY

Burch et al. [1997] first described the TNX-deficient pheno- and genotype in a male patient (26 years old) with hyperextensible skin, hypermobile joints, easy bruising and 21-hydroxylase deficiency. A heterozygous 30 kb deletion was found on chromosome 6 involving both the *CYP21B* gene and the *TNX* gene as a causative explanation for all symptoms. They also reported that TNX in serum, skin and muscle was measured and absent. The authors concluded that a small deletion or missense mutation had probably remained undetected on the maternal allele [Burch et al., 1997].

The second patient with this Continuous gene syndrome was included in the initial series of Schalkwijk et al. [2001] (Patient 3) and subsequent cohorts [Egging et al., 2008; Voermans et al., 2009b; Gerrits et al., 2013]. She was homozygous for the 30-kb deletion detected in the index case reported by Bristow. Both her parents and two siblings were heterozygous for the deletion and were clinically normal, providing evidence of recessive inheritance in this family. This 32-year female patient was described to have recurrent (sub) luxations, hypermobile joints, hyperextensible, and velvety skin that easily bruises, musculoskeletal pain, and CAH. Besselink-Lobanova et al. [2010] presented the follow-up of this case in order to draw attention to the severe complications encountered

during intubation. During elective surgery at the age of 41 years (because of a luxation of the left knee joint) a tracheal rupture developed, despite the initially uneventful intubation. The authors acknowledge that the patient had other anatomic risk factors for a tracheal rupture (obesity and short stature for example). However, the authors also state that TNX alters the characteristics of the ECM and therefore advise caution when intubating patients with TNX-deficiency, or even to refrain from intubating entirely [Besselink-Lobanova et al., 2010].

### *TNXB* Haploinsufficiency

Zweers et al. [2003] studied the 20 heterozygous family members of the index cases in Schalkwijk et al. [2001] regardless of clinical symptoms. In all of these individuals, significantly reduced serum TNX levels were detected, and in 17 of them, they confirmed heterozygosity for a truncating *TNXB* mutation. Clinical examination in these family members showed generalized joint hypermobility (GJH) in nine family members (45%; all female). Skin hyperextensibility and easy bruising, frequently seen in the individuals with complete TNX deficiency, were absent. Subsequently, they measured serum TNX levels (by ELISA) in an unselected cohort of 80 patients with hEDS. Six of these patients (7.5%; all female) had serum TNX levels more than 2.5 SD below the mean for unaffected individuals. Clinically, patients with reduced TNX levels showed hypermobile joints, often associated with joint subluxations and chronic musculoskeletal pain. The authors concluded that *TNXB* haploinsufficiency is associated with hEDS [Zweers et al., 2003].

Merke et al. [2013] investigated the prevalence of a Continuous gene syndrome in a cohort of 192 consecutive unrelated CAH patients. *TNXB* haploinsufficiency, here termed CAH-X syndrome, was present in 13 patients (and two sibs). Twelve of 91 patients carrying a *CYP21A2* deletion (13%)

carried a contiguous deletion that extended into *TNXB*. One patient carried a *TNXB* premature stop codon. Twelve of 13 patients with CAH-X had EDS clinical features. Patients with CAH-X were more likely than age-matched controls to have joint hypermobility ( $P < 0.001$ ), chronic joint pain ( $P = 0.003$ ), multiple joint dislocations ( $P = 0.004$ ), a structural cardiac valve abnormality by echocardiography ( $P = 0.02$ ), and reduced TNX expression by Western blot and immunostaining. Piezogenic papules on the feet were also observed. A subset of parents was investigated (five mothers, two fathers), of which three had GJH with a Beighton score of 5 or more [Merke et al., 2013].

Morissette et al. [2015] investigated the genetic background of this cohort in the same natural history study ( $n = 246$ ). Seven families (10 patients) harbored a novel *TNXB* missense variant c.12174C>G,p.(Cys4058Trp) and had a clinical phenotype consistent with hEDS. Fourteen CAH probands carried previously described *TNXA/TNXB* chimeras, resulting in a CAH-X prevalence of 8.5%. This highly conserved pseudogene-derived variant in the *TNX* fibrinogen-like domain is predicted to be deleterious and disulfide-bonded, resulting in reduced dermal elastin and fibrillin-1 staining and altered TGF-1 binding, and represents a novel *TNXA/TNXB* chimera. TNX protein expression was normal in dermal fibroblasts, suggesting a dominant-negative effect. They concluded that the CAH-X syndrome is commonly found in CAH due to 21-hydroxylase deficiency and may result from various etiological mechanisms [Morissette et al., 2015]. Patients in this cohort were considered to be heterozygous for the *TNXB* deletion, and had reduced but not absent TNX levels in serum. Therefore, this phenotype is likely be more in line with what is reported by Zweers et al. [2003] in patients with reduced serum levels of TNX, and with the reported features in some of the sibs who are obligate carriers of the mutation in their affected family member.

## CARDIAC-VALVULAR EDS (cvEDS)

### History of Cardiac-Valvular EDS

In 1987 and 1988, Sasaki et al. [1987], Kojima et al. [1988], and Hata et al. [1988] reported two Japanese patients with complete absence of the pro $\alpha$ 2(I) collagen chains, both presenting EDS-like features, including joint hypermobility, skin hyperextensibility, abnormal wound healing, but also cardiac-valvular problems. Nicholls et al. [2001] reported a total absence of  $\alpha$ 2(I) collagen chains in a 9-year-old girl with phenotypic manifestations of both OI and EDS, but without cardiovascular anomalies. A homozygous splice site mutation led to the introduction of a premature termination codon (PTC). Schwarze et al. [2004] reported four patients from three independent families (including the patient reported by Kojima et al. [1988]) with a rare, recessively inherited form of EDS, characterized by joint hypermobility, skin hyperextensibility, and severe cardiac-valvular defects, resulting from biallelic *COL1A2* mutations, leading to complete absence of pro $\alpha$ 2(I)-chains. Because of the severe cardiac valve problems in most of the adult patients, this phenotype was called “cardiac-valvular EDS” [Schwarze et al., 2004]. One additional child with this condition was subsequently reported by Malfait et al. [2006].

The exact prevalence of this rare condition is unknown.

### Mechanism of Disease

The biallelic *COL1A2* mutations result in the complete absence of pro $\alpha$ 2(I)-chains. Cells from affected individuals produce type I collagen molecules that contain only pro $\alpha$ 1(I) chains. The mRNA from the mutant alleles is unstable and degraded so that no protein is produced. Nicholls et al. [1984] reported a 5-year-old boy with severe, progressively deforming OI (OI type III). No cardiac abnormalities were reported in this patient [Nicholls et al., 1984]. A homozygous 4-nucleotide frame shift deletion within the carboxy (C)-terminal propeptide of pro- $\alpha$ 2(I) collagen was identified. This mutation

escapes nonsense-mediated mRNA decay (NMD) and the mRNA is stable, but the pro $\alpha$ 2(I) chains fail to fold normally and are degraded, which eventually also results in the production of pro- $\alpha$ 1(I) homotrimers [Pihlajaniemi et al., 1984]. Given that pro $\alpha$ (I) homotrimer formation alone does not lead to OI, the OI phenotype in the latter patient suggests that the intracellular accumulation of mutant pro $\alpha$ 2(I) chains and the cellular alterations, resulting from a high rate of degradation of these chains, contributes to the skeletal phenotype. In contrast, the EDS phenotype that results from unstable mRNA and no pro $\alpha$ 2(I) chains reflects what appears to be a more limited response in the ECM.

### Allelic Heterogeneity

Seven different mutations have been reported in five independent cardiac-valvular EDS patients. These include one homozygous nonsense mutation (c.213dupC,p.(Arg99\*)), and six splice site mutations (two homozygous (c.3105+2T>C and c.3601G>T)) and two compound heterozygous (c.70+717A>G; c.1404+1G>A and c.540+5G>A; c.1404G>C). The patient reported by Sasaki et al. [1987] was only analyzed at the biochemical level and no molecular data were reported.

### Clinical Description

To date, six patients from five independent families have been reported (Table S1). Their age at diagnosis ranged from 6 to 65 years [Hata et al., 1988; Kojima et al., 1988; Nicholls et al., 2001; Schwarze et al., 2004; Malfait et al., 2006].

The hallmark of the condition is the severe cardiac-valvular disease, necessitating valve replacement surgery at adult age, in conjunction with variable skin hyperextensibility, atrophic scarring and joint hypermobility, and autosomal recessive inheritance.

#### • Musculoskeletal system

Three patients were reported to have GJH, whereas in the other three the hypermobility was restricted to the

small joints. One patient had shoulder dislocations, pectus excavatum, and muscle and tendon tears. One patient had recurrent patellar dislocations. Foot deformities were reported in three patients: A 6-year-old boy displayed pes planus with valgus heels, hallux valgus, and subluxations of the toes; a 9-year-old girl had pedes palonvalgi with secondary shortening of Achilles tendon; and in an adult man pes planus and calcaneovalgus were reported. One patient had increased bone fragility.

#### • Skin and integument

Reported skin abnormalities included: Skin hyperextensibility (n = 4) (ranging from mild to severe), soft skin (n = 2), atrophic scar formation (classical EDS-like) (n = 2), easy bruising (n = 2) delayed wound healing (n = 1), thin skin (n = 1), striae (n = 1). Inguinal hernia was reported in two males, including congenital bilateral inguinal hernia in one.

#### • Ocular involvement

Myopia and astigmatism was reported in one patient; one patient was reported to have blue sclerae.

#### • Cardiovascular system

All four reported adults had severe cardiac-valvular problems, resulting in valve replacement surgery. A 45-year-old male had severe mitral valve regurgitation due to MVP, resulting in left atrium and ventricle dilatation and mild ventricular hypertrophy. He also had aortic valve insufficiency, eventually necessitating mitral and aortic valve replacement. Post-surgery, there was dehiscence of the mitral annulus from the ventricle, and of the aortic valve from the atrioventricular groove. Finally, there was massive leakage through the left ventricular myocardium with disintegration of the entire left ventricle, from which the patient died. A 65-year-old woman had mitral valve insufficiency with uncomplicated replacement surgery. A 30-year-old male had a secundum-type atrial septum defect (ASD), MVP with regurgitation, and aortic valve regurgitation. He underwent mitral and aortic valve replacement with no complications. His 25-year-old brother had aortic valve replacement because of aortic insufficiency [Schwarze et al., 2004].



The two reported children had no severe cardiovascular features, although mitral valve bulging was noted in one [Nicholls et al., 2001; Malfait et al., 2006].

### Genotype–Phenotype Correlations and Penetrance

All mutations result in complete absence of the pro $\alpha$ 2(I) chains. There are no reported genotype–phenotype correlations. Obligate carriers displayed no overt symptoms. Penetrance is presumably complete.

### Management

Key management guidelines focus on the cardiovascular system.

Specific management guidelines include:

- Measurement of aortic root size and assessment of heart valves by echocardiogram at the time of diagnosis or by age 5 years
- Yearly echocardiogram, even if the initial echocardiogram is normal
- Cardiac valve replacement surgery
- Consider bone densitometry evaluation

Management guidelines for musculoskeletal problems, skin, ophthalmologic and dental follow-up, and pregnancy should follow those formulated for other forms of EDS (for reference: See “management guidelines for the classical Ehlers–Danlos syndrome,” by Bowen et al., this issue).

### Differential Diagnosis

- Classical EDS
- Hypermobile EDS

## ARTHROCHALASIA EDS (aEDS)

Synonyms: Ehlers–Danlos syndrome, type VII (VIIA, VIIB); Arthrochalis multiplex congenita

### The History of EDS Arthrochalis Type

Hass and Hass [1958] proposed presence of a distinct entity of congenital flaccidity of the joints, which they called “arthrochalis multiplex congenita” and which may or may not involve skin changes. In 1973, three patients with the condition “EDS VII” were reported with accumulation of procollagen in their skin and tendon [Lichtenstein et al., 1973a]. The disorder was therefore supposed to be caused by a defect in the conversion of procollagen to collagen, resembling dermatosparaxis in cattle, and the activity of the converting proteinase in the cultured fibroblasts from these patients was found to be reduced to between 10 and 40% of normal [Lichtenstein et al., 1973b]. However, Steinmann et al. [1980] demonstrated, through reinvestigation of the fibroblasts from one of the patients, the presence of mutant pN $\alpha$ 2(I) collagen chains (precursor procollagen chains in which the (C)- but not the N-propeptide is cleaved off) in collagen extracted from skin or produced by fibroblasts and the normal activity of procollagen N-proteinase in cell extracts. They concluded the condition to be caused by a structural abnormality in the portion of the pro- $\alpha$ 2(I) chain that is normally cleaved by N-proteinase (and other proteinases) [Steinmann et al., 1980]. Subsequently, Cole et al. [1987] found mutant pN $\alpha$ 1(I) in a patient with similar features. EDS VII was, therefore, subdivided into types VIIA and type VIIB, depending on whether the  $\alpha$ 1(I) or the  $\alpha$ 2(I) chain is affected, respectively [Beighton et al., 1998].

Prevalence of this condition is unknown.

### Mechanism of Disease

Arthrochalis EDS is caused by heterozygous mutations in either *COL1A1* (previously EDS type VIIA) or *COL1A2* (previously EDS type VIIB). Heterozygous mutations

that lead to entire or partial loss of exon 6 of either *COL1A1* or *COL1A2* determines lack of the N-telopeptide linking the N-propeptide to the major triple helical domain of the  $\alpha$ 1(I) or the  $\alpha$ 2(I) chain. Deletion of the respective 24 and 18 amino acid residues in the pro- $\alpha$ 1(I) and the pro- $\alpha$ 2(I) chain results in loss of the small globular region of the N-propeptide (present only in the pro- $\alpha$ 1(I) chain), the procollagen N-proteinase cleavage site (Pro-Gln and Ala-Gln at positions 4–5, respectively), the cross-linking lysine residue at position 13 and 9, respectively, of the N-telopeptide and the first triplet of the main helical Gly-X-Y domain [Giunta et al., 1999].

### Allelic Heterogeneity

Most of the mutations are splice site mutations leading to skipping of exon 6 in *COL1A1* (intron 5–2A>G/T; intron 5–1G>A/C/T; exon 6–1G>A/C) or *COL1A2* (intron 5–2A>G; intron 5–1G>A/C; exon 6–1G>A; intron 6+1G>A/T/C; intron 6+2T>C/G) [Steinmann et al., 2002]. Genomic deletions of exon 6 [Byers et al., 1997] and exon 5+6 [Nicholls et al., 2000] were also reported.

There is a registry of reported *COL1* gene variants [Dagleish, 1998].

### Clinical Description

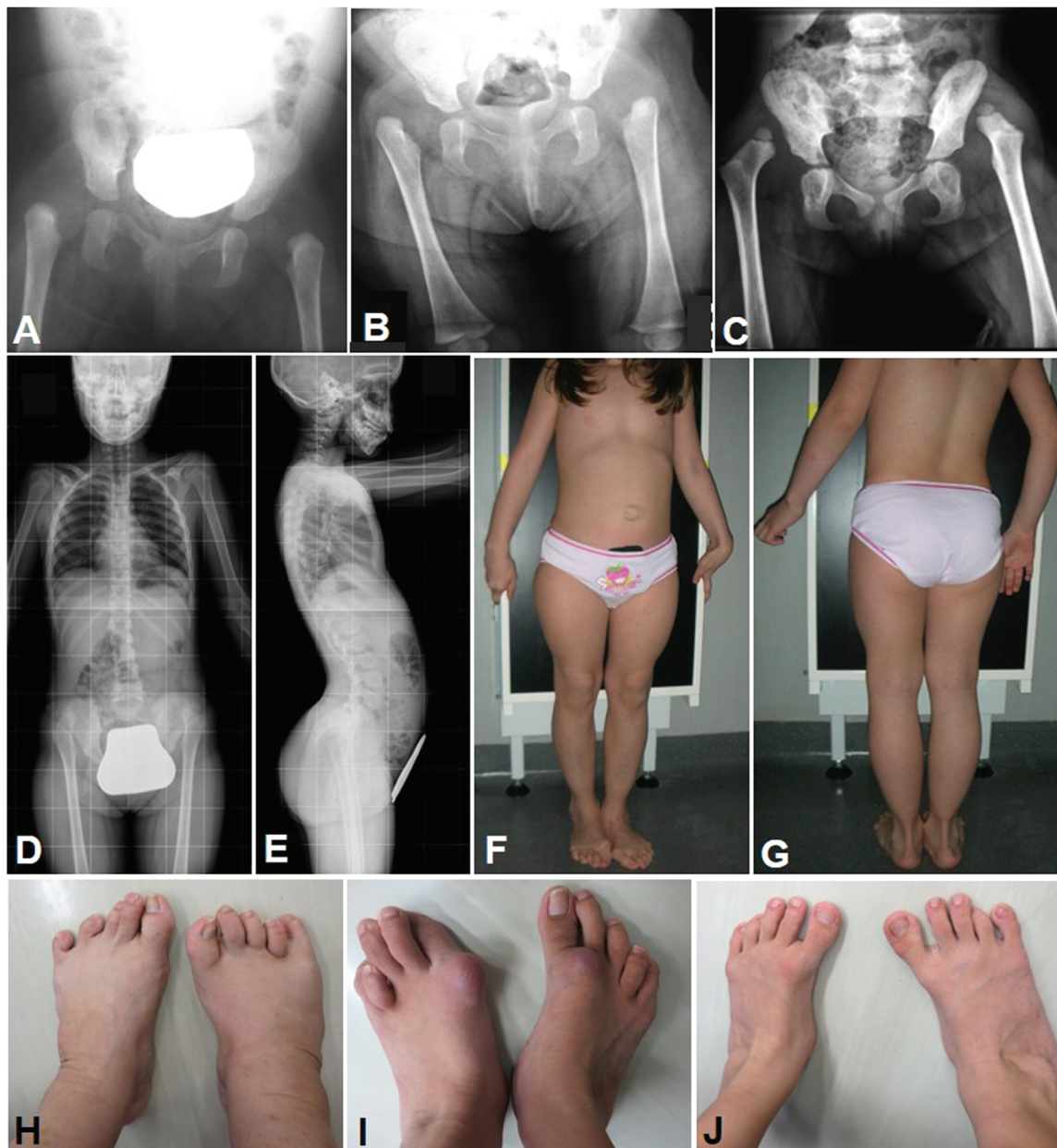
At present, 49 patients from 36 families have been published (Table S1). The ages at the publication ranged from 2.5 months to 46 years (n = 35; Median age, 7.5 years of age) [Steinmann et al., 1980; Eyre et al., 1985; Cole et al., 1986; Viljoen et al., 1987; D’Alessio et al., 1991; Nicholls et al., 1991; Vasani et al., 1991; Chiodo et al., 1992; Pope et al., 1992; Carr et al., 1994; Ho et al., 1994; Lehmann et al., 1994; Byers et al., 1997; Giunta et al., 1999; Hudgins et al., 1999; Nicholls et al., 2000; Whitaker et al., 2009; Giovannucci Uzielli et al., 2011; Klaassens et al., 2012; Giunta and Steinmann, 2014; Hatamochi et al., 2014].

The hallmarks of the disorder are severe generalized joint hypermobility, congenital bilateral hip dislocation, and recurrent subluxations and dislocations of both small and large joint [Steinmann et al., 2002] (Representative

picture of the phenotype are given in Fig. 1).

- Reproductive, including pregnancy  
At least four affected women were reported to be pregnant, and to deliver

a total of 11 children, including seven affected ones. Pregnancy or delivery-related complications included breech presentation ( $n = 4$ ), PPRM ( $n = 2$ ), polyhydramnios ( $n = 2$ ), and decreased fetal movement ( $n = 2$ ).



**Figure 1.** Clinical photographs and radiological images of patients with aEDS. A girl (A–G) with a  $c.279+1G>$  mutation in *COL1A2* and a mother and her two sons (H–J) with a  $c.279+2T>C$  in *COL1A2*. (A) X-rays of the hip at age 7 days showing congenital bilateral hip dislocation and femoral and acetabular deformities. (B and C) Status of the hip dislocation at age 5 months and 3 years, respectively. (D) An anteroposterior total body radiograph at age 11 years showing unsuccessful treatment of the hip dislocation with harness and bracing. (E) A left lateral total body radiograph at age 11 years showing lumbar lordosis. (F and G) The patient at age 9 years with umbilical hernia, lordotic posture of the spine, and foot deformities. Foot deformities of an affected mother at age 38 years (H), her first son at age 14 years (I), and her second son at age 5 years (J) (Images A–G kindly provided by Prof. Maria Luisa Giovannucci-Uzielli, with permission).

- **Craniofacial characteristics**  
Characteristic craniofacial features include large fontanelle (n = 6), frontal bossing (n = 9), hypertelorism (n = 4), blue sclerae (n = 3), epicanthal folds (n = 3), depressed nasal bridge (n = 6), midfacial hypoplasia (n = 3), and micrognathia (n = 6).
- **Musculoskeletal features**  
Congenital bilateral hip dislocation was described in all reported patients (n = 41). One unreported patient is known to have had a unilateral congenital hip dislocation (Byers et al, personal communication). Joint hypermobility (n = 29) and recurrent dislocations or subluxations affecting both large and small joints (n = 26) were frequent. Foot deformities (n = 15), including pes equinovarus (n = 8), pes planus (n = 7), pes valgus (n = 2) and hallux valgus (n = 6) (Fig. 1), and spinal deformities (n = 13), including scoliosis (n = 9), kyphoscoliosis (n = 2), and lordosis (n = 3), were also frequent. Swan neck deformity of hands was described in several adults (n = 3). Pectus excavatum was observed in some (n = 3). Fractures (n = 9) and Wormian bones on cranial radiographs (n = 5) suggested bone fragility, similar to patients to mild OI.
- **Skin and integument**  
The skin was often described to be hyperextensible; hyperelastic or redundant (n = 26); soft, doughy, or velvety (n = 17); and/or fragile (n = 9). Easy bruising (n = 12), atrophic scarring (n = 10), abnormal wound healing (n = 3), and crisscross patterning of palms/soles (n = 4) were also noted. Umbilical hernia was sometimes described (n = 8).
- **Ocular involvement**  
Blue sclerae (n = 3) and ectopia lentis (n = 1) were recorded.
- **Dental involvement**  
Dentinogenesis imperfecta was recorded in a few patients (n = 3).
- **Neuromuscular features and motor development**  
Motor developmental delay was recorded in 16 patients, six of whom were not ambulatory at the time of publication because of hypotonia and/or foot deformity.

- **Neurological features and neurodevelopment**  
Mild learning disability was recorded in a patient with leptomeningocele and intracranial hemorrhage, presenting with seizures, the 3rd cranial nerve palsy, left hemiparesis, and left homonymous hemianopsia.

### Genotype–Phenotype Correlations and Penetrance

Interfamilial and intrafamilial variability seems to be slight [Steinmann et al., 2002]. Type I collagen consists of two  $\alpha 1$  (I) and one  $\alpha 2$ (I) chains, thus three quarters of the collagen I molecules in aEDS due to *COL1A1* mutations contain one or two mutant pN $\alpha$ 1(I) chains, whereas only half of the collagen I molecules in aEDS due to *COL1A2* mutations are affected [Steinmann et al., 2002; Giunta et al., 2008a]. This difference in the stoichiometry suggests that *COL1A1*-associated aEDS might be more severe than *COL1A2*-associated aEDS. Because the number of patients with a *COL1A1* mutation is small, it is uncertain whether this correlation holds true [Giunta et al., 2008a]. Severe phenotypes in patients with a *COL1A1* mutation have been described: A patient with a *COL1A1* “intron 5–1G>A” leading to complete loss of exon 6 presented with dermatosparaxis EDS-like features [Nicholls et al., 2000] and a patient with a *COL1A1* mutation “intron 5–2A>T” had multiple congenital dislocations and dermatosparaxis EDS-like skin features (doughty, redundant) [Giunta et al., 2008a].

Penetrance is complete.

### Management

Key management guidelines focus on the musculoskeletal system and the skin.

- The advice to management of the musculoskeletal system is:
  - At diagnosis a whole body skeletal survey is recommended
  - Management of orthopedic problems is the center of care for patients with

the disorder. Stable reductions of congenital hips dislocations were not achieved frequently through closed reductions with orthoses or hip spica. Anterolateral open reductions with capsular plication were also ineffective. In contrast, open reductions with an iliac osteotomy, with or without femoral osteotomy, were favorable in some patients. Appropriate surgical intervention is therefore difficult to plan but is crucial for reducing the risk of recurrence of hip dislocations, avascular necrosis, and premature osteoarthritis [Giunta et al., 1999; Steinmann et al., 2002; Giunta and Steinmann, 2014]

- Recurrent and/or persistent dislocations and hypermobility of upper limb joints are also disabling, but operative intervention is rarely considered because limited effectiveness of operative procedures is predicted [Giunta et al., 1999; Steinmann et al., 2002; Giunta and Steinmann, 2014]
- Orthotic management and early intervention, including physical and occupational therapy are recommended to assist standing, walking, and activities of daily living [Giunta et al., 1999; Steinmann et al., 2002; Giunta and Steinmann, 2014]
- Contact sports should be avoided to prevent dislocations
- Consider bone mineral density studies
- The advice to management of the skin, cardiovascular, and ophthalmological features is similar to that for patients with classical EDS (see “management guidelines for the classical Ehlers–Danlos syndrome,” by Bowen et al., this issue)
- **Pregnancy management**
  - Follow-up throughout pregnancy is warranted
  - Delivery should be performed in a medical center where intensive treatment could be given to an affected pregnant woman and an affected neonate
  - Breech presentation is frequent if the fetus is affected. Though not described previously, affected pregnant women might be predisposed to tearing of the perineal skin and to have postpartum extension of

episiotomy incisions as well as prolapse of the uterus and/or bladder, as described in cEDS

### Differential Diagnosis

- Larsen syndrome
- Classical EDS
- Dermatosparaxis EDS
- Kyphoscoliotic EDS
- Musculocontractural EDS
- Loeys–Dietz syndrome
- PYCR1-related autosomal recessive cutis laxa

### DERMATOSPAXIS EDS (dEDS)

Synonyms: Dermatosparaxis; Ehlers–Danlos dermatosparaxis type; EDS–VIIC; EDS7C

#### The History of Dermatosparaxis EDS

Dermatosparaxis was first reported in cattle [Lenaers et al., 1971; Hanset and Lapiere, 1974] and subsequently in sheep [Fjølstad and Helle, 1974], cats [Counts et al., 1980; Holbrook and Byers, 1982] and dogs [Holbrook and Byers, 1982]. Affected animals display a loose and extremely fragile skin (dermatosparaxis means “tearing of the skin”), resulting in large skin lacerations during delivery or early in life, with subsequent infections and premature death. Early ultrastructural studies showed alterations in the dermis of these animals, with loosely packed, thin, and twisted ribbon-like collagen fibrils that displayed a typical “hieroglyphic aspect” on cross sections, pointing toward impaired collagen biosynthesis and fibrillogenesis. Subsequent biochemical studies performed on dermatosparactic cattle revealed that these abnormal collagen molecules were composed of incompletely processed type I procollagen precursor molecules, in which the N-propeptide was insufficiently cleaved [Lenaers et al., 1971]. Deficient activity of the endopeptidase that excises the N-propeptide of

procollagen chains was eventually demonstrated [Smith et al., 1992]. Although animal dermatosparaxis was the first recognized collagen disorder, it took more than 20 years to confirm the existence of a human counterpart for the disorder. In 1992, three independent infants were reported with clinical signs resembling dermatosparaxis [Nusgens et al., 1992; Smith et al., 1992]. Ultrastructural studies of the dermis demonstrated the same “hieroglyphic” pattern of the collagen fibrils as those observed in dermatosparactic animals, and biochemical studies on cultured human fibroblasts confirmed deficient cleavage of the pro $\alpha$ 1(I) and pro $\alpha$ 2(I) N-propeptides. Seven additional patients were identified subsequently, all of them displaying a severe phenotype with an extremely fragile and lax skin, severe bruising, and a characteristic dysmorphic face, leading to a diagnosis usually within the first few months of life. It lasted until 1999 to identify the first biallelic mutations in *ADAMTS2*, the gene encoding the procollagen I N-proteinase ADAMTS-2 (a disintegrin and metalloproteinase with thrombospondin motifs 2), in several patients with the dermatosparaxis type of EDS as well as in a strain of dermatosparactic cattle [Colige et al., 1999].

The exact prevalence of this rare condition is unknown.

#### Mechanism of Disease

Dermatosparaxis EDS (dEDS) is caused by homozygous or compound heterozygous mutations in *ADAMTS2*, the gene that encodes ADAMTS-2. ADAMTS-2 is a metalloproteinase containing properdin repeats and a cysteine-rich domain with similarities to the disintegrin domain of reprolysin. This enzyme is the main procollagen I N-proteinase, but it can also cleave the N-propeptides of type II and type III procollagens [Colige et al., 2005]. The mutations result in decreased activity of ADAMTS-2, which leads to defects in processing of type I procollagen to mature type I collagen [Colige et al., 1999; Colige et al., 2004; Van Damme et al., 2016]. There is an accumulation of

pN-collagen type I, resulting in polymerization of abnormal collagen fibers that appear thin, irregular, branched and “hieroglyphic” in cross-section.

#### Allelic Heterogeneity

Up till now eight biallelic mutations have been reported in 15 patients (14 independent families), including a recurrent homozygous nonsense mutation p.(Gln225\*) in six patients, a unique homozygous nonsense mutation p.(Trp795\*), a homozygous in-frame skip of exons three to five a homozygous in-frame skip of exon 17 and compound heterozygosity for an out-of-frame exon-skip of exon 3 and an in-frame skip of exons 14–16, three homozygous loss-of-function mutations (c.2927\_2928delCT, p.(Pro976Argfs\*42); c.669–670dupG, p.(Pro224Argfs\*41); c.2751–2A>T) and one compound heterozygous mutation (c.2T>C, p.? and c.888–891delTGAA, p.(Met295Thrfs25\*)). All mutations result in deficient activity of ADAMTS-2.

There is a registry of reported *ADAMTS2* gene variants [Dalgleish, 1998]

#### Clinical Description

To date 15 patients from 14 independent families have been reported. Three patients were born to known consanguineous parents (Table S1). Age at diagnosis ranged from birth to 13 years. [Nusgens et al., 1992, Smith et al., 1992; Wertelecki et al., 1992; Reardon et al., 1995; Fujimoto et al., 1997; De Coster et al., 2003; Malfait et al., 2004; Bar-Yosef et al., 2008; Solomons et al., 2013; Van Damme et al., 2016]. Clinical follow-up into puberty and early adulthood is reported for only two patients.

The hallmark of the disorder is the extreme skin fragility with redundant, almost lax skin, and the severe susceptibility of bruising.

- Reproductive, including pregnancy  
Preterm birth was reported in nine patients, and was preceded by PPRM in six. Mean gestational age was 34 weeks and 4 days (n = 14, range: 28–41 weeks). The umbilical cord was noted to be friable in two infants; one of them

also had a short umbilical cord that ruptured after clamping. Perinatal complications were reported in several patients. One patient died shortly after birth (39 weeks gestation) due to severe hemorrhage and shock. A boy with a gestational age of 33 weeks was born with multiple skull fractures and an extensive subgaleal hemorrhage. A dural tear at the site of the skull fracture led to the development of a large cerebrospinal fluid collection, and he died due to secondary infection at 145 days of age. Three other prematurely born infants were admitted to a neonatal intensive care unit for several weeks for a range of complications, including cerebral hemorrhage ( $n=2$ ); pneumothorax and respiratory distress ( $n=1$ ); hydronephrosis ( $n=1$ ); and hypoglycemia, hypocalcemia, and hypothyroidism ( $n=1$ ). No pregnancies have been reported in affected individuals.

- **Craniofacial involvement**

Most patients were born with a severe and recognizable facial gestalt, including prominent and protuberant eyes with puffy, edematous eyelids and excessive periorbital skin, large fontanels and/or wide cranial sutures, a hypoplastic chin and bluish or greyish discoloration of the sclerae ( $n=12$ ). Less frequent findings included gingival hyperplasia ( $n=6$ ), dental lamina cysts ( $n=2$ ), and generalized hypertrichosis ( $n=6$ ). These patients also presented extreme skin fragility with tearing of the skin, either at birth ( $n=2$ ) or within the first few years of life ( $n=10$ ). In addition, they had a lax and sagging skin with redundant skin folds, especially in the neck, and around wrists and ankles ( $n=10$ ). Together, these findings led to an early diagnosis in these patients. A number of patients displayed a strikingly milder phenotype, with absence of obvious congenital facial dysmorphic features, skin fragility or redundancy. Mild dysmorphic features, skin fragility, and features of generalized connective tissue fragility however gradually became more apparent during childhood and adolescence.

- **Musculoskeletal system**

Whereas height, weight, and orofacial circumference were usually within normal limits at birth, postnatal growth

retardation was reported in all patients, except for those that died shortly after birth ( $n=13$ ). Eleven patients presented with non-rhizomelic shortening of the limbs and short, plump hands and feet with stubby fingers and toes. Joint hypermobility was a consistent finding ( $n=11$ ), but often mild at birth. Follow-up data in older patients demonstrated that the joint hypermobility becomes more pronounced later on. Four patients had a history of fractures, including (congenital) skull fractures in three. Several other skeletal abnormalities were reported, including delayed ossification of the cranial vault ( $n=3$ ), Wormian bones ( $n=2$ ), delayed bone age ( $n=2$ ), and persistence of woven bone in the ribs of one patient 15. Osteopenia was reported in only two patients.

- **Skin and integument**

Frequently occurring skin features, apart from the severe skin fragility ( $n=14$ ) and loose, lax, or hyperextensible skin ( $n=15$ ), included a soft and doughy skin texture ( $n=12$ ), increased palmar wrinkling ( $n=6$ ), and atrophic scarring ( $n=5$ ). One of the most consistent clinical findings was an umbilical hernia at birth ( $n=14$ ).

- **Ocular involvement**

One patient had severe, congenital myopia, whereas several others presented with early onset and progressive myopia ( $n=5$ ). Three others had astigmatism, and one patient developed severe glaucoma at very young age.

- **Dental involvement**

In addition to gingival hyperplasia and dental lamina cysts, several other dental abnormalities have been reported. These include microdontia ( $n=6$ ) or even agenesis of several permanent teeth ( $n=3$ ), and tooth discoloration ( $n=3$ ). Abnormal morphology of the molars ( $n=2$ ) and severe enamel attrition of the deciduous teeth ( $n=2$ ) have been reported in a limited number of patients [De Coster et al., 2003; Malfait et al., 2004].

- **Cardiovascular system**

Easy bruising was frequent ( $n=11$ ) and often very severe with the formation of large subcutaneous hematomas. Bleeding problems were encountered in

seven patients, ranging from severe epistaxis and gum bleeds to internal and (congenital) cerebral hemorrhages. Arterial rupture or aortic dilatation has not been reported so far.

- **Neuromuscular features and motor development**

A mild delay in gross motor development was reported in about half of the patients ( $n=8$ ).

- **Visceral complications**

A 9-year-old girl ruptured her diaphragm due to postoperative vomiting. She subsequently developed a paraesophageal hernia with incarceration of the stomach that was further complicated by the occurrence of a large abdominal hematoma after reduction. Two patients had bladder diverticula, complicated by spontaneous bladder rupture, and two other patients developed rectal prolapse with profuse anal bleeding in puberty.

### **Genotype–Phenotype Correlations and Penetrance**

The patients harboring the *c.2927\_2928delCT* and the *c.2751-2A>T* mutations have a relatively milder phenotype compared to the others. These mutations lead to introduction of a PTC. The milder phenotype could be due to the fact that NMD might be only partially efficient for these alleles and some transcripts can escape NMD. As such, these alleles could produce truncated ADAMTS-2 enzymes lacking either the last thrombospondin 1 (TSP1) domain and the PLAC (protease and lacunin) domain (*c.2927\_2928delCT*), or the two most C-terminal TSP1 domains and the PLAC domain (*c.2751-2A>T*) [Van Damme et al., 2016]. Interestingly, investigation of the N-endopeptidase activity of various forms of recombinant ADAMTS-2 has previously shown that removal of one or two of the most C-terminal TSP1 and the PLAC domain results in an enzyme which is still significantly active [Colige et al., 2005]. In addition, two other members of the ADAMTS family, ADAMTS-3 and ADAMTS-14, have been shown to possess procollagen

N-endopeptidase activity, and could compensate for the reduced activity of ADAMTS-2 [Fernandes et al., 2001; Colige et al., 2002; Le Goff et al., 2006].

Penetrance is presumably complete.

### Management

No specific guidelines for management of patients with dEDS are available. Management guidelines should follow those formulated for other forms of EDS (for reference: See “management guidelines for the classical Ehlers–Danlos syndrome,” by Bowen et al., this issue).

### Differential Diagnosis

- Classical EDS
- Cutis laxa syndromes
- OI
- Arthrochalasia EDS
- RIN2 syndrome
- Achondroplasia

### KYPHOSCOLIOTIC EDS (kEDS) DUE TO LYSYL HYDROXYLASE 1 DEFICIENCY (kEDS-*PLOD1*)

Synonyms: Kyphoscoliotic EDS; EDS Type 6; EDS Type VIA; Ocular-scoliotic EDS; Nevo syndrome; Cutis hyperelastica; Lysyl hydroxylase-deficient EDS

### The History of Kyphoscoliotic EDS

Kyphoscoliotic EDS was the first inborn error of human collagen metabolism to be defined at the biochemical level, as early as 1972. Based on a family study in which two sisters had marked muscular hypotonia, severe progressive scoliosis from birth, marked joint hypermobility, and recurrent joint dislocations [Krane et al., 1972; Pinnell et al., 1972], the authors found lysyl-hydroxylase deficiency in fibroblasts from the two siblings that produced hydroxylysine-deficient collagen. Because the sisters also presented microcornea, fragility of ocular tissues, and a distinct biochemical

defect, it was suggested that they be classified as a new subtype, EDS VI, the ocular type or the ocular-scoliotic type [McKusick, 1972]. Later, it was recognized that the ocular signs, though dramatic, were far less frequent than initially reported, prompting the Villefranche Nosology to reclassify the disorder as the kyphoscoliotic type of EDS [Beighton et al., 1998]. At that time it was recognized that in the majority of cases, the condition was caused by the lysyl hydroxylase 1 enzyme deficiency and specified as the kyphoscoliotic form of EDS (EDS VIA), whereas a rarer, clinically similar condition with normal lysyl hydroxylase activity was designated EDS VIB [Steinmann et al., 2002; Walker et al., 2004b]. Thereafter, it was recognized that the Nevo Syndrome, first reported in 1974, was an allelic condition to kEDS [Giunta et al., 2005a].

Recently, a number of rare autosomal recessive entities with distinct molecular and biochemical abnormalities that clinically overlap with kEDS have been described, and are discussed below: kEDS due to *FKBP14* mutations, the Brittle cornea syndrome (BCS) (*ZNF469* and *PRDM5*), the spondylo-dysplastic form of EDS caused by *SLC39A13* mutations (previously called spondylocheirodysplastic EDS), and musculocontractural EDS (*CHST14* and *DSE*).

The exact prevalence of kEDS due to lysylhydroxylase 1 deficiency is unknown.

### Mechanism of Disease

kEDS-*PLOD1* is caused by deficiency of the collagen-modifying enzyme procollagen-lysine, 2-oxoglutarate 5-dioxygenase 1 (*PLOD1* or LH1 [lysylhydroxylase1]) due to homozygosity or compound heterozygosity for mutated *PLOD1* alleles. Lysylhydroxylase 1 (LH1) plays an important role as a post-translational modifying enzyme in collagen biosynthesis through (1) hydroxylation of helical lysyl residues in Xaa-Lys-Gly collagen sequences to hydroxylysyl residues which serve as sites of attachment for carbohydrate units (either galactose or

glucosyl-galactose), and (2) in the formation of intra- and intermolecular collagen cross-links. LH1 deficiency results in underhydroxylation of lysyl residues and underglycosylation of hydroxylysyl residues in collagens and, hence, impaired cross-link formation with consequent mechanical instability of the affected tissues [Rohrbach et al., 2011].

### Allelic Heterogeneity

A total of 139 mutations in *PLOD1* have been identified in the 84 confirmed cases, of these there are 39 different mutations. The 8.9 kb duplication of 7 exons (exons 10–16; c.1067\_1846 dup) is the most common and has been reported in 42/139 (30%) mutations (20 individuals homozygotes; 2 patients compound heterozygotes). Nine patients from six families are homozygous for the nonsense mutation p.Arg319\*, all of Arab descent. The nonsense mutation p.Tyr511\* has been identified in five patients, two of whom are homozygous.

There is a registry of reported *PLOD1* gene variants [Dalglish, 1998].

### Clinical Description

At present, 84 patients from 73 families with confirmed kEDS-*PLOD1* (either by demonstration of biallelic *PLOD1* mutations or by urinary analysis) have been identified [Beighton, 1970b; Krieg et al., 1979; Ihme et al., 1983; Dembure et al., 1984; Chamson et al., 1987; Wenstrup et al., 1989; Hyland et al., 1992; Hautala et al., 1993; Ha et al., 1994; Al-Gazali et al., 1997; Yeowell and Walker, 1997; Brinckmann et al., 1998; Heikkinen et al., 1999; Walker et al., 1999, 2004a, 2005; Yeowell et al., 2000a, b, 2005; Brunk et al., 2004; Giunta et al., 2005b; Yis et al., 2008; Esaka et al., 2009; Voermans et al., 2009a; Kariminejad et al., 2010; Rohrbach et al., 2011; Gok et al., 2012; Busch et al., 2014; Tosun et al., 2014; Abdalla et al., 2015]. The ages at publication ranged from 5 months to 54 years. Clinical features were adequately reported in 74 patients with kEDS-*PLOD1* (either by demonstration of biallelic *PLOD1* mutations or by urinary analysis) (Table S1).

The hallmarks of the disorder include (congenital) kyphoscoliosis, muscle hypotonia, and joint hypermobility (Representative pictures of the phenotype are given in Fig. 2).

- **Reproductive, including pregnancy**  
Pregnancy of an affected fetus is usually uneventful although reduced fetal movements have been reported (n = 7). PPRM was reported in three cases, four patients were known breech presentation and there were three reports of oligohydramnios. Affected pregnant women may be at increased risk for spontaneous abortions, premature rupture of membranes, and rupture of arteries. The patient reported by Esaka et al. [2009] experienced minor trauma at 29 weeks gestation resulting in a stillbirth and maternal death. Post mortem autopsy showed a spontaneous rupture of the right iliac artery [Esaka et al., 2009]. Two affected women had a total of seven pregnancies resulting in three miscarriages and four healthy children, three of whom were born vaginally at term and one of whom was born at 24 weeks; there were no maternal complications [Steinmann, unpublished].

- **Craniofacial features**

A number of dysmorphic features have been reported. However, individual case series often report the same feature in a number of patients. As a result, the occurrence of certain features may be over-represented. The most frequently observed features are high palate, epicanthal folds, down-slanting palpebral fissures, synophrys and low-set ears.

- **Musculoskeletal system**

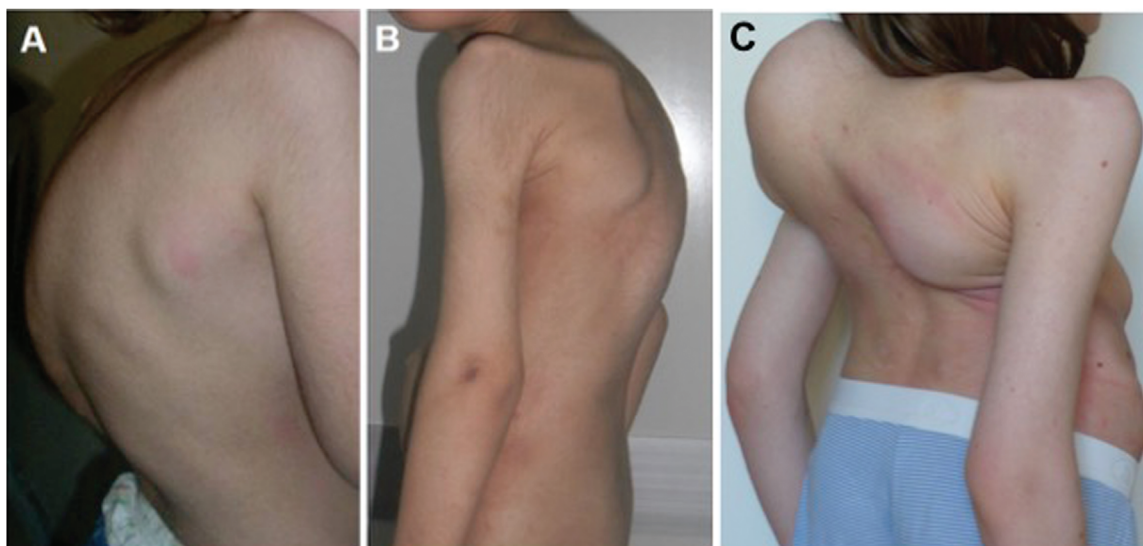
Kyphoscoliosis is present and usually severe and progressive. In most patients, this is congenital (n = 55) but postnatal kyphoscoliosis (n = 12) or scoliosis alone (n = 1) have been reported. Almost all patients have joint hypermobility (n = 69). Joint dislocations/subluxations are common. Congenital hip dislocations has been reported in 15 patients and post-natal hip dislocation in an additional three patients. Besides hip, shoulder (n = 12), knee (n = 5), and wrist (n = 2) were the most commonly noted dislocations/subluxations. Generally, recurrent dislocations were noted in 18 patients. Hand deformities were noted in 13 patients. Foot deformities were also noted in 17, which included four cases of talipes

equinovarus. Pes planus was reported in 11 patients.

A marfanoid habitus has been reported in 19/74 patients. Arachnodactyly was reported in eight separate patients. High palate was recorded in 11 patients, and in association with arachnodactyly in five, or with marfanoid habitus in three patients. A pectus deformity was observed, with pectus excavatum being more common (n = 12) than pectus carinatum (n = 2). Osteopenia (n = 17) or osteoporosis (n = 2) was sometimes seen on X-ray; however, fractures were not reported in any patients.

- **Skin and integument**

Skin abnormalities are almost universally described. Skin hyperextensibility (n = 48) and soft, doughy, or velvety skin (n = 43) were most frequently observed. Fragility was reported (n = 26) with easy bruising (n = 26); thin, translucent skin (n = 8); and abnormal wound healing (n = 17). Atrophic scarring was reported in 35 patients. Criss-cross patterning of the palms was only reported in one patient. Hernia was reported in 12 patients including six umbilical and five inguinal.



**Figure 2.** Clinical findings in patients with kEDS: (A and B) Kyphoscoliosis in two unrelated patients homozygous for causative mutations in *PLOD1*. (C) Severe kyphoscoliosis in a patient homozygous for a causative mutation in *FKBP14* (Images kindly provided by Prof. Ebtesam Abdalla and Dr. Matthias Baumann, with permission).

- **Ocular involvement**  
Ophthalmic features are variable and include bluish sclerae (n = 18), microcornea (n = 16), and myopia (n = 16). Rupture of the eye globe, following minimal trauma has been reported in five individuals including one patient with both eyes affected [Beighton, 1970a; Pinnell et al., 1972; Ihme et al., 1983; Kariminejad et al., 2010].

- **Cardiovascular system**  
Medium-sized vessel rupture has been reported in several individual case reports. These events appear to be more prevalent from teenage years and into adulthood; however, there have been six cases of antenatal/neonatal brain haemorrhage [Wenstrup et al., 1989; Yeowell and Walker, 1997; Yeowell et al., 2000a; Giunta et al., 2005b; Rohrbach et al., 2011; Tosun et al., 2014]. Arterial rupture has been reported in various locations and during pregnancy as mentioned above [Esaka et al., 2009].

In one of the first reported siblings [Beighton, 1970b], the sister died from a dissecting aortic aneurysm at the age of 50 and the brother had a cerebral bleed in the distribution of the right middle cerebral artery at the age of 19. Dembure et al. [1984] reported a patient, who was then followed up by Ha et al. [1994] who had a spontaneous arterial rupture into his upper thigh at the age of 15. In Wenstrup et al. [1989], one patient had a rupture of a vertebral artery and another patient had multiple ruptures of the femoral artery and two episodes of spontaneous intrathoracic arterial rupture. Brinckmann et al. [1998] reported two patients with vascular complications; one suffered a stroke at the age of 15 years and subsequently at age 30 years, he had spontaneous bleeding of minor pancreatic arteries; and at age 32 years, he had spontaneous bleeding from branches of the right profundal femoral artery [Brinckmann et al., 1998; Busch et al., 2014]; another patient had an aneurysm of the mesenteric artery age 12 years.

The patient reported by Yeowell et al. [2000a] had an intracranial haemorrhage and brachial plexus injuries at birth; he had dextrocardia and mild aortic root dilatation with mild aortic insufficiency due to a bicuspid aortic valve and he died from arterial rupture age 14 years. Voermans et al. [2009a] followed-up patient 7 from Giunta et al. [2005b] who had a ruptured aneurysm of the left popliteal artery at the age of 15 years. Rohrbach et al. [2011] reported a 27-year-old man with chest symptoms who during coronary angiography had a spontaneous dissection of the ramus interventricularis anterior (RIVA) and main coronary artery causing acute cardiac failure. A Turkish boy presented with a left brachial artery pseudo-aneurysm at the age of 12 [Gok et al., 2012]. There has been one report of aortic stenosis [Ihme et al., 1983]. Another three patients were reported to have MVP [Pinnell et al., 1972; Rohrbach et al., 2011].

- **Neuromuscular features and motor development**  
One of the key features of the condition is congenital muscular hypotonia (n = 56), with associated feeding problems (n = 17). Gross motor delay is common (n = 54) with varying severity, but with only one non-ambulatory case.
- **Neurological features and neurodevelopment**  
Intelligence is usually normal but learning disabilities have been reported in eight patients (two of these patients were reported to have antenatal or perinatal intracranial bleeds) [Wenstrup et al., 1989; Rohrbach et al., 2011].

### Genotype–Phenotype Correlation and Penetrance

A range of clinical severity is observed in individuals with kEDS-*PLOD1* for each of the systems discussed as detailed in the above section [Steinmann et al., 2002; Rohrbach et al., 2011]. No specific work has been carried out looking at genotype–phenotype correlations. There are two siblings with the same mutations

reported by Hyland et al. [1992]. The younger sibling appears to be much less severely affected. Intrafamilial variation has been observed.

Penetrance is complete.

### Management

Key management guidelines focus on the musculoskeletal system, skin, and the cardiovascular system.

- The advice to management of the musculoskeletal system is:
  - According to that for patients with classical EDS (for reference: See “management guidelines for the classical Ehlers–Danlos syndrome,” by Bowen et al., this issue)
  - Photographic and radiologic documentation of the spine is recommended in view of the progressive kyphoscoliosis. Regular follow-up by an orthopedic surgeon for management of kyphoscoliosis is appropriate
  - Any surgery should be carried out with caution due to the risk of vascular complications
  - Consider bone densitometry evaluation
  - Consider sleep study to assess for nocturnal hypoxemia, and nighttime ventilation in case of severe muscle hypotonia
- The advice to management of the skin is:
  - According to that for patients with classical EDS (see “management guidelines for the classical Ehlers–Danlos syndrome,” by Bowen et al., this issue)
  - Routine examination for hernia and surgical referral as necessary
- The advice for management of the cardiovascular system is:
  - Measurement of aortic root size and assessment of heart valves by echocardiogram at the time of diagnosis or by age 5 years
  - Echocardiogram at 5-year intervals, even if the initial echocardiogram is normal
  - Vigilant observation and control of blood pressure can reduce the risk of arterial rupture



- Further vascular surveillance ought to be considered
- The advice for management of the ophthalmologic system is:
  - Formal ophthalmologic evaluation at diagnosis for myopia, astigmatism, and potential for retinal detachment
  - Routine ophthalmologic examination for management of myopia and early detection of ophthalmic complications
  - Myopia and/or astigmatism may be corrected by glasses or contact lenses
  - Laser treatment of the retina is indicated in case of imminent detachment
- Pregnancy management
  - Follow-up throughout pregnancy and delivery should be performed in a specialized fetal medicine center
  - Measurement and monitoring of aortic root size by echocardiogram during pregnancy

### Differential Diagnosis

- Kyphoscoliotic EDS-*FKBP14*
- Brittle cornea syndrome
- Spondylodysplastic EDS
- Musculocontractural EDS
- Classical EDS
- Congenital myopathies, including collagen VI and collagen XII-associated myopathies (myopathic EDS)
- Metabolic disorders
- Vascular EDS
- Marfan syndrome
- Loeys–Dietz syndrome

### KYPHOSCOLIOTIC EDS (kEDS) DUE TO *FKBP22*-DEFICIENCY (kEDS-*FKBP14*)

Synonyms: *FKBP14*-related EDS, *FKBP22*-deficient EDS

### The History of Kyphoscoliotic EDS (kEDS) due to *FKBP22*-Deficiency (kEDS-*FKBP14*)

Baumann et al. [2012] reported five families with an autosomal recessive

variant of EDS, characterized by severe congenital muscle hypotonia, joint hypermobility, skin hyperextensibility, progressive kyphoscoliosis, and sensorineural hearing loss. The condition was shown to be caused by biallelic mutations in *FKBP14* [Baumann et al., 2012]. In view of the major clinical overlap of this phenotype with kEDS-*PLOD1*, both conditions are grouped within the same clinical entity (“Kyphoscoliotic EDS” in the new EDS classification).

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***Baumann et al. [2012] reported five families with an autosomal recessive variant of EDS, characterized by severe congenital muscle hypotonia, joint hypermobility, skin hyperextensibility, progressive kyphoscoliosis, and sensorineural hearing loss. The condition was shown to be caused by biallelic mutations in *FKBP14*.***

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The prevalence of kEDS-*FKBP14* is unknown.

### The Mechanisms of Disease

*FKBP14* encodes FKBP22, a member of the F506-binding family of peptidyl-prolyl cis–trans isomerases found in the lumen of the endoplasmic reticulum (ER), where it is thought to catalyze cis–trans-isomerization of peptidyl-prolyl peptide bonds and to accelerate protein folding, particularly of procollagens [Galat, 2003]. FKBP22 interacts with types III, VI, and X collagen, but does not show direct binding to other types of collagen, such as type I or V collagen [Ishikawa and Bachinger, 2014]. Deficiency of FKBP22 was shown to result in enlarged ER cisterns in dermal fibroblasts, and an altered assembly of the ECM [Baumann et al., 2012].

### Allelic Heterogeneity

Four different *FKBP14* mutations have been identified to date [Baumann et al., 2012; Aldeeri et al., 2014; Murray et al., 2014; Dordoni et al., 2016]. The c.362dup, p.(Glu122Argfs\*7) has been identified in homozygous state in five independent families. Furthermore, this mutation was twice identified in compound heterozygosity with another mutation: One with a nonsense mutation (c.42\_60del, p.(Thr15\*)) and once with a 3-bp deletion (c.573\_576del, p.(Gly191del)). A homozygous deletion of four amino acids was recently reported: c.197+5\_197+8delGTAA [Alazami et al., 2016].

### Clinical Description

To date, 10 patients with kEDS-*FKBP14* from nine independent families have been described: Five pediatric (<12 years), three adolescents (16 years), and two adults (42- and 48-year-old) (Table S1) [Baumann et al., 2012, Aldeeri et al., 2014, Murray et al., 2014, Alazami et al., 2016, Dordoni et al., 2016]. Sufficient clinical data are available for nine patients.

The hallmarks of the disorder include kyphoscoliosis (either progressive or non-progressive), severe congenital muscle hypotonia with muscle atrophy, joint hypermobility, and congenital hearing loss (sensorineural, conductive, or mixed) (Representative pictures of the phenotype are given in Fig. 2).

- Craniofacial features
  - Facial dysmorphism is not always described and a facial “gestalt” is not recognizable. Some patients had epicanthal folds (n = 3), micrognathia (n = 3), hypotelorism (n = 1), square nasal root (n = 1), or long-narrow face (n = 1).
- Musculoskeletal
  - Kyphoscoliosis was noted at a mean age of 12 months (range 2–18 months) and was either non-progressive (n = 3) or progressive (n = 7). Orthotic treatment seemed successful in case of non-progressive kyphoscoliosis, progressive kyphoscoliosis required a

surgical approach. Atlantoaxial instability was reported in one patient, the other patients presented uncomplicated joint hypermobility without recurrent dislocations/sprains or chronic pain (mean value of Beighton score 7/9). Height was generally within the normal range, but at lower level (10th–25th centile in 4 of 8), two patients had short stature (lower than third centile).

- **Skin and integument**  
The most distinctive cutaneous features in kEDS-*FKBP14* are soft skin (n = 8), hyperextensible skin (n = 7) and hyperkeratosis follicularis (n = 5). Other skin features include atrophic scarring, umbilical skin redundancy, and multiple merging comedones in a few patients. Four patients had a hernia, including umbilical hernia in three and inguinal hernia in one.
- **Ocular features**  
Ophthalmologic features include myopia, hypermetropia, and blue sclerae.
- **Hearing**  
Hearing impairment was noted in most patients. It varied from sensorineural (n = 6), to conductive (n = 2), or mixed. In one patient, because of the mixed origin of this sign, hearing improved after transtympanic drains.
- **Cardiovascular system**  
Vascular complications were described in an adult patient, who presented a celiac artery pseudoaneurysm rupture at the age of 41 years, and in the older likely affected sister of a patient, likely affected but without molecular confirmation, who died due to unspecified aortic rupture at age 12 years. Celiac artery pseudoaneurysm rupture was observed in a child at age 6 years.
- **Neuromuscular features and motor development**  
Myopathic signs include muscle hypotonia and atrophy, poor head control in infancy, and delayed motor development. Muscular weakness seemed to regress with age and all of the subjects—but one—became able to walk at the mean age of 33 months. The outcome was very variable and

the final ability to walk ranged from myopathic gait, impossibility of walking without aids, to a motor self-sufficiency from 200 m to 1 km. Muscle biopsy showed pathological results in six patients, with myopathic changes and/or fiber atrophy; creatine kinase was generally within the normal range or slightly elevated and electromyographic patterns were usually normal at very young age, but sometimes myopathic later on [Baumann et al., 2012].

- **Visceral complications**  
Large bladder diverticula (n = 3) or rectal prolapse (n = 1) were reported.

### Genotype–Phenotype Correlation and Penetrance

No genotype–phenotype correlations have been described. Penetrance is presumably complete.

### Management

Key management guidelines focus on the musculoskeletal, cardiovascular, and auditory systems. No specific guidelines for management of patients with kEDS-*FKBP14* are available. Guidelines for management of musculoskeletal problems, skin involvement, cardiovascular involvement, ophthalmologic and dental follow-up, and pregnancy should follow those formulated for kEDS-*PLOD1*.

Specific management guidelines should also include hearing evaluation at initial diagnosis and annual hearing evaluation.

### Differential Diagnosis

- Kyphoscoliotic EDS-*PLOD1*
- Musculocontractural EDS
- Spondylodysplastic EDS
- Congenital myopathies, including collagen VI and collagen XII-associated myopathies (myopathic EDS)
- Vascular EDS
- Classical EDS

- Marfan syndrome
- Loeys–Dietz syndrome

## BRITTLE CORNEA SYNDROME (BCS)

Synonyms: EDSVIB

### History of Brittle Cornea Syndrome

Brittle cornea syndrome is a rare autosomal recessive generalized HCTD, hallmarked by a thin and fragile cornea that tends to perforate spontaneously or after minor trauma. It was originally described as a constellation of brittle cornea, blue sclerae, and red hair [Ticho et al., 1980; Cameron, 1993]. On the basis of overlapping clinical features, BCS and EDS kyphoscoliosis type were previously considered to represent the same disorder [Cameron, 1993]. Because of subtle clinical differences, this claim was later questioned and proven wrong on the basis of biochemical studies. In kEDS-*PLOD1* there is deficient activity of LH1 whereas in BCS the LH1-activity is normal [Royce et al., 1990; Al-Hussain et al., 2004].

Abu et al. [2008] first mapped the BCS gene to a 4.7 Mb region on chromosome 16q24 and later identified recessive mutations in *ZNF469* (MIM 612078). The single exon gene *ZNF469* encodes a C2H2 zinc finger protein of which the function is yet to fully be understood. Because not all BCS patients harbored *ZNF469* mutations, a second locus for BCS was suspected. This was confirmed by the discovery of mutations in *PRDM5* (MIM 614161) [Burkitt Wright et al., 2011]. *PRDM5* encodes a C2H2 zinc finger protein of the PR/SET family of proteins. *PRDM5* was first characterized as a potential tumor suppressor gene in the development of several types of cancer [Watanabe et al., 2007, 2009; Cheng et al., 2010], but has now been shown to regulate transcription of collagen and several other ECM genes in a mouse osteoblast cell line (MC3T3) [Galli et al., 2012]. In addition, expression profiling studies suggest that both *PRDM5* and *ZNF469* might be part of a common pathway regulating the

expression of ECM genes such as fibrillary collagens, and several studies have suggested a role for *ZNF469* normal corneal development [Lu et al., 2010; Vitart et al., 2010; Vithana et al., 2011; Rohrbach et al., 2013].

The report of a single BCS family with mutations in neither *PRDM5* or *ZNF469* suggests the existence of a third genetics locus, but the large majority of cases is probably attributable to mutations in *ZNF469* or *PRDM5* [Rohrbach et al., 2013].

The exact prevalence of BCS is unknown.

### Mechanism of Disease

*ZNF469* encodes ZNF469, a zinc finger protein of unknown function, but limited homology (~30%) with a number of collagens suggests that ZNF469 could be involved in collagen transcription and fibrillogenesis. Genome-wide association studies have consistently associated single nucleotide polymorphisms (SNPs) in the vicinity of the *ZNF469* locus with central corneal thickness (CTT) [Lu et al., 2010; Vitart et al., 2010; Vithana et al., 2011; Hoehn et al., 2012; Ulmer et al., 2012], and pathogenic *ZNF469* alleles have been identified as the single most significant genetic risk factor in the development of keratoconus (relative risk of 12) [Lechner et al., 2014].

*PRDM5* encodes a protein of the PR/SET protein family that lacks the intrinsic histone methyltransferase activity of other PR-domain containing proteins, but suppresses or activates the transcription of its target genes by recruiting the histone methyltransferase G9a and class I histone deacetylases [Duan et al., 2007]. In line with its role in BCS, *PRDM5* was shown to regulate transcription of ECM genes, including several collagen genes and small leucine-rich proteoglycans (SLRP) in pre-osteoblastic mouse cells. More specifically, it regulates collagen transcription and fibrillogenesis by binding collagen genes and maintaining RNA polymerase II occupancy [Galli et al., 2012]. The role of *PRDM5* does not appear to be limited to ECM development. Early studies focused on hypermethylation of the *PRDM5* promoter in

several types of cancer and its tumor suppressor activity by modulating the Wnt signaling pathway and expression of oncogenes [Watanabe et al., 2007, 2009; Meani et al., 2009; Cheng et al., 2010]. Its involvement in vertebrate development has been addressed in zebrafish studies; *prdm5* was shown to be essential for convergent extension movements through regulation of Wnt signaling [Meani et al., 2009].

### Allelic Heterogeneity

The following *ZNF469* mutations have been reported: 13 frameshift mutations: c.9611del, p.(Gln3206Argfs\*23); c.9483delG, p.(His3162Thrfs\*20); c.8901\_8914dup, p.(Glu2972Glyfs\*50); c.6647delA, p.(Gln2216Argfs\*19); c.6444delG, p.(Gln2149Serfs\*51); c.6638del, p.(Leu2210Trpfs\*27); c.6027delA, p.(Gly2011Alafs\*16); c.5787ins, p.(Gln1902Profs\*13); c.5787delG, p.(Gln1902Argfs\*6); c.3476del, p.(Gly1159Alafs\*15); c.2234del, p.(Phe717Serfs\*15); c.2150delT, p.(Phe717Serfs\*15); and c.350dupC, p.(Gln118Thrfs\*32), five missense mutations: c.10106G>C, p.(Arg3369Pro); c.10100G>A, p.(Cys3339Tyr); c.7508C>A, p.(Arg2478Glu); c.7424C>A, p.(Arg2478Glu); and c.5686C>G, p.(Pro1896Ala), and four nonsense mutations: c.5353C>T, p.(Gln1785\*); c.4258G>T, p.(Glu1420\*); c.3304G>T, p.(Glu1109\*); and c.2029G>T, p.(Gly677\*).

The following *PRDM5* mutations have been reported: Three frameshift mutations: c.1517\_1527del11, p.(Val506Glu fs\*5); c.974delG, p.(Cys325Leu fs\*2); and c.711\_714delTGT, p.(Val238Alafs\*35), one nonsense mutation (c.1768C>T, p.(Arg590\*)), two missense mutations (c.320A>G, p.(Tyr107Cys) and c.17T>G, p.(Val6Gly)), one splice site mutation (c.93+1G>A), and one multiple-exon deletion (exons 9–14) [Burkitt Wright et al., 2011; Avgitidou et al., 2015a].

### Clinical Description

To provide a comprehensive overview of the clinical phenotype of BCS, we reviewed the data on 51 patients (*ZNF469*: n = 32; *PRDM5*: n = 19)

(Table S1) [Al-Hussain et al., 2004; Abu et al., 2008; Christensen et al., 2010; Khan et al., 2010; Burkitt Wright et al., 2011; Al-Owain et al., 2012; Aldahmesh et al., 2012; Rohrbach et al., 2013; Ramappa et al., 2014; Avgitidou et al., 2015b; Porter et al., 2015]. Only those patients with individual clinical and molecular data were included in the review. Their age ranged between 6 months and 48 years.

The hallmarks of the condition in the thin, fragile cornea, with an increased risk for spontaneous corneal rupture (Representative pictures of the phenotype are given in Fig. 3).

#### • Craniofacial involvement

In the experience of the authors, patients with BCS present with a somewhat recognizable facial gestalt, including frontal bossing, high palate, depressed nasal bridge, and/or prominent chin. These features may however be mild.

#### • Musculoskeletal system

Joint hypermobility was a frequent finding (n = 40), and was sometimes complicated by joint dislocations (n = 6), but appeared mostly limited to small joints. Other frequent features include developmental dysplasia of the hip (DDH, n = 16), kyphoscoliosis (n = 22), foot deformities (n = 22) including pedes planovalgi and hallux valgus, and arachnodactyly (n = 6). Fractures (n = 5) and osteopenia/osteoporosis (n = 2) have been reported in a limited number of patients.

#### • Skin and integument

Patients with BCS have a mild skin phenotype with soft, velvety (n = 16), and transparent (n = 11) skin. A hyperextensible skin was noted in a minority of patients (n = 3), and is often mild. Wound healing and easy bruising was sometimes delayed (n = 4), but atrophic scarring was absent.

Soft connective tissue herniations were reported in five patients.

#### • Ocular

BCS is associated with a severe ocular phenotype. Its most striking feature is a high risk of corneal perforation (n = 36), either spontaneously or after minor trauma, due to extreme corneal thinning (central corneal thickness or CCT: 220–450  $\mu\text{m}$ , normal range 520–560  $\mu\text{m}$ ), and often leading to



**Figure 3.** Clinical findings of a 13 year old female BCS patient homozygous for a causative mutation in *PRDM5*: (A) Marfanoid habitus with height on P75 and weight on P3; velvety skin, hematomas lower leg and hallux valgus bilaterally, pectus excavatum. Shoulder symmetrical, spine straight. Facial: depressed nasal bridge and/ prominent chin. (B) Blue sclerae. (C) Current protective spectacles after bilateral successful cornea-crosslinking (Images kindly provided by Dr. Marianne Rohrbach, with permission).

irreversible blindness. Ocular rupture frequently occurred at young age, but several adults without ocular rupture have been described. Prior to rupture, visual acuity in BCS patients was often affected by keratoconus and/or keratoglobus ( $n = 27$ ) and high myopia ( $n = 17$ ). The most consistent ophthalmic feature was blue sclerae ( $n = 49$ ). Secondary glaucoma was reported in several patients ( $n = 5$ ), particularly those with extensive corneal damage following rupture. Retinal detachment and neovascularization were both reported once. Of note and in contrast to *kEDS-PLOD1*, microcornea was never observed. Megalocornea, on the other hand, was reported in three cases. BCS usually presents as a generalized connective tissue disorder with multi-tissue involvement, but one adult case with isolated ocular findings has been described [Khan et al., 2012]. This suggests that recessive mutations in *ZNF469* and *PRDM5* could be a rare cause of isolated keratoconus or corneal rupture. It should be noted however that the majority of BCS cases has been reported in ophthalmological journals, and that extraocular findings might be underestimated.

- **Hearing**  
Hearing loss has been recognized as a predominant feature, but has not yet

been comprehensively studied. Approximately, one third was affected with hearing loss ( $n = 19$ ). The most frequent type was mixed conductive/sensorineural hearing loss ( $n = 11$ ) with a predominance of conductive hearing loss in childhood. Both inter- and intrafamilial variability with respect to age of onset and progression of deafness were observed. The combined hearing loss and decreased visual acuity often led to severe sensorineural disability.

- **Cardiovascular**  
Cardiovascular defects were uncommon, but mitral valve insufficiency has been described ( $n = 3$ ). Notably and in contrast to *kEDS*, vascular and visceral fragility has not been described in the context of BCS.

#### Genotype–Phenotype Correlation and Prevalence

There is currently no evidence of a clear genotype–phenotype correlation: All types of mutations scattered across both genes appear to cause indistinguishable clinical phenotypes.

Penetrance is presumably complete. Individuals heterozygous for BCS-associated mutations have been reported to have blue sclerae and small joint hypermobility. These are not always

present, and in particular may not be striking in adult carriers. Heterozygous carriers may have very mild corneal thinning (e.g., CCT around  $500 \mu\text{m}$ ). Keratoconus has also been diagnosed in a young adult, heterozygous for a *PRDM5* mutation [Burkitt Wright et al., 2011].

#### Management

Early diagnosis (prior to ocular rupture) is possible and desirable to make anticipatory management as effective as possible. The distinctive syndromic features of BCS, such as DDH, kyphoscoliosis, blue sclerae, soft and/or translucent skin, and hypercompliant tympanic membranes, serve as important diagnostic clues in the early recognition of patients with this condition, particularly where they are the only affected individual in their family.

Key management guidelines focus on the ocular system, with primary prevention of corneal rupture by provision of protective polycarbonate eyeglasses and careful screening of vision, but also hearing. An overview of clinical management strategies for BCS patient is given in Burkitt Wright et al. [2013].

Guidelines for management of musculoskeletal problems, skin involvement, cardiovascular problems, and pregnancy should follow those formulated for other forms of EDS (for reference: See “management guidelines for the classical Ehlers–Danlos syndrome,” by Bowen et al., this issue).

#### Differential Diagnosis

- Kyphoscoliotic EDS
- Spondylodysplastic EDS
- Musculocontractural EDS
- OI

#### SPONDYLODYSPLASTIC EDS DUE TO B4GALT7-DEFICIENCY (spEDS-B4GALT7)

Synonyms: EDS progeroid type, EDS progeroid type 1, EDS with short stature and limb anomalies

### **The History of Spondylodysplastic EDS due to $\beta$ 4GalT7-Deficiency (spEDS-*B4GALT7*)**

Hernandez et al. [1979, 1981, 1986] reported five patients with EDS-features and features of early aging, including wrinkled facies, significant growth failure, fine/curly hair, periodontitis, bilateral cryptorchidism, apparent intellectual deficit. Kresse et al. [1987] reported a patient with a similar phenotype, and showed its skin fibroblasts converted only half of the core protein of the small dermatan sulfate proteoglycan decorin to a mature glycosaminoglycan (GAG) bearing proteoglycan. This defective proteoglycan biosynthesis was shown to result from biallelic mutations in *B4GALT7*, encoding galactosyltransferase I [Quentin et al., 1990; Okajima et al., 1999]. Cartault et al. [2015] detected a homozygous missense mutation (c.808C>T, p.(Arg270Cys)) in *B4GALT7* in a series of 22 patients with Larsen of Reunion Island syndrome (LRS), a skeletal dysplasia with clinically overlaps with spEDS-*B4GALT7*.

In view of the major clinical overlap of EDS caused by *B4GALT7* mutations with the phenotypes caused by *B3GALT6* and by *SLC39A13* mutations, these three conditions are now grouped within the same clinical entity ("Spondylodysplastic EDS") in the new EDS classification.

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The prevalence of spEDS-*B4GALT7* is unknown.

### **Mechanism of Disease**

*B4GALT7* encodes galactosyltransferase I ( $\beta$ 1,4-galactosyltransferase 7 or  $\beta$ 4GALT7), a Golgi-resident enzyme, that is involved in synthesizing the GAG linker region of proteoglycans. GAGs are long, unbranched polysaccharides composed of repeating disaccharide units, which consist of alternating uronic acids and amino sugars. Most GAGs are covalently attached to specific serine residues of core proteins via a defined linker region of xylose, two galactoses and one glucuronic acid, thus assembling to proteoglycans (PG). Alternative addition of N-acetylglucosamine or N-galactosylglucosamine to the terminal glucuronic acid of the linker region leads to the formation of heparan sulfate (HS) or chondroitin/dermatan sulfate (CS/DS), respectively (Fig. 4). Cosynthetic modifications such as epimerization and sulfation result in the formation of diverse motifs in the GAG chains, that allow binding of a variety of ligands, thus regulating growth factor signaling, cell adhesion, proliferation, differentiation, and motility. The  $\beta$ 1,4-galactosyltransferase 7 is a glycosyltransferase catalyzing the transfer of the first galactose onto the xylose residue of the PG core protein-GAG linker region.

Seidler et al. [2006] studied fibroblasts of a patient harboring the homozygous p.(Arg270Cys) substitution and showed reduced  $\beta$ 1,4-galactosyltransferase 7 activity, reduced glycanation of decorin and biglycan, and reduced epimerization of the decorin GAG chain. In addition, morphological alterations and intracellular accumulation of degradative vacuoles were seen in the patient's fibroblasts. Analysis of the collagen fibrils showed that the  $\beta$ 4GalT7-deficient cells had an altered suprastructure, no banded collagen fibrils and an altered ratio of  $\alpha$ 1- $\alpha$ 2 collagen chains. Finally the  $\beta$ 4GalT7-deficient cells showed reduced proliferation rates compared to controls. Gotte et al. [2008] analyzed structural alterations in HS and their functional consequences

in fibroblasts harboring this mutation. They showed a reduced sulfation degree of HS, delayed in vitro wound repair, and increased fibronectin adhesion, impaired actin stress fiber formation, delayed collagen gel extraction with reduced formation of pseudopodia and filopodia, and finally diminished formation of collagen suprastructures.

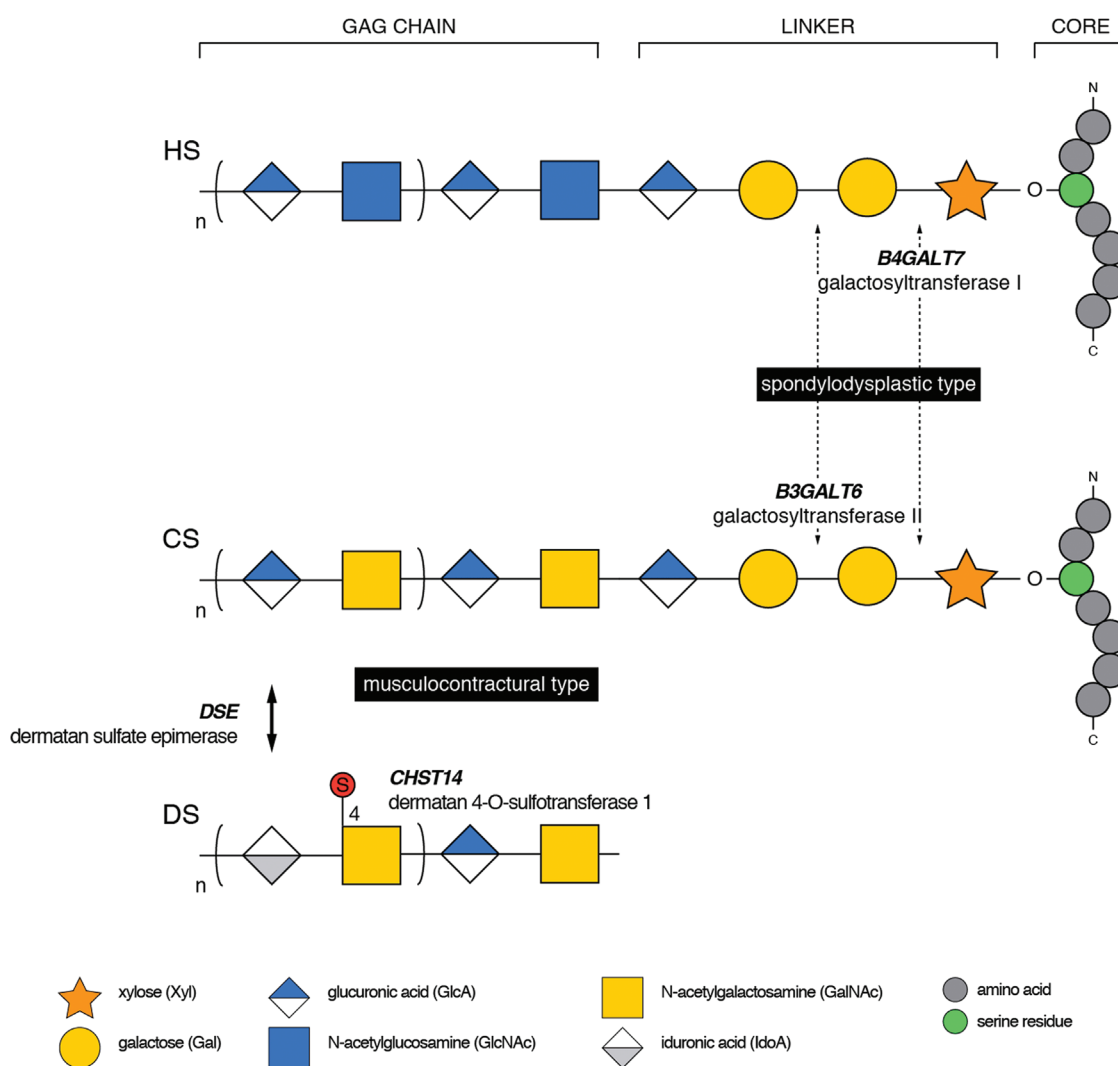
Bui et al. [2010] studied the ability of mutant  $\beta$ 4GalT7 harboring either the homozygous p.(Arg186Asp), p.(Leu206Pro), and p.(Arg270Cys) substitution to prime GAG biosynthesis in recombinant cells. Whereas the p.(Arg185Asp) did not affect GAG biosynthesis severely, the p.(Leu206Pro) mutation led to complete inhibition and the p.(Arg270Cys) to significant reduction of GAG biosynthesis. Molecular modeling predicted that the p.(Leu206Pro) mutation located in a conserved secondary structure affected the overall structure of the protein, whereas p.(Arg186Asp) is located in a less structural critical domain and the p.(Arg270Cys) in the vicinity of the substrate active site.

### **Allelic Heterogeneity**

In total, seven missense and two frameshift mutations have been reported for *B4GALT7*. The c.808C>T, p.(Arg270Cys) is most frequent. It has once been reported in homozygous state, once in compound heterozygosity with c.122T>C, p.(Leu41Pro), and once in compound heterozygous state with c.421C>T, p.(Arg141Trp). Furthermore, the c.557C>A, p.(Ala186Asp) mutation was identified in compound heterozygosity with the c.617T>G, p.(Leu206Pro) mutation, the c.641G>A, p.(Cys214Tyr) with c.277dup, p.(His93Profs\*73) and finally a homozygous c.970T>A, p.(Cys324Ser) was reported. All mutations are localized in the luminal catalytic domain, except for the p.(Leu41Pro), which is localized in the transmembrane domain.

### **Clinical Description**

At present seven patients from six families with molecularly confirmed



**Figure 4.** Biosynthesis of the HS and CS/DS GAG chains is initiated by the attachment of a common tetrasaccharide linker region to a specific serine residue of the core protein. This linker region is synthesized by the stepwise action of specific enzymes: Xylosyltransferase I/II (encoded by *XYLT1* and *XYLT2*, respectively), galactosyltransferase I ( $\beta$ 4GalT7, encoded by *B4GALT7*) and II ( $\beta$ 3GalT6, encoded by *B3GALT6*), and glucuronyltransferase I (encoded by *B3GAT3*). Following completion of the linker region, the addition of the next residue determines whether HS or CS/DS is synthesized. CS is formed by the alternating addition of N-acetylgalactosamine (GalNAc) and glucuronic acid (GlcA) residues, which are subsequently modified by several sulfotransferases. The formation of DS requires the epimerisation of GlcA residues to iduronic acid (IdoA), an event catalyzed by dermatan sulfate epimerases I and II (DS-epi1 encoded by *DSE* and DS-epi2 encoded by *DSEL*, respectively), and subsequent 4-O-sulfation of the adjacent GalNAc residue by dermatan 4-O-sulfotransferase-1 (D4ST1, encoded by *CHST14*). Defects in the initiation and modification of the GAG chains are associated with different EDS subtypes (indicated in black boxes). Defects in linker enzymes *B4GALT7* and *B3GALT6* lead to spEDS and affect the formation of both HS and CS/DS whereas alterations in *DSE* and *CHST14* result in mcEDS and compromise the formation of DS.

spEDS-*B4GALT7* have been identified (Table S1). The ages at publication ranged from 2 years to 33 years [Kresse et al., 1987, Faiyaz-Ul-Haque et al., 2004, Guo et al., 2013, Arunrut et al., 2016, Salter et al., 2016]. The clinical features reported in the patients with LRS are not included in this review.

The hallmarks of the disorder include short stature, muscle hypotonia,

radio-ulnar synostosis, and intellectual disability.

- Reproductive, including pregnancy  
Antenatal ultrasonography showed asymmetrical ventriculomegaly in one patient and severe intrauterine growth retardation in another patient. No pregnancies have been reported in affected individuals.

- Craniofacial features

The most consistent craniofacial features include triangular face ( $n=7$ ), wide-spaced eyes ( $n=6$ ), proptosis ( $n=6$ ), narrow mouth ( $n=5$ ), low-set ears ( $n=5$ ), sparse scalp hair ( $n=4$ ), abnormal dentition ( $n=4$ ), flat face ( $n=4$ ), wide forehead ( $n=4$ ), blue sclerae ( $n=3$ ), cleft palate/bidif uvula ( $n=2$ ), high palate ( $n=1$ ), small jaw

(n = 1). Of note, none of the patients was described to have progeroid features.

- **Musculoskeletal features**

The most consistent musculoskeletal features include severe growth retardation (n = 7), present at birth but progressing later on, generalized joint hypermobility (n = 7), which was noted to be quite severe in several patients, bowing of limbs (n = 5), and foot deformities (pes planus (n = 4), pes equinovarus (n = 1)). Other reported symptoms include dislocations/subluxations (n = 3), bilateral elbow contractures or limited elbow movement (n = 3), syndactyly (n = 2), pectus carinatum (n = 2), scoliosis (n = 1), long fingers (n = 4), thin fingers with bulbous tips and broad thumbs (n = 1). One patient with low-impact rib and vertebral fractures in infancy received bisphosphonate treatment, with improvement of bone pain and muscle function [Salter et al., 2016].

- **Skeletal X-ray imaging**

Reported abnormalities include: Radio-ulnar synostosis (n = 6), metaphyseal flaring (n = 4), osteopenia (n = 4), radial head subluxation or dislocation (n = 3), short clavicles with broad medial ends (n = 3), anterior splaying of ribs (n = 2), swedish key feature of the femur (n = 1), bulbous appearance of distal phalangeal tufts (n = 1), coxa valga (n = 1), reduced height of vertebral bodies (n = 1).

- **Skin and integument**

Hyperextensible (n = 6), single transverse palmar crease (n = 5), loose skin (n = 3), atrophic scarring (n = 3), soft and doughy skin (n = 2), reduced subcutaneous fat (n = 1), prominent scalp veins (n = 1), prominent venous pattern on chest (n = 1).

- **Ocular features**

Hypermetropia at very young age was reported in 5/7 patients. In most of them, it was severe. One patient was operated at age 3 months for unilateral ptosis. He also had astigmatism and intermittent exotropia. Small optic nerves (n = 1), and strabismus (n = 1) were reported. Arunrut et al. [2016] reported a patient with congenital cloudy cornea, bilateral high

hypermetropia, pendular nystagmus, coloboma of iris and optic nerves, and posterior subcapsular cataracts.

- **Dental features**

Yellowish teeth with defective enamel was reported in one patient.

- **Hearing**

Mild conductive hearing loss was reported in one patient, likely related to cleft palate.

- **Neuromuscular features and motor development**

Muscle hypotonia was reported in all patients but ranged from mild to very severe. Three patients were reported to be “floppy” at birth, one had mild congenital hypotonia, and the other three were reported to be mildly hypotonic later in childhood. Delayed motor development was reported in 6/7 patients, but none of them remained non-ambulatory. In none of the reported patients, a muscle biopsy was taken.

- **Neurological features and neurodevelopment**

Five patients were reported to have mild intellectual deficit. This included speech delay in three patients, mild learning difficulties in one, and a somewhat more severe delay in one patient.

### **Genotype–Phenotype Correlation and Penetrance**

Although marked differences in ability to prime GAG biosynthesis have been described for different missense substitutions (see “Mechanism of Disease” section), no genotype–phenotype correlations have emerged to date. It remains also unclear why the p.(Arg270Cys) is associated with either an EDS phenotype or with LRS. Cartault et al. [2015] hypothesized this could be due to the high levels of homozygosity among the LRS population and modification by interaction with other variants in close linkage disequilibrium to *B4GALT7*.

Penetrance is presumably complete. Obligate carriers display no overt clinical symptoms.

### **Management**

No specific guidelines for management of patients with spEDS-*B4GALT7* are available. Management guidelines should be tailored to the individual’s specific problems and should follow those formulated for other forms of EDS.

Specific management guidelines may include:

- **Musculoskeletal:**

- At diagnosis a whole body skeletal survey and bone densitometry studies are recommended
- In patients with recurrent fractures, bisphosphonate therapy should be considered, with treatment protocols following those formulated for patients with OI

### **Differential Diagnosis**

- Spondylodysplastic EDS-*B3GALT6*
- Spondylodysplastic EDS-*SLC39A13*
- Musculocontractural EDS
- kEDS (*PLOD1* and *FKBP14*)
- Chondrodysplasia

### **SPONDYLODYSPLASTIC EDS DUE TO *B3GALT6*-DEFICIENCY (spEDS-*B3GALT6*)**

Synonyms: EDS progeroid type 2

#### **The History of $\beta$ 3GalT6-Deficient EDS**

In 2013, two independent research studies identified biallelic mutations in *B3GALT6*, encoding  $\beta$ 3GalT6 (galactosyltransferase II or  $\beta$ 1,3-galactosyltransferase 6), in two different conditions. Nakajima et al. [2013] identified *B3GALT6* mutations in seven Japanese families with spondyloepimetaphyseal dysplasia with joint laxity type 1 (SEMD-JL1 or SEMD-JL Beighton type) by whole exome sequencing. In three other families with a phenotype resembling  $\beta$ 4GalT7-deficient EDS,

targeted *B3GALT6* sequencing subsequently identified causal mutations in all of them. Coincidentally, Malfait et al. [2013] identified *B3GALT6* mutations in three unrelated families with a pleiotropic EDS-like connective tissue disorder, characterized by severe kyphoscoliosis, joint hypermobility and contractures, multiple early-onset fractures, SEMD, skin fragility, and intellectual disability. Following the identification of *B3GALT6* as the causal gene for SEMD-JL1, Vorster et al. [2015] identified the same mutations and a novel p.(Thr79Ala) mutations, all located in or in the vicinity of the stem region, in eight prototype South African families. A few additional patients with an EDS/SEMDJL1 overlap phenotype have also been reported [Sellars et al., 2014; Ritelli et al., 2015; Alazami et al., 2016].

In view of the major clinical overlap of EDS caused *B3GALT6* mutations, with the phenotypes caused by *B4GALT7* and by *SLC39A13* mutations, these three conditions are grouped within the same clinical entity (“Spondylodysplastic EDS”) in the new EDS classification.

The exact prevalence of spEDS-*B3GALT6* is unknown.

### Mechanisms of Disease

*B3GALT6* encodes galactosyltransferase II ( $\beta$ 1,3-galactosyltransferase 6 or  $\beta$ 3GalT6), a membrane Golgi-resident enzyme, that catalyzes the addition of the third galactose onto the second galactose of the GAG linker region (for introduction, see also “Mechanism of Disease for spEDS-*B4GALT7*” section). Malfait et al. [2013] showed, using cultured dermal fibroblasts, that GAG synthesis and activity was strongly reduced by the homozygous or compound p.(Asp207His) mutation found in two patients, as well as by the homozygous p.(Gly217Ser) mutation present in one of the patients. Expression of both HS and CS GAG chains was affected in these patients. This study also showed that the GAG defects were associated with abnormal collagen structure and delayed migration in a wound healing assay, emphasizing the role of GAG in

collagen fibril formation, as well as in various physiological functions of connective tissue, such as cicatrization. Nakajima et al. [2013] showed that a recombinant mutant, lacking the initiation codon (p.Met1) produced a truncated protein that was mislocalized to the cytoplasm and nucleus, presumably inactive. Also using recombinant proteins, they reported that both enzyme mutants (p.(Ser65Gly), p.(Pro67Leu)) were inactive, emphasizing the role of the conserved N-terminal end of the catalytic domain in the functional activity of the enzyme. Other mutants studied, harboring mutations in the central or C-terminal part of the catalytic domain, that is, p.(Asp156Asn) and p.(Cys300Ser), also exhibited severely impaired enzyme activity except for p.(Glu174Asp) (50% loss in activity). Importantly, in the lymphoid cells of three SEMD-JL1 patients, the amount of HS was reduced whereas CS/DS was increased (2–5-fold). The molecular basis of these observations remains to be established. In two patients harboring a compound deletion and catalytic domain mutation [Ritelli et al., 2015] showed by micro-array transcriptome and immunofluorescence analyses a reduced expression of cartilage oligomeric matrix protein (*COMP*) and osteopontin (*SPP1*). Interestingly, these authors reported reduced expression and disassembly of HS GAG chains and of the HS-matrix PG perlecan.

### Allelic Heterogeneity

In total, 22 missense mutations, eight frameshift mutations, two in-frame deletions, two start codon mutations, one splice site, and one in-frame duplication have been reported for *B3GALT6*.

The most frequent mutation is the p.(Pro67Leu) substitution, which is frequent among South African patients, but which was also reported in a Vietnamese patient, followed by the p.(Thr79Ala) mutation, identified seven times among South African patients. Other recurrent substitutions include p.(Arg232Cys) (n = 4 families), p.(Asp207His) (n = 3 families), p.(Phe186Leu) (n = 3 families), p.(Arg6Trp)

(n = 2 families), p.(Glu265Asp) (n = 2 families), p.(Ser309Thr) (n = 2 families), and p.(Glu174Alafs\*266) (n = 2 families). The p.(Met1?) was reported in five families.

Other reported missense mutations include: p.(Val61Leu), p.(Ser65Gly), p.(Asp144Asn), p.(Asp156Asn), p.(Ser158-Tyr), p.Tyr182Cys, p.(Pro211Ser), p.(Gly217Ser), p.(Arg256Trp), p.(Arg261His), p.(Cys300Ser), p.(Tyr310Cys), and p.(Pro318Leu).

Other reported frameshift mutations are: p.(Ile76Thrfs\*202), p.(Ala108Glyfs\*163), p.(Asp118Alafs\*160), p.(Met 139Ala141del), p.(Phe180Serfs\*118), and p.(Arg197Alafs\*81). Finally, p.(Arg179\_Arg180dup), and p.(Ala66\_Arg84del) were each reported once.

Out of 36 families, 25 were compound heterozygous and 11 were homozygous. Except for the homozygous p.(Arg179\_Arg180dup), compound heterozygosity always included a missense mutation on one of the two alleles.

Four highly deleterious mutations are found outside the catalytic domain, one mutant lacking the initiation Met codon (p.(Met1?)), one mutant in the cytoplasmic tail (p.(Arg6Trp)), and two in the stem region (p.(Ser65Gly) and p.(Pro67Leu)). Other mutations are located in the luminal catalytic domain.

### Clinical Description

At present, 47 patients from 36 families with molecularly confirmed spEDS-*B3GALT6* have been identified (Table S1). The ages at publication ranged from birth to 33 years [Malfait et al., 2013; Nakajima et al., 2013; Sellars et al., 2014; Ritelli et al., 2015; Alazami et al., 2016; Honey, 2016, Van Damme et al., unpublished]. Detailed clinical data are available for 36 patients. This overview includes the SEMD-JL1 patients reported by Nakajima et al. [2013], but not those reported by Vorster et al. [2015], since detailed clinical data of the latter patients were not available.

The hallmarks of the disorder include: (1) Characteristic craniofacial features, (2) kyphoscoliosis, (3) joint hypermobility, mostly of distal joint,



(4) joint contractures, (5) short stature, (6) muscle hypotonia, (7) osteoporosis with multiple fractures, (8) radiographic skeletal abnormalities compatible with SEMD, and (9) intellectual disability (Representative pictures of the phenotype are given in Fig. 5).

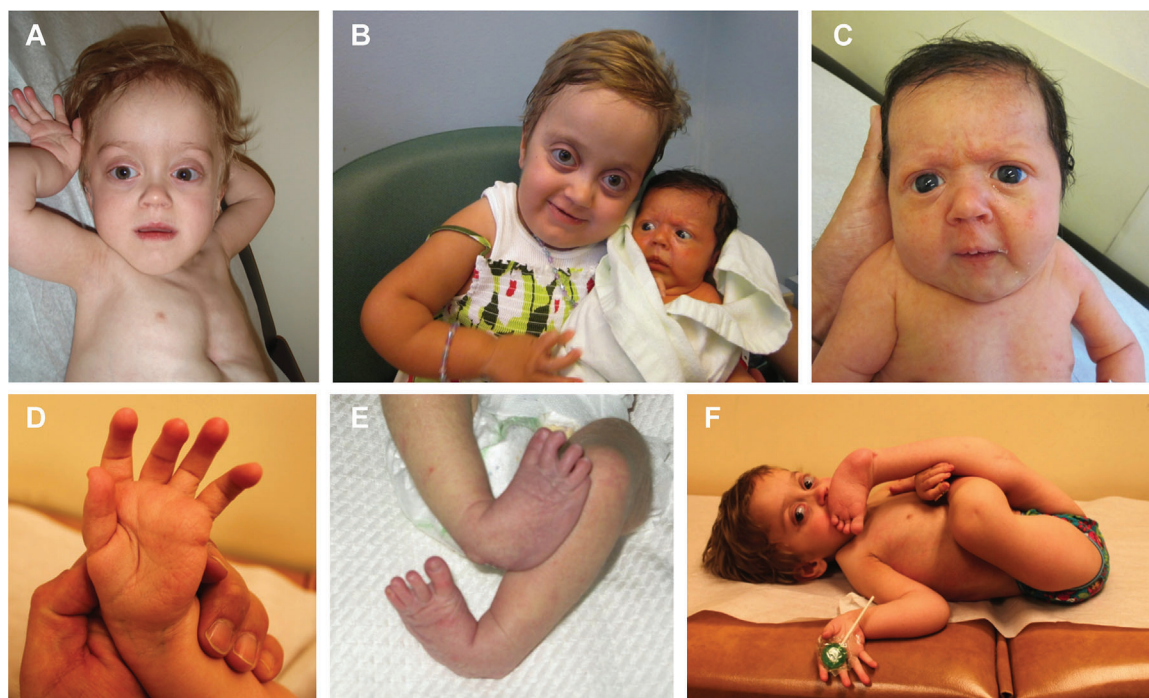
- **Reproductive, including pregnancy**  
Antenatal ultrasound abnormalities were reported in eight pregnancies. Reported abnormalities included prenatal kyphoscoliosis (n = 2), shortening of tubular bones (n = 2), contractures of wrists and clubfeet (n = 1), oligohydramnios (n = 1); polyhydramnios (n = 1), decreased fetal movements (n = 1), and small cerebellum (n = 1). For two siblings, the pregnancy was terminated around 22 weeks of gestation because of “severe skeletal dysplasia.”  
Reported perinatal complications include breech presentation (n = 3), congenital kyphoscoliosis (n = 3), congenital bilateral hip dislocation (n = 1), bilateral clubfeet (n = 3), congenital fractures (n = 1), cloudy cornea (n = 1), unilateral unilateral agenesis of the kidney (n = 1), open foramen ovale (n = 1), cleft palate (n = 3), Pierre-Robin sequence (n = 1), severe congenital muscle hypotonia or floppy infant (n = 2), and a cerebral hemorrhage following vaginal delivery (n = 1)
- **Craniofacial features**  
Characteristic craniofacial features include blue sclerae (n = 24), frontal bossing (n = 21), midfacial hypoplasia (n = 20), downslanting palpebral fissures (n = 4), low-set, sometimes posteriorly rotated, ears (n = 19), prominent eyes/proptosis (n = 15), long philtrum (n = 15), micrognathia (n = 11), depressed nasal bridge (n = 9), small nose (n = 6), tooth discoloration (n = 5), hypoplastic teeth (n = 4), sparse hair (n = 4), high arched palate (n = 3), prominent chin (n = 2), cleft palate (n = 3), Pierre-Robin sequence (n = 1), asymmetrical skull (n = 2), large anterior fontanel (n = 1), short neck with low hairline (n = 1)
- **Musculoskeletal system**  
Kyphoscoliosis is very frequent (n = 32), and may be congenital or

develop during the first 24 months of age, and is usually progressive. Severe short stature was frequently reported (n = 26). While stature may be short at birth, growth restriction usually evolved postnatally. Patients present joint hypermobility (n = 27), either generalized or restricted to distal joints, and sometimes complicated by dislocations of large and small joints (n = 14). Joint contractures were frequent (n = 21); they were either congenital, such as talipes equinovarus (n = 21), and/or evolved postnatally, and mostly affected fingers (e.g., adducted thumbs, camptodactyly), wrists, elbows, feet, and knees. Pectus deformities, either carinatum or excavatum, were reported in 8 patients. A total of 13 patients had a history of multiple spontaneous bone fractures and 12 had documented generalized osteoporosis or osteopenia. Finger shapes were characteristically described as “slender,” “arachnodactyly,” or “tapering,” with “spatulate or broad distal phalanges” (n = 13).

- **Skeletal X-ray imaging**  
Reported abnormalities include: Short ilia (n = 17), platyspondyly (n = 16) (described as becoming less conspicuous over time by Nakajima et al. [2013]), ovoid vertebra (n = 1), metaphyseal flaring (n = 13), osteopenia (n = 12), anterior beak of vertebral body (n = 12), prominent lesser trochanter (n = 11), elbow malalignment (n = 10), epiphyseal dysplasia femoral head (n = 10), metacarpal shortening (n = 7), overtubulation (n = 6), radial head dislocation (n = 5), advanced carpal ossification (n = 5), bowing of long bones (n = 3), narrowing of long bones (n = 3), acetabular dysplasia (n = 2), vertebral listhesis (n = 2), radioulnar synostosis (n = 1), craniosynostosis (n = 1), coxa valga (n = 1), coxa valga (n = 1), wedged vertebral bodies (n = 1), bony fusion of proximal ends of ulna and radius (n = 1), carpal fusion (n = 1). One patient had a severe torticollis at 12 months due to a posterior displacement of the vertebral column, with atlanto-occipital and atlanto-axial dislocation. Two patients had an invagination of the atlas into the foramen magnum and anterior

atlanto-axial subluxation. In two patients, the cervical instability was associated with hydrocephalus [Van Damme et al., unpublished].

- **Skin and integument**  
The skin was usually described as hyperextensible or loose (n = 19), soft and doughy (n = 18), and/or thin or translucent (n = 8). In 10 patients, increased palmar wrinkling of the hands was reported. Atrophic scarring (n = 5) and easy bruising (n = 3) were rarely reported. Other reported skin features include wrinkling of the skin on the dorsum of the hands (n = 1), and loose, redundant skin folds on wrists and ankles (n = 1). Bilateral inguinal hernia was described in one patient.
- **Ocular involvement**  
Refractive errors were reported in five patients. Other ocular problems include microcornea (n = 1); intermittent glaucoma (n = 1); congenital corneal clouding with sclerocornea (n = 1), and repetitive retinal detachment (n = 1). One patient had optic nerve atrophy.
- **Dental involvement**  
Dental involvement was reported in a number of patients and includes tooth discoloration (n = 5), hypoplastic teeth (n = 4), and early decay of teeth (n = 1).
- **Hearing**  
Hearing impairment with a conductive component was described in one patient.
- **Cardiovascular system**  
Two patients had a dilation of the ascending aorta in infancy, two patients had a mitral valve prolapse, two patients had an atrial septum defect, one patient had a patent ductus arteriosus, and one patient had a patent foramen ovale. Two patients suffered from a cerebral hemorrhage. One patient was reported with severe bruising, spontaneous scalp hematomas, and multiple hemorrhagic blisters.
- **Gastro-intestinal system**  
Constipation was described in one patient, and gastro-oesophageal reflux in another.
- **Urogenital system**  
Hydronephrosis was detected in one patient, unilateral renal agenesis in one, dilatation of renal pelvis ureterocoele in one and bladder atonia in one. One patient developed a Wilms tumor.



**Figure 5.** Clinical characteristics of patients with spEDS-*B3GALT6*. Facial characteristics at 2 years of age (A), 6 years of age (B) and 2 months of age (younger sister in panel C) include long face with mild micrognathia in infancy, proptotic eyes with shallow orbits and blue tint to the sclerae. The palpebral fissures are downslanting. The nasal bridge is broad and there is a low nasal ridge. Chest shows moderate pectus excavatum. The limbs show significant hyperextensibility, reduced movement of distal joints that leads to absent creases of the distal interphalangeal joints (D), increased creases of the skin of the palms (D) and club feet (E). There is muscle hypotonia, which is compounded by the hyperextensibility of the joints and makes anti-gravitational movements difficult (F) (Images kindly provided by Dr. Roberto Mendoza-Londono, with permission).

- Pulmonary involvement

Several pulmonary problems were reported: Three patients were reported with restrictive lung disease; in one of them this was due to lung hypoplasia and a diaphragmatic hernia. Respiratory distress due to lung hypoplasia was reported in another patient. Other reports included mild respiratory deficiency (n = 1), sleep apnea (n = 1), chronic aspiration and pneumonia (n = 1), and asthma (n = 1).

- Neuromuscular features and motor development

Muscle hypotonia was reported in 16 patients, and four of them had documented muscle hypoplasia. Gross motor developmental delay, with a delay in sitting and walking, was described in seven patients mainly because of muscle hypotonia. The age at which unassisted walking occurred in patients who accomplished ranged between 2 and 7 years. Three patients remained

non-ambulatory during childhood. In three patients, feeding problems, due to muscle hypotonia, were reported.

- Neurological features and neurodevelopment

Two patients were reported with brain atrophy, and two patients had hydrocephalus.

Mild to moderate cognitive delay was suggested in 11 patients.

### Genotype–Phenotype Correlation and Penetrance

No genotype–phenotype correlations have been described.

Penetrance is complete. Obligate carriers display no overt clinical symptoms.

### Management

No specific guidelines for management of patients with spEDS-*B3GALT6*

are available. Management guidelines should be tailored to the individual's specific problems and should follow those formulated for other forms of EDS.

Specific management guidelines may include:

- Musculoskeletal:

- At diagnosis a whole body skeletal survey and bone densitometry studies are recommended
- In patients with recurrent fractures, bisphosphonate therapy should be considered, with treatment protocols following those formulated for patients with OI
- Physical therapy for the contractures and muscle hypotonia, and monitoring for any signs of feeding or respiratory difficulties, in particular nocturnal hypoventilation. If the latter is present then assisted non-interventional ventilation at night may be indicated

- Cardiovascular system:
  - Measurement of aortic root size and assessment of heart valves by echocardiogram at the time of diagnosis or by age 5 years.
  - Echocardiogram at 5-year intervals, even if the initial echocardiogram is normal
  - Further vascular surveillance ought to be considered

### Differential Diagnosis

- Spondylodysplastic EDS-*B4GALT7*
- Spondylodysplastic EDS-*SLC39A13*
- Kyphoscoliotic EDS (*PLOD1* and *FKBP14*)
- Musculocontractural EDS
- OI
- Cutis laxa syndromes
- Chondrodysplasia
- Congenital myopathies

### SPONDYLODYSPLASTIC EDS DUE TO *SLC39A13* MUTATIONS (spEDS-*SLC39A13*)

Synonyms: spondylocheirodysplastic EDS (SCD-EDS)

#### History of Spondylodysplastic EDS due to *SLC39A13* Mutations (spEDS-*SLC39A13*)

Giunta et al. [2008b] reported a “new” clinical entity caused by a mutation in the zinc transporter gene *SLC39A13*. The clinical features of six patients from two unrelated consanguineous families were similar to those of kEDS-*PLOD1*, but lack (kypho)scoliosis and in addition presented distinct phenotypic components, including platyspondyly, osteopenia, short stature and widened metaphyses, tapered fingers, and a tendency to develop contractures of small joints. Because of features affecting mainly the spine (spondylo) and the hands (cheiro) this variant was termed the Spondylocheirodysplastic form of EDS (SCD-EDS) [Giunta et al., 2008b]. The six

patients presented with EDS-like features, short stature, finger contractures, distinct radiological features, elevated ratios of lysyl pyridinoline to hydroxylysyl pyridinoline (LP/HP) (but to a lesser degree than in EDS type VIA), and underhydroxylated collagens in culture despite normal in vitro activities of lysyl hydroxylase and prolyl 4-hydroxylase, respectively. The underhydroxylation was a generalized process which occurs along the entire molecule, and is not confined to specific residues as shown by tandem mass spectrometry of the  $\alpha 1(I)$ - and  $\alpha 2(I)$ -chain derived peptides of collagen type I and involves at least collagen types I and II [Giunta et al., 2008a]. Subsequently, Fukada et al. [2008] reported a third family with two affected siblings presenting with similar clinical findings who were homozygous for a missense mutation in *SCL39A13* [Fukada et al., 2008]. The authors furthermore generated a *Slc39a13*<sup>-/-</sup> knockout mouse that recapitulated defects observed in the patients, thereby demonstrating that mutations in *SLC39A13* cause Spondylocheiro-dysplastic EDS [Fukada et al., 2008].

In view of the clinical overlap of EDS caused by *SLC39A13* mutations, with the phenotypes caused by *B3GALT6* and by *B4GALT7* mutations, these three conditions are grouped within the same clinical entity (“Spondylodysplastic EDS”) in the new EDS classification.

The exact prevalence of spEDS-*SLC39A13* is unknown.

#### Mechanisms of Disease

SpEDS-*SLC39A13* is caused by homozygous loss-of-function mutations in the zinc transporter gene *SLC39A13*. This gene encodes the homodimeric transmembrane Zrt/irt-like protein 13 (ZIP13) protein, a member of the SLC39A/ZIP family that regulates the influx of zinc ( $Zn^{++}$ ) into the cytosol [Bin et al., 2011]. This protein is a member of the LIV-1 subfamily of ZIP zinc Transporters (LZT), a highly conserved group of

eight transmembrane domain proteins known to transport zinc and/or other metal ions from the extracellular space or from the organellar lumen into the cytoplasm [Eide, 2006]. Mutant ZIP13 proteins are easily degraded, and disturb the intracellular  $Zn^{++}$  homeostasis [Bin et al., 2014a,b]. It has been shown that ZIP13 loss-of-function leads to a generalized disturbed hydroxylation of lysyl and prolyl residues in collagen  $\alpha$  chains [Giunta et al., 2008b]. Since  $Zn^{++}$  was found to be an effective competitive inhibitor with respect to iron ( $Fe^{++}$ ) for prolyl 4-hydroxylase and for lysyl hydroxylase, it was initially suggested that the generalized underhydroxylation of collagen was likely due to  $Zn^{++}$  overload in the ER.  $Zn^{++}$  competes with  $Fe^{++}$  for binding to lysyl hydroxylase, prolyl 4-hydroxylase, and prolyl 3-hydroxylase, thus impairing hydroxylation of lysyl and prolyl residues [Giunta et al., 2008b]. Further studies however have disputed this hypothesis. One study proposed that trapping of  $Zn^{++}$  in vesicular stores reduces the availability of Zn in the ER and other cellular components and induces ER stress [Jeong et al., 2012]. Another study showed that ZIP13 is required for full activation of BMP/TGF- $\beta$  signaling via regulation of the intracellular localization of Smad proteins in connective tissue forming cells; this study put forward the hypothesis that incomplete activation of BMP/TGF- $\beta$  signaling is responsible for the observed phenotype [Fukada et al., 2013].

#### Allelic Heterogeneity

Three mutations have been identified so far in a total of eight patients from three independent families.

A homozygous 9-bp in-frame deletion in exon 4, c.483\_491del9 was found in two unrelated consanguineous families originating from North-Western Iraq and the Southeastern part of Turkey, respectively. At the protein level, the c.483\_491del9 mutation leads to the deletion of the highly conserved amino acid residues Phe-Leu-Ala from the third transmembrane domain of

ZIP13 [Giunta et al., 2008b]. The second mutation, a homozygous missense variant c.221G>A, p.(Gly64Asp) [Bin et al., 2011] has been identified in two siblings from Portugal [Fukada et al., 2008]. It is localized in the second transmembrane domain of *SLC39A13* and is conserved through all vertebrate species down to fish.

### Clinical Description

To date, eight patients with spEDS-*SLC39A13* from three independent families have been described: Three pediatric (<12 years), two adolescents (12.5 and 14.5 years), and three adults (>20 years) (Table S1) [Fukada et al., 2008, Giunta et al., 2008b].

The hallmarks of the disorder include: (1) Moderate short stature; (2) hyperelastic, velvety, thin skin with an easily visible venous pattern, and bruiseability which leads to atrophic scars; (3) slender, tapering fingers, wrinkled palms, and considerable thenar (and hypothenar) atrophy; (4) distal joint hypermobility which later results in contractures; (5) characteristic radiographic abnormalities; and (6) a ratio of urinary pyridinolines, LP/HP, of ~1.0 [Giunta et al., 2008b] (Representative pictures of the phenotype are given in Fig. 6).

- Reproductive, including pregnancy  
All affected individuals were born at term from uncomplicated pregnancies. No pregnancies have been reported in the affected individuals.
- Craniofacial features  
Protuberant eyes and down-slanting palpebral fissures were described in the majority of affected from the three families.
- Musculoskeletal system  
Short stature with height at the third centile or below was reported for all patients but one, whose height was at the 10th centile at age 8.5 years. The adult patients presented with mildly shortened trunk. Slender tapering fingers were also reported in the majority of the affected.
- Skeletal X-ray imaging  
Reported features include: platyspondyly, osteopenia of the axial skeleton,

widening of the ends with relative narrowing of the diaphyses and flat epiphyses of metacarpals and phalanges, small ileum, mildly flat proximal epiphyses, and short and wide femoral necks.

- Skin and integument  
The most distinctive cutaneous features were thin, velvety and fragile, easy bruisable skin with atrophic, cigarette paper-like scars. The skin of the palm of the hands was wrinkled in all affected individuals. In some individuals the skin was translucent particularly on legs and feet with easily visible veins.
- Ocular features  
Ophthalmologic features included myopia, hyperopia, astigmatism, and blue sclerae.
- Dental features  
Hypodontia of one or few teeth in permanent dentition or abnormally shaped teeth were described in all affected individuals, but one.
- Cardiovascular system  
Vascular complications were described in the male patient from Portugal who suffered from a cerebral hemorrhage posteriorly to the left putamen at age 21 years, from which he recovered completely. The adult patients had venous varicosities on their feet and legs in adulthood.
- Neuromuscular features and motor development  
The most distinctive muscular feature was atrophy of the thenar and the hypothenar and muscle weakness of the fingers. This feature was not reported in the two adult siblings described by Fukada et al. [2008]. Motor development was normal.

### Genotype–Phenotype Correlation and Penetrance

No genotype–phenotype correlations have been described. Penetrance is presumably complete. Obligate carriers have no overt phenotype.

### Management

No specific management guidelines have been reported. Guidelines for management of musculoskeletal problems, skin

involvement, cardiovascular problems, and pregnancy should follow those formulated for other forms of EDS (for reference: See “management guidelines for the classical Ehlers–Danlos syndrome,” by Bowen et al., this issue).

### MUSCULOCONTRACTURAL EDS (mcEDS)

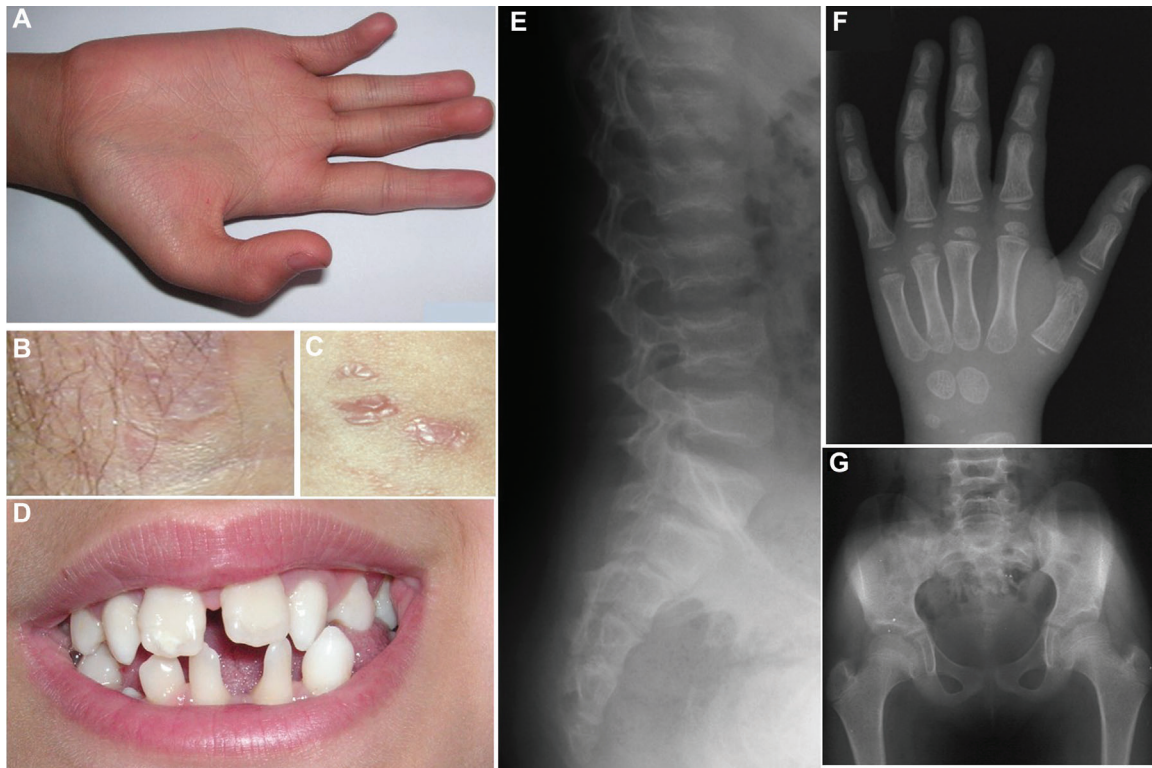
Synonyms: Adducted thumb, clubfoot, and progressive joint and skin laxity syndrome; Adducted thumb–club foot syndrome (ATCS); Dündar syndrome; EDS Kosho type (EDS-KT); EDS musculocontractural type 1 (EDS-MC); EDS type VIB, EDS6B; Distal arthrogyposis with peculiar facies and hydronephrosis

### The History of Musculocontractural EDS

EDS caused by D4ST1 deficiency has initially been reported as three independent conditions: A rare type of arthrogyposis syndrome, “adducted thumb–clubfoot syndrome (ATCS)”; a specific type of EDS, “EDS, Kosho Type (EDSKT)”; and a subset of kEDS without lysyl hydroxylase deficiency, “musculocontractural EDS (MCEDS),” all of which are now concluded to be a single clinical entity [Kosho et al., 2005, Malfait et al., 2010, Janecke et al., 2011, Kosho et al., 2011, Shimizu et al., 2011].

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***EDS caused by D4ST1 deficiency has initially been reported as three independent conditions: A rare type of arthrogyposis syndrome, “adducted thumb–clubfoot syndrome (ATCS)”; a specific type of EDS, “EDS, Kosho Type (EDSKT)”; and a subset of kEDS without lysyl hydroxylase deficiency, “musculocontractural EDS***



**Figure 6.** Clinical and radiological findings in patients with spEDS-*SLC39A13*. (A) Appearance of the hand in a 10.1 years old patient. Excessively wrinkled palm, thenar and hypothenar atrophy, tapering fingers, and contracted thumb. (B and C) Abnormal scar formation in two patients. (D) Abnormal dentition and hypodontia. (E) Radiograph of the low thoracic and lumbar spine in a patient aged 11.5 year with flattening, irregular endplates, and osteopenia of the vertebral bodies. (F) Radiograph of the hand of a 3.5 years old patient showing alterations of shape and flat epiphyses of the short tubular bones. (G) Radiograph of the pelvis of a 10 years old patient showing small ilia, mild flattening of the proximal epiphyses, short and wide femoral necks (Images kindly provided by Prof. Beate Albrecht and Prof. Nursel Elçioglu, with permission).

**(mcEDS),” all of which are now concluded to be a single clinical entity.**

The prevalence is unknown.

- Adducted thumb–clubfoot syndrome Dündar et al. [1997] originally reported on two cousins from consanguineous Turkish family, both having developmental delay, ocular abnormalities, characteristic facial features, generalized joint laxity, arachnodactyly, camptodactyly, and distal arthrogyriposis with adducted thumbs and clubfeet. The authors named this condition adducted thumb–clubfoot syndrome (ATCS) [Dündar et al., 1997]. They subsequently reported a similar patient from another consanguineous Turkish family with three other similarly affected

siblings who died of an unknown etiology in early infancy [Dündar et al., 2001]. The authors suggested that two brothers from a Japanese consanguineous family, manifesting multiple distal arthrogyriposis, characteristic facial features, cleft palate, short stature, hydronephrosis, cryptorchidism, and normal intelligence, also had the syndrome [Sonoda and Kouno, 2000]. Janecke et al. [2001] described two affected brothers from another consanguineous Austrian family, and concluded that the syndrome would represent a new type of arthrogyriposis with central nervous system involvement, congenital heart defects, urogenital defects, myopathy, connective tissue involvement (GJH), and normal or subnormal intellectual development [Janecke et al., 2001]. Dündar et al. [2009] reported that loss-of-function mutations in *CHST14* was causal for

the syndrome, through homozygosity mapping using samples from previously described consanguineous families. They also described follow-up clinical findings of previously reported patients, including GJH, delayed wound healing, ecchymoses, hematomas, and osteopenia/osteoporosis, from which the authors categorized the syndrome into a generalized connective tissue disorder.

- EDS, Kosho Type In 2000, Kosho and colleagues encountered the first patient with a specific type of EDS, and the second unrelated patient with parental consanguinity in 2003. Both patients were Japanese girls with characteristic craniofacial features, skeletal features (multiple congenital contractures, marfanoid habitus, pectus excavatum, GJH recurrent dislocations, progressive talipes, and spinal deformity), cutaneous features (hyperextensibility, bruisability, and fragility with

atrophic scars), recurrent large subcutaneous hematomas, and hypotonia with mild motor developmental delay [Kosho et al., 2005] which were strikingly similar manifestations observed in Pakistani siblings classified as having a rare variant of kEDS with normal lysyl hydroxylase activity (“EDS type VIB”) [Steinmann et al., 1975]. Kosho et al. [2005] proposed that these patients represented a clinically recognizable subgroup of EDS, tentatively classified as EDS type VIB. Kosho et al. [2010] described four additional unrelated Japanese patients with similar features, including a patient with parental consanguinity and a patient reported by Yasui et al. [2003]. They concluded that these patients represented a new clinically recognized form of EDS with distinct craniofacial features, multiple congenital contractures, and multisystem fragility-related manifestations [Kosho et al., 2010]. The syndrome was registered as “EDS, Kosho type” in the London Dysmorphology Database (<http://www.lm.databases.com/index.html>) and also in POSSUM (<http://www.possu.net.au/>). Miyake et al. [2010] identified *CHST14* as the causal gene for this condition through homozygosity mapping using the two consanguineous families.

- Musculocontractural EDS

Malfait et al. [2010] found mutations in *CHST14* through homozygosity mapping in two Turkish sisters and an Indian girl, both with parental consanguinity. The patients shared characteristic craniofacial features, joint contractures, and wrinkled palms in addition to common features of kEDS, including kyphoscoliosis; joint hypermobility; muscular hypotonia; hyperextensible, thin, and bruisable skin with atrophic scarring; and ocular complications. The authors concluded that these patients and those diagnosed with ATCS or EDS, Kosho type had a single clinical condition, which they termed “Musculocontractural EDS (MCEDS)” [Malfait et al., 2010].

- EDS caused by DSE deficiency

Janecke and colleagues identified a homozygous loss-of-function *DSE*

mutation, through positional candidate gene approach, in a boy from a consanguineous Indian family, who had characteristic facial features, congenital contractures of the thumbs and the feet, joint hypermobility, muscle weakness, and atrophic scars and was diagnosed with MCEDS [Müller et al., 2013]. Malfait and colleagues found a missense *DSE* mutation in two affected sisters from a Spanish family, who shared hyperextensible and fragile skin, recurrent large hematomas, long slender fingers, and clubfeet, but no adducted thumbs [Syx et al., 2015]. In OMIM, the syndrome is termed as EDS, musculocontractural type 2 (EDSMC2) to be distinguished from EDS caused by *D4ST1*-deficiency termed as EDS, musculocontractural type 1 (EDSMC1).

### Mechanisms of Disease

- *D4ST1* deficient EDS

EDS caused by *D4ST1*-deficiency results from recessive mutations in the carbohydrate sulfotransferase 14 gene (*CHST14*), localized at 15q14. *CHST14* is a single-exon gene encoding carbohydrate sulfotransferase 14 or dermatan 4-O-sulfotransferase 1 [Evers et al., 2001]. *D4ST1* is a Golgi-resident enzyme which is involved in the biosynthesis of the GAG dermatan sulfate, where it catalyzes 4-O-sulfation of N-acetylgalactosamine (GalNAc) in the sequence “L-iduronic acid (IdoA)-GalNAc,” immediately after epimerization of D-glucuronic acid (GlcA) to IdoA by dermatan sulfate epimerase (*DSE*) [Evers et al., 2001].

The sulfotransferase activity of cos-7 cells transfected with *CHST14* containing p.(LysK69\*), p.(Pro281Leu), p.(Cys289Ser), or p.(Tyr293Cys) mutations was decreased at almost the same level, suggesting that loss-of-function mutations in *CHST14* (i.e., *D4ST1* deficiency) are the basis of this disorder [Miyake et al., 2010].

Sulfotransferase activity toward DS in mutant skin fibroblasts was significantly decreased to 6.7% in a patient with the compound heterozygous mutation

p.(Pro281Leu)/p.(Tyr293Cys) and to 14.5% in a patient with the homozygous mutation p.(Pro281Leu), compared with each age- and sex-matched control [Miyake et al., 2010]. Disaccharide composition analysis of CS/DS chains isolated from the affected skin fibroblasts in these two patients showed a negligible amount of DS and excess amount of CS, which was suggested to result from impaired 4-O-sulfation lock due to *D4ST1* deficiency, followed by back-epimerization from IdoA to GlcA [Dündar et al., 2009; Miyake et al., 2010; Syx et al., 2015]. Decorin, a major DS-PG in the skin, consists of a core protein and a single GAG chain that plays an important role in assembly of collagen fibrils, possibly through an electrostatic interaction between decorin DS chains and adjacent collagen fibrils. GAG chains of decorin from the affected skin fibroblasts contained exclusively CS and no DS disaccharides, while those from the controls contained mainly DS disaccharides (approximately 95%) [Miyake et al., 2010; Syx et al., 2015]. Light microscopy of the affected skin specimens using hematoxylin and eosin staining showed that fine collagen fibers were predominantly present in the reticular to papillary dermis with marked reduction of normally thick collagen bundles [Miyake et al., 2010]. Transmission electron microscopy showed that collagen fibrils in the affected skin specimens were dispersed in the reticular dermis in contrast to the regularly and tightly assembled collagen fibrils observed in the controls and that each collagen fibril in affected skin specimens was smooth and round, not varying in size and shape, similar to that in the controls [Miyake et al., 2010].

In view of these findings, skin fragility in patients with EDS caused by *D4ST1* deficiency is postulated to result from impaired assembly of collagen fibrils caused by the replacement of a DS with a CS chain of decorin through alterations in the electrostatic binding of decorin to collagen fibrils followed by difference in the spatial relationship between collagen fibrils and decorin [Kosho, 2013; Kosho et al., 2014; Kosho, 2016].

- DSE-deficient EDS

EDS caused by DSE deficiency results from recessive mutations in the dermatan sulfate epimerase gene (*DSE*). DSE is a Golgi-resident enzyme that catalyzes the epimerization of D-glucuronic acid (GlcA) toward iduronic acid (IdoA). This allows D4ST1 to catalyze the 4-O-sulfation of the adjacent GalNAc, which then prevents back-epimerization of the IdoA to GlcA.

Two homozygous missense mutations (p.(Arg267Gly); p.(Ser268Leu)) have been detected [Müller et al., 2013; Syx et al., 2015]. Heterologous expression of mutant full-length and soluble recombinant DSE proteins harboring the p.(Ser268Leu) substitution showed a loss of activity towards partially desulfated DS, and patient-derived fibroblasts also showed a significant reduction in epimerase activity. The amount of DS disaccharides was markedly decreased in the conditioned medium and cell fraction from cultured patient fibroblasts compared to control. No difference was seen in CS chains from the conditioned media, though the total amount of CS disaccharides in the cell fraction from the patient was increased approximately 1.5-fold, consistent with increased synthesis or reduced conversion of CS chains [Müller et al., 2013].

Syx et al. [2015] analyzed fibroblasts from a patient harboring the p.(Arg267-Gly) substitution and could show that a minor fraction of decorin DS was present, consisting of IdoA-containing disaccharides, which could be attributed to residual DSE activity, or compensating DSE2 activity. In this patient, no pronounced ultrastructural abnormalities of dermal collagen fibrils were noted, but immunofluorescent stainings of collagen types I, III, and V and fibronectin showed evidence of abnormal ECM assembly [Syx et al., 2015].

### Allelic Heterogeneity

- *CHST14*

Mutations have been detected throughout the *CHST14* gene. These include

11 missense mutations, six frameshift mutations, and two nonsense mutations.

The p.(Pro281Leu) was most frequent (n = 9), other recurrent mutations include p.(Val49\*) (n = 3), p.(Arg213Pro) (n = 2), and p.(Trp293Cys) (n = 4). All other mutations were reported once: p.(Arg29Gfs\*113), p.(Lys69\*), p.(Gln113Argfs\*14), p.(Arg135Gly), p.(Leu137Gln), p.(Cys152Leufs\*10), p.(Phe209Ser), p.(Arg218Ser), p.(Gly228Leufs\*13), p.(Glu262Lys), p.(Arg274Pro), p.(Met280-Leu), p.(Cys289Ser), p.(Trp327Cfs\*29), and p.(Glu334Glyfs\*107).

- *DSE*

Two homozygous *DSE1* missense mutations (p.(Arg267Gly) and p.(Ser268-Leu)) have been detected.

A registry with *CHST14* and *DSE* gene variants is available [Dalglish, 1998].

### Clinical Description

At present, 39 patients (18 females, 21 males) from 26 families have been published with recessive *CHST14* mutations (Table S1). The ages of patients with *CHST14* mutations at the latest publication ranged from 0 day to 59 years. [Dündar et al., 1997; Sonoda and Kouno, 2000; Dündar et al., 2001; Janecke et al., 2001; Yasui et al., 2003; Kosho et al., 2005; Kosho et al., 2010; Malfait et al., 2010; Shimizu et al., 2011; Mendoza-Londono et al., 2012; Winters et al., 2012; Voermans et al., 2012; Syx et al., 2015; Janecke et al., 2016; Mochida et al., 2016].

Three patients (one child, two adult women) from two families have been published with recessive *DSE1* mutations [Müller et al., 2013; Syx et al., 2015].

The hallmarks of the disorder include: (1) Characteristic craniofacial features, (2) congenital multiple contractures, including adducted thumbs and talipes equinovarus, (3) characteristic cutaneous features including fine palmar creases, (4) peculiar finger shapes, (5) progressive spinal and foot deformities, (6) large subcutaneous hematomas, and (7) ophthalmological and urogenital

involvement (Representative pictures of the phenotype are given in Fig. 7).

- Reproductive, including pregnancy

Pregnancy-related findings include hand/foot anomalies (n = 3), oligohydramnios (n = 2), and decreased fetal movement (n = 2). No deliveries have been described in female patients with EDS caused by D4ST1 deficiency. Two deliveries were described in a female patient with EDS caused by DSE deficiency, followed by uterine and bladder prolapse [Syx et al., 2015].

- Craniofacial features

Characteristic craniofacial features include a large fontanelle (n = 24), hypertelorism (n = 36), downslanting palpebral fissures (n = 35), blue sclerae (n = 28), short nose with hypoplastic columella (n = 17), ear deformities (n = 35) including low-set (n = 22) and posteriorly rotated (n = 14) ears, high palate (n = 21), long philtrum and/or thin upper lip vermilion (n = 24), and small mouth and/or micro-retrognathia (n = 16) at birth to early childhood. Slender facial shapes with protruding jaws (n = 11) and facial asymmetry (n = 8) are evident from adolescence.

- Musculoskeletal system

Mild prenatal growth restriction was suggested: Mean birth length  $-0.5$  SD and median  $-0.6$  SD (n = 9; range, from  $-1.6$  to  $+1.3$  SD); mean birth weight  $-0.6$  SD and median  $-0.67$  SD (n = 11; range, from  $-2.0$  to  $+0.5$  SD); and mean birth occipital frontal circumference  $-0.2$  SD and median  $-0.5$  SD (n = 8; range, from  $-1.0$  to  $+1.0$  SD) [Shimizu et al., 2011]. Mild postnatal growth restriction was suggested with slenderness and relative macrocephaly: Mean height  $-0.9$  SD and median  $-0.6$  SD (14 data points from 12 patients; range, from  $-3.9$  to  $+1.2$  SD); mean weight  $-1.5$  SD and median  $-1.4$  SD (11 data points from 9 patients; range, from  $-2.4$  to  $-0.4$  SD); and mean occipital frontal circumference  $-0.2$  SD and median  $0.0$  SD (10 data points from 8 patients; range, from  $-1.2$  to  $>2.0$  SD) [Shimizu et al., 2011].

Multiple congenital contractures were cardinal features and typically included adduction-flexion contractures of the thumbs (n = 32; no adducted



**Figure 7.** A female patient with mcEDS-*CHST14*. Facial characteristics at age 23 days (A) and 24 years (B). (A and C) Congenital contractures of fingers including adducted thumbs (A) and cylindrical fingers at age 24 years (C). (D) Characteristic wrinkling palmar creases. (E) Left talipes equinovarus. (F) progressive foot deformities at age 15 years. (G) A large subcutaneous hematoma at age 6 years (Images kindly provided by Dr. Tomoki Kosho, with permission. A and E, originally published in Kosho et al. [2010], in American Journal of Medical Genetics; G, originally published in Kosho et al. [2005], in American Journal of Medical Genetics).

thumbs in seven and data not available in two) and talipes equinovarus ( $n = 42$ ). Finger shapes were characteristically described as “arachnodactyly,” “tapering,” “slender,” or “cylindrical” ( $n = 36$ ). Progressive talipes deformities (planus, valgus, or severer) ( $n = 27$ ) and spinal deformities (decreased physiological curvature, scoliosis, or kyphoscoliosis) ( $n = 23$ ) were observed. Marfanoid habitus ( $n = 13$ );

recurrent, chronic or easy joint dislocations ( $n = 19$ ), and pectus deformities (flat and thin, excavatum, or carinatum) ( $n = 18$ ) were also noted.

- Skin and integument

Skin hyperextensibility (from childhood) and redundancy (from adolescence) ( $n = 24$ ), bruisability ( $n = 22$ ), and fragility with atrophic scars ( $n = 23$ ) were common. Acrogeria-like fine

palmar creases or wrinkles were characteristic and became evident with aging ( $n = 29$ ). Hyperalgesia to pressure was suggested ( $n = 8$ ) because patients disliked being hugged in infancy or disliked blood pressure measurement in the upper arms. Recurrent subcutaneous infections with fistula formation were also observed ( $n = 8$ ).

- Ocular involvement

Refractive errors, typically myopia ( $n = 12$ ) followed by astigmatism ( $n = 5$ ) and hyperopia ( $n = 4$ ), were described in 16 patients; strabismus in 13 patients; microcornea in 13; glaucoma or elevated intraocular pressure in eight; and retinal detachment in seven. Progressive visual field loss was described in a 31-year-old female [Kosho et al., 2010], deterioration in vision of the right eye because of the lacquer crack in Bruch’s membrane adjacent to right fovea in a 15-year-old male [Syx et al., 2015], and right-sided blindness because of retinal detachment in a 45-year-old female [Janecke et al., 2016].

- Hearing

Hearing impairment was described in nine patients (specified for high-pitched sounds in five).

- Cardiovascular system

Recurrent large subcutaneous hematomas (skull, extremities, or hips) are a serious complication that can, even after minor trauma, progress acutely and massively to hemorrhagic shock requiring intensive treatment (hospital admission, blood transfusion, or surgical drainage) ( $n = 21$ ). Intranasal administration of 1-desamino-8-D-arginine vasopressin (DDAVP) after trauma effectively prevented large subcutaneous hematomas in three patients [Kosho et al., 2010; Janecke et al., 2016]. Congenital heart defects, typically ASD, were detected in seven patients. Valve abnormalities and/or aortic root dilatation were also detected in seven patients. Infectious endocarditis occurred in two patients: One was successfully treated with surgical resection of the vegetation [Kosho et al., 2010] and the other expired [Janecke et al., 2016].



- **Gastro-intestinal system**  
Constipation was described in nine patients. Two adults and one adolescent patient had colonic diverticula perforation, corrected surgically [Kosho et al., 2010; Kono et al., 2016]. One adolescent patient had a severe progressive gastric ulcer, treated with partial gastrectomy [Kosho et al., 2010]. Complications associated with gastro-intestinal malformations included a common mesentery, spontaneous volvulus of the small intestine associated with absent gastrocolic omentum [Janecke et al., 2001], and duodenal obstruction due to malrotation [Malfait et al., 2010].
- **Urogenital system**  
Hydronephrosis was detected in eight patients. It was caused by renal ptosis in one patient, who underwent laparoscopic placement of a ureteral stent, complicated by severe hemorrhage due to tissue fragility [Malfait et al., 2010]. Another patient had a pelviureteric junction obstruction requiring nephrostomy in the neonatal period [Syx et al., 2015]. Nephrolithiasis or cystolithiasis was described in six patients, and recurrent urinary tract infection in three. Cryptorchidism was observed in most male patients. One patient who underwent orchidopexy developed hypogonadism in adulthood.
- **Pulmonary**  
Pneumothorax or pneumohemothorax occurred in three adult patients, who were treated with chest tube drainage [Kosho et al., 2010].
- **Neuromuscular features and motor development**  
Muscle hypotonia or weakness was described in 17 patients. A myopathic process was suggested as the cause of the muscle weakness in a patient. Electromyographic examination demonstrated muscle action potentials with reduced amplitude but with a normal distal latency time and nerve conduction velocity, and muscle biopsy revealed no histological abnormalities [Dündar et al., 1997]. In another patient, quantitative muscle ultrasonography showed increased echo intensity in the forearm extensors and anterior tibial muscles as well as marked bilateral atrophy

of the forearm flexors, forearm extensors, and quadriceps. Nerve conduction studies showed low compound muscle action potential amplitudes in the distal muscles. Needle electromyography showed an abnormal and mixed pattern of short-duration, low-amplitude, polyphasic motor units, as well as polyphasic motor units with a longer duration and higher amplitude, reflecting an increase in fiber size diameter. Muscle biopsy showed fiber type 1 predominance without fiber type grouping, increased variation in the diameter of both type 1 and type 2 fibers, and some type 1 fibers in close proximity to lobulated fibers. These findings were compatible with a myopathy, similar to other EDS types [Voermans et al., 2012]. Elevation of serum CK (creatine kinase) level was described in four patients (277, 698, 1838, 3000 IU/L) [Janecke et al., 2016].

Gross motor developmental delay was described in 23 patients mainly because of muscle hypotonia. The median age at which unassisted walking occurred in patients who accomplished it was 2 years 4 months ( $n = 10$ ; range, from 1 year 5 months to 4 years). One adult patient could not walk unassisted because of severe foot deformities and leg muscle weakness [Kosho et al., 2010].

- **Neurological features and neurodevelopment**  
Ventricular enlargement was described in six patients and asymmetry in three on brain ultrasonography, computed tomography, or magnetic resonance imaging. Additional minor findings were also recorded: Absence of the left septum pellucidum [Janecke et al., 2001], a short corpus callosum with lack of an isthmus and well-defined rostrum, mild prominence of the Sylvian fissures, and a few small gray matter heterotopias along the lateral walls of the temporal horns of the lateral ventricles [Mendoza-Londono et al., 2012], absence of the septum pellucidum, hypoplasia of the inferior vermis with a normally sized posterior fossa (Dandy-Walker variant), hypoplasia of the hippocampi and splenium of the corpus callosum, and hypoplasia of the optic nerves (septo-optic dysplasia) [Winters et al., 2012] and mild cerebellar hypoplasia,

hypoplasia of the cerebellar vermis (reminiscent of Dandy-Walker variant), and absence of the septum pellucidum [Syx et al., 2015]. Spinal cord tethering was noted in three patients, two of whom underwent corrective surgery.

Mild intellectual delay was suggested in three patients; one reportedly had global psychomotor delay in infancy, but his IQ was around 90 at the age of 7 years 2 months [Dündar et al., 1997; Janecke et al., 2011].

- **Other**  
Poor breast development was noted in seven female patients beyond adolescence.

### **Genotype-Phenotype Correlation and Penetrance**

Penetrance is complete, whereas differences in phenotypic severity among affected siblings have been suggested. No apparent genotype-phenotype correlations have been described among patients with EDS caused by D4ST1 deficiency. Phenotypic features in three patients with EDS caused by DSE deficiency seemed to be milder than those in patients with EDS caused by D4ST1 deficiency, presumably associated with the glycobiochemical finding that some DS moieties were present in the decorin GAG in the fibroblasts derived from patients with EDS caused by DSE deficiency, whereas DS was completely replaced by CS in the fibroblasts derived from patients with EDS caused by CHST14/D4ST1 deficiency [Syx et al., 2015].

### **Management**

Management should be comprehensive especially focusing on musculoskeletal, cutaneous, cardiovascular, visceral, and ocular complications [Shimizu et al., 2011; Kosho, 2016]. No specific guidelines for management of patients with D4ST1/DSE-deficient EDS are available. Guidelines for management of musculoskeletal problems, skin involvement, cardiovascular involvement, ophthalmologic and dental follow-up, and pregnancy should follow those formulated for other forms of EDS (for

reference: See “management guidelines for the classical Ehlers–Danlos syndrome,” by Bowen et al., this issue).

Specific management guidelines may include:

- At diagnosis:
  - Screening for congenital heart defects through a cardiac ultrasonography
  - Screening for ocular malformations through an examination by a pediatric ophthalmologist
  - Screening for malformations in the renal system through a renal ultrasonography
  - Screening for hearing impairment by an automated auditory brainstem response (aABR) as well as an examination of a pediatric otologist
- Musculoskeletal:
  - Orthopedic intervention (e.g., serial plaster casts or surgery) for talipes equinovarus and physical therapy for motor developmental delay as the center of care for patients with the disorder
  - After walking independently, special attention to progressive foot deformities and trauma that could cause skin lacerations, joint dislocations, or large subcutaneous hematomas
  - Assessment of spinal deformities (scoliosis, kyphoscoliosis)
- Skin: A wrist-type sphygmomanometer for patients with hyperalgesia to pressure
- Ophthalmological: Regular check-ups for strabismus, refractive errors, glaucoma
- ORL: Regular check-up for otitis media with effusion, hearing impairment
- Urological: Regular check-up for urination problems, bladder enlargement. Surgical fixation for cryptorchidism in males
- Cardiovascular: Regular check-up for valve abnormalities, aortic root dilation
- Gastro-intestinal: Regular check-up for constipation and evaluation for need of laxatives and/or enemas
- Assessment of secondary sex characteristics (breast development in females and gonadal function in males)
- Emergent treatment for pneumothorax or pneumohemothorax, large subcutaneous hematomas, and diverticular perforation

## Differential Diagnosis

- Spndylodysplastic EDS
- Kyphoscoliotic EDS (*PLOD1*, *FKBP14*)
- Freeman–Sheldon syndrome
- Loeys–Dietz syndrome

## MYOPATHIC EDS (mEDS)

Synonyms: EDS/Myopathy overlap syndrome

### The History of Myopathic EDS (mEDS)

The spectrum of diseases characterized by muscle weakness, hypotonia, myopathy, and connective tissue symptoms was first associated with mutations in the genes that code for collagen type VI. These conditions have a wide spectrum of severity that ranges from the most severe Ullrich congenital muscular dystrophy to the milder Bethlem myopathy. Zou et al. [2014] and Hicks et al. [2014] investigated groups of patients that had some symptoms of these myopathies but presented with a distinctive phenotype and did not have mutations affecting type VI collagen. Through these studies they identified eight patients in four families that presented with autosomal recessive (two patients, one family) and dominant (six patients, three families) forms of EDS/myopathy. Since then, one additional patient has been described [Punetha et al., 2016]. Prevalence of this condition is unknown.

### Mechanism of Disease

Collagen XII is synthesized as a homotrimer made up of three  $\alpha 1$  collagen chains coded by *COL12A1*. It is found along the surfaces of the fibers of collagen type I in tissues that express that collagen, and it is presumed to act as a bridge between the collagen I fibers and other extracellular components including decorin, fibromodulin, and TNX [Zou et al., 2014]. Deficiency of this collagen results in lax tissues due to a mechanical problem with the ECM. Because collagen XII is expressed in the

muscle ECM, its absence results in disorganized patterning, abnormal force transmission, and other biomechanical alterations resulting in myopathy. Its absence in skin and tendons would explain the overlapping EDS manifestations.

### Allelic Heterogeneity

To date, nine patients from five families with myopathic EDS have been reported [Hicks et al., 2014; Zou et al., 2014; Punetha et al., 2016]. The mutations include four heterozygous missense mutations that have an autosomal dominant mode of inheritance: c.7167 T>C, p.(Ile2334Thr), c.C5893T, p.(Arg1965Cys), 8329G>C, p.(Gly2777Arg), and c.G8357A, p.(Gly2786Asp), and one homozygous frameshift mutation, introducing a PTC, associated with autosomal recessive inheritance (c.8006 +1 G>A, p.(2567Asp>Phefs\*2).

### Clinical Description

At present, nine patients from five families have been reported (Table S1). Their age at publication ranged from birth to 48 years (Table S1) [Hicks et al., 2014; Zou et al., 2014; Punetha et al., 2016]. The severity ranges from a severe autosomal recessive neonatal form that was described in two boys born to a consanguineous couple, to a milder autosomal dominant form that presents in childhood with muscle weakness, large joint contractures, and variable degrees of joint hypermobility and hypertrophic scarring. The phenotype may not be fully understood as there are so few reported cases.

The hallmark of the disorder is muscle weakness that is present in infancy or childhood and is associated with proximal large joint contractures and distal joint hypermobility. Characteristically, the muscle weakness tends to get better with age until young adulthood with some deterioration in the 4th decade.

- Reproductive, including pregnancy
  - One patient was born after a selective caesarian section because of oligohydramnios, intrauterine growth restriction, and breech presentation.

- **Craniofacial features**  
One patient displayed facial asymmetry with skull flattening at birth, and mild dysmorphic facial features, including micrognathia, high palate, short nose, big dysplastic ears. High arched palate was reported in two other patients.
- **Musculoskeletal**  
Congenital proximal joint contractures in combination with distal joint hypermobility were reported in three patients. One patient was reported to have proximal contractures at birth without mentioning of distal joint hypermobility. At age 7 years, contractures had disappeared but she had joint hypermobility in childhood. All patients tended to have congenital kyphosis, in conjunction with torticollis in one, and some developed scoliosis. The patients reported by Hicks et al. [2014] (n = 5) developed long finger flexions (n = 4), rigid spine (n = 2), flexion contractures of knee (n = 2), elbow (n = 1), and wrist (n = 1). Hip dislocation or subluxation was reported in two patients. One patient had a pectus excavatum.
- **Skin and integument**  
Reported skin features include hypertrophic scars (n = 3), atrophic scars (n = 2), hyperkeratosis pilaris (n = 1).
- **Neuromuscular features and motor development**  
The siblings reported by Zou et al. [2014] (homozygous mutation) had the most severe phenotype with profound muscle hypotonia at birth, poor feeding and swallowing, and night-time hypoventilation, with need for tube feeding and non-invasive night-time ventilation. They had a severe delay in motor development, and they eventually were able to reach a sitting position, but were never able to stand or walk [Zou et al., 2014]. All other patients had congenital muscle hypotonia, with delayed gross motor development, but symptoms seemed to improve over time. None of these patients remained non-ambulatory. In one adult, muscle strength was reported to deteriorate again in his late 30s.  
Reported abnormalities on muscle biopsy include myopathy with variability in fiber diameter, without overt signs of degeneration or regeneration. In one instance, there was decreased laminin

B1. CK was elevated in several, but not all patients (of note: It was normal in the siblings with the most severe, autosomal recessive form).

### Genotype–Phenotype Correlation and Penetrance

There appears to be a clear genotype–phenotype correlation. In general, from the five mutations described so far it seems that missense mutations that affect critical residues in the molecule have a dominant-negative effect and result in variable degrees of severity. There are no documented instances of non-penetrance. There is significant variability, within and between families, with individuals carrying the same mutation presenting with different degrees of severity.

The one family with the autosomal recessive condition harbors a homozygous loss of function mutation. The affected siblings have a more severe form of the condition. Reportedly carrier parents walked late (almost at 2 years of age); therefore there appears to be a mild phenotype (dominant) in the carriers and a severe phenotype (recessive) in the individuals that are homozygous [Zou et al., 2014].

Penetrance is unknown.

### Management

There are no described treatments for this group of disorders. Anticipatory guidance should focus on preventing complications and improving the presenting symptoms. This may include physical therapy for the contractures (since they tend to resolve over time), and monitoring for any signs of feeding or respiratory difficulties, in particular nocturnal hypoventilation. If the latter is present, then assisted non-interventional ventilation at night may be indicated.

### Differential Diagnosis

- Bethlem and Ullrich myopathies (Collagen type VI-related disorders)
- Kyphoscoliotic EDS
- Hypermobility EDS

- Classical EDS
- Classical-like EDS

### PERIODONTAL EDS (pEDS)

Synonyms: EDS type VIII, Ehlers–Danlos Syndrome periodontitis type, Ehlers–Danlos Syndrome periodontosis type.

#### The History of Periodontal EDS

McKusick [1972] described a “unique condition” in a patient with EDS-like features, that is, lesions on the shins, slow-healing breaks in the skin, atrophic scars, and absorptive periodontitis with early loss of the teeth. Five years later, Stewart et al. [1977] published a similar case and classified this new variant as EDS VIII. Since then, pEDS has been reported in 32 case reports and seven pedigree analyses. Extensive periodontal destruction with early onset is a core finding of periodontal EDS, mostly in combination with striking pre-tibial plaques and tissue fragility. There is greater variability in other clinical features.

In the past, the delineation of pEDS as a specific phenotype was hampered by the relatively high prevalence of chronic periodontitis, with an estimated range from 19 to 83% depending on age and severity [Demmer et al., 2010]. Therefore, periodontal disease in vascular and other EDS types could be a coincidence of two unrelated diseases. These historical overlaps between pEDS and other EDS subtypes have confused the phenotype. While the majority of pEDS patients had vEDS excluded through collagen protein analysis, this does not completely exclude a *COL3A1* variant being causative in some of the older cases.

Also, joint hypermobility is a common feature in the general population, which complicates accurately ascertaining co-segregation of these two traits. The Villefranche EDS nosology group noted these difficulties in distinguishing this rare disorder from other hereditary disorders of connective tissue [Beighton et al., 1998]. In 2003, pEDS was mapped to a 7 cM (5.8 MB) interval on

chromosome 12p13 in three families [Rahman et al., 2003], but no candidate gene was identified. In 2016, an international consortium published 19 independent families comprising 107 individuals with pEDS to identify the genetic locus [Kapferer-Seebacher et al., 2016]. Included were samples of eight previous case reports and pedigree studies [Stewart et al., 1977, Hartsfield and Kousseff, 1990, Rahman et al., 2003, Reinstein et al., 2011, Reinstein et al., 2012, Reinstein et al., 2013, Cıkla et al., 2014, George et al., 2016]. In 17 of these families, heterozygous missense or in-frame insertion/deletion mutations in *C1R* (15 families) or *C1S* (two families) were identified. *C1R* and *C1S* are contiguous genes in the previously reported linkage region on chromosome 12p13, and encode subunits C1r and C1s of the first component of the classical complement pathway.

The prevalence of pEDS is unknown.

### The Mechanisms of Disease

Complement is a major element of antimicrobial host defense through its ability to recognize pathogens and limit infection in the early phase after exposure to microorganisms. The classical pathway of complement is triggered by C1, a complex comprising a recognition subunit C1q and two modular serine proteases (SPs) C1r and C1s [Budayova-Spano et al., 2002]. C1r and C1s are assembled into a Ca(2+)-dependent C1s–C1r–C1r–C1s tetramer which associates with the recognition protein C1q [Gaboriaud et al., 2014; Rossi et al., 2014]. After C1 binding to immune complexes, C1r auto-activates and then cleaves C1s which in turn cleaves C4 (into C4a and C4b) and C2 (into C2a and C2b) to form the classical pathway C3 convertase (C4b2a) [Patrick et al., 1970; Thielens et al., 1982; Amano et al., 2008].

One attractive hypothesis for the pathomechanism of pEDS is altered binding of the C1r–C1s tetramer or prematurely cleaved fragments to C1q or soluble procollagens. This could affect C1 function or interfere with procollagen processing within the ER.

Moderate enlargement of the ER cisterns documented in vitro in dermal fibroblasts may reflect retention of malprocessed molecules but could also reflect boosted expression/production of collagen as a feed-back response to decreased deposition of mature collagen into the matrix. The exact pathomechanism of pEDS remains to be clarified.

### Allelic Heterogeneity

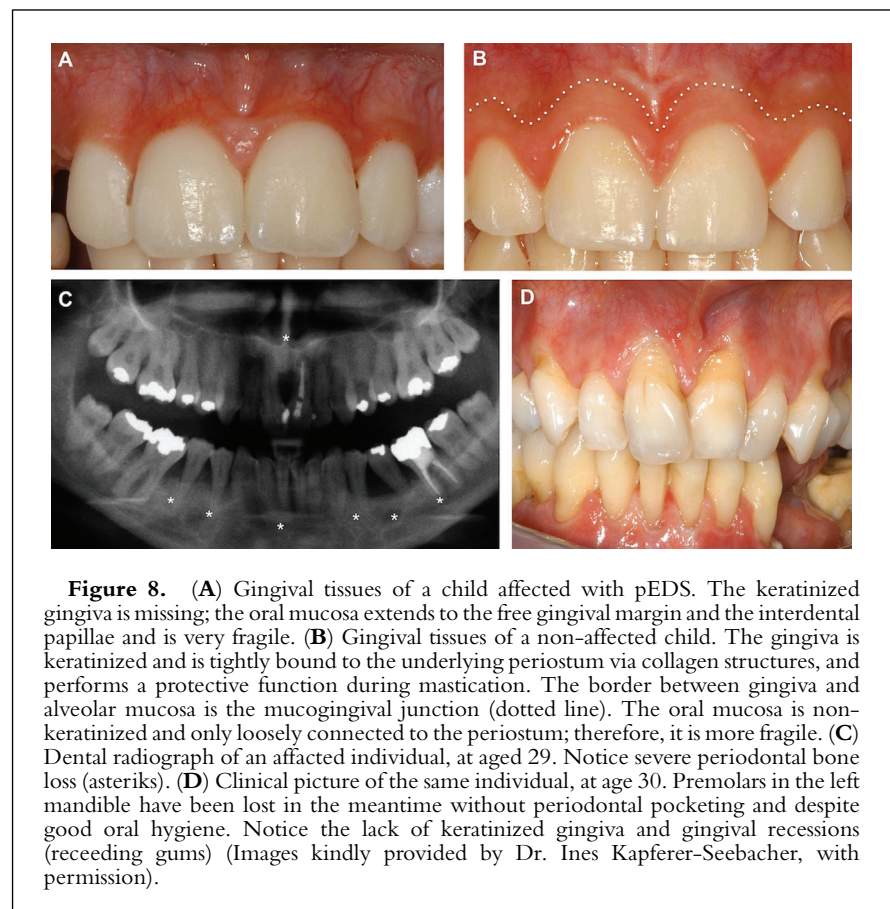
Heterozygous missense or in-frame insertion/deletion variants in *C1R* were detected in 15 families (comprising 76 affected individuals), and heterozygous missense or in-frame insertion/deletion variants in *C1S* were detected in two families (comprising 16 affected individuals). These variants involve the C1r/C1s, C1r/C1r, or C1r/C1q interfaces or the hinges between interaction and catalytic domains of C1r and C1s. Variants never involved the catalytic domain of C1r or C1s [Kapferer-Seebacher et al., 2016].

### Clinical Description

In the review on clinical features, we distinguished between individuals with confirmed mutations in *C1R* or *C1S* and other case reports. See Table S1 for the prevalence of clinical features in the molecularly confirmed patients (Representative pictures of the phenotype are given in Fig. 8).

#### • Craniofacial features

Stewart et al. [1977] included a photograph of the reported patient with an “aesthetic build.” Dysmorphic facial features were not described by Rahman et al. [2003]. In the dysmorphology literature, Cunniff and Williamson-Kruse [1995] described a triangular face, prominent eyes, long nose, and short philtrum. Biesecker described a paucity of subcutaneous fat with prominent nose and larynx [Biesecker et al., 1991]. Hartsfield noted the phenotypic overlap with vEDS [Hartsfield and Kousseff, 1990].



**Figure 8.** (A) Gingival tissues of a child affected with pEDS. The keratinized gingiva is missing; the oral mucosa extends to the free gingival margin and the interdental papillae and is very fragile. (B) Gingival tissues of a non-affected child. The gingiva is keratinized and is tightly bound to the underlying periostum via collagen structures, and performs a protective function during mastication. The border between gingiva and alveolar mucosa is the mucogingival junction (dotted line). The oral mucosa is non-keratinized and only loosely connected to the periostum; therefore, it is more fragile. (C) Dental radiograph of an affected individual, at aged 29. Notice severe periodontal bone loss (asterisks). (D) Clinical picture of the same individual, at age 30. Premolars in the left mandible have been lost in the meantime without periodontal pocketing and despite good oral hygiene. Notice the lack of keratinized gingiva and gingival recessions (receding gums) (Images kindly provided by Dr. Ines Kapferer-Seebacher, with permission).

- Musculoskeletal system

In 93 individuals with *C1R* or *C1S* mutations reported by Kapferer-Seebacher et al. [2016] joint hypermobility was reported in 56% of patients, mostly affecting the fingers (30%), the elbows (19%), knees (11%), hips, wrist, and ankle (3%). Marfanoid habitus, scoliosis, osteoarthritis, flat feet, and hernia were not consistent features [Kapferer-Seebacher et al., 2016].

- Skin and integument

Pretibial plaques with or without haemosiderosis are a consistent feature in most reports. Clinically, the lesions resemble necrobiosis lipoidica, but generally differ histologically. Fibrosis and haemosiderin deposits were reported, as opposed to interstitial and palisade granulomas and/or microangiopathy [19]. Buckel and Zaenglein [2007] confirmed similar findings. Easy bruising, skin fragility, and (mild) hyperelasticity of the skin have been reported in the majority of cases. There is a single report of an associated vasculitis in the absence of pretibial lesions [Hoffman et al., 1991]. In 93 individuals with *C1R* or *C1S* mutations reported by Kapferer-Seebacher et al. [2016], consistent cutaneous findings were pretibial discolorations (83%), easy bruising (96%), skin fragility (83%), and (mild) hyperelasticity of the skin (73%). Atrophic scars were present in 50% of cases [Kapferer-Seebacher et al., 2016].

- Cardiovascular involvement

Until recent, pEDS has not been associated with catastrophic vascular complications or hollow visceral rupture that arise in vEDS. It is of note that the father of the case reported by Stewart et al. [1977] had duodenal rupture and subsequent collagen protein analysis showed a normal collagen (I:III) ratio. Recently, a 42-year-old woman was reported with pEDS and a ruptured “blood blister” shaped aneurysm of her left middle cerebral artery [Cikla et al., 2014].

Kapferer-Seebacher et al. [2016] reported a prevalence of 16% for vascular complications (cerebral aneurysms or aortic dissection), and one individual with three events of organ

rupture (once duodenum, twice lung). As pEDS patients are not routinely checked for aneurysms, there might be also unrecorded cases. Additionally, many of the reports describe pediatric patients and there is a great need for longterm follow-up.

- Dental involvement

Early-onset periodontitis with extensive periodontal destruction and loss of teeth, starting in childhood or adolescence, is one of the defining hallmarks of pEDS, and was present in all reported cases and in 99% of individuals with confirmed *C1R/C1S* mutations. A clear demarcation to the diagnosis of chronic or aggressive periodontitis is essential. Typically, periodontal destruction in pEDS is not accompanied by periodontal pocket formation but by receding gums (personal observation IK). Prior to periodontitis, affected individuals may present with extensive gingival inflammation in response to mild dental plaque accumulation. A characteristic recently described feature is a striking lack of attached gingiva causing oral tissue fragility (Fig. 1) [Kapferer-Seebacher et al., 2016].

- Additional laboratory findings

- Biochemistry:

Biochemical analysis of collagen in cultured skin fibroblasts did not show abnormalities in the production and secretion of type I, III, and V collagens in several case reports and also in eight individuals with confirmed mutations in *C1S/C1R* [Biesecker et al., 1991; Cunniff and Williamson-Kruse, 1995; Kapferer-Seebacher et al., 2016]. In contrast, Mataix et al. [2008] reported a reduced rate of collagen (I) and collagen (III) synthesis compared to control samples. Lapiere and Nusgens [1981] initially reported a family with pEDS and reduced collagen III protein from skin, but later reported that a repeat analysis had not been able to replicate this.

- Skin histology and ultrastructure:

It is of note that there is variation in collagen ultrastructure depending on the site of biopsy in the general population (Pope and Vandersteen, personal observations). Many patients with pEDS in the literature

had biopsy from pre-tibial lesions, others at the standard site. There were no consistent findings, but evidence of collagen fibril size, packing, morphology, and ER dilatation are all reported.

Electron microscopy examination of skin reported in seven individuals with *C1R/C1S* mutations showed decreased collagen content, abnormal variation in collagen fibril diameter and some abnormally shaped fibrils [Rahman et al., 2003; Reinstein et al., 2012, 2013; Kapferer-Seebacher et al., 2016], which is in line with further reports [Kobayasi, 2004; Mataix et al., 2008]. Dermal collagen fibers showed some variability in the density of the packing and a few areas of kinking. The size uniformity does not support vEDS, while the kinking is non-specific. Additionally, patients' fibroblasts showed an increased proportion of dilated rough ER (RER) cisternae [Reinstein et al., 2012; Kapferer-Seebacher et al., 2016].

Dyne et al. [1993] reported collagen depletion and a larger number of small elastin fibrils; ultrastructure showed occasional serrated collagen fibrils, with normal fibril diameter.

- Immunology:

Hoffman et al. [1991] reported a patient with severe periodontitis, valvular heart disease, osteocondylar and phalangeal osteolysis in the absence of pretibial hyperpigmentation. This patient had intractable vasculitis and had a T cell response to type I collagen. Other patients have not had evidence of vasculitis. The case has some similarities to patients described with Singleton Merton syndrome, (premature dental loss, aortic calcification, and osteoporosis, OMIM 182250). It is doubtful whether this patient had true pEDS.

Kapferer-Seebacher et al. [2016] reported a prevalence of 40% for recurrent infections like otitis media, herpes zoster, bladder infections, empyema, kidney infections, or

pneumonia. There were single patients with autoimmune disorders like Sjögren syndrome, rosacea, and Crohn's disease [Kapferer-Seebacher et al., 2016].

## Management

There is no curative treatment for this disorder. A recent review of the dental management of EDS recommended special care regarding mucosal fragility, bleeding, temporomandibular joint hypermobility, and local anaesthetic resistance [Tulika and Kiran, 2015]. Management of periodontal disease requires lifelong biofilm management including intense oral hygiene instructions and nonsurgical debridement about every 3 months. Systemic antibiotics may be indicated. Conservative surgical management is recommended because of difficulties with fragile periosteal skin flaps and suture tears.

## Differential Diagnosis

- Vascular EDS
- Classical EDS
- Hypermobility EDS
- Periodontal diseases

Periodontal diseases range from mild and reversible gingivitis to irreversible loss of periodontal attachment resulting in tooth loss. "(Plaque-induced) gingivitis" is an inflammation solely of the gums in response to bacterial biofilms, and is characterized by bleeding on probing. "Periodontitis" is characterized by inflammation of the gingiva and destruction of the periodontal tissues, which are the gingiva, the cementum, the periodontal ligament, and the alveolar bone. Periodontal bone loss is diagnosed radiologically or clinically with a periodontal probe. "Gingival recession" (receding gums) in general is the exposure of the tooth roots. It might be an accompanying feature of periodontitis, and in this case the tooth root is exposed also

interdentally. Gingival recession might also present only on the buccal or lingual aspects of the tooth. In this case, it can, for example, be associated with a thin gingival biotype and intense tooth brushing, but not with periodontitis.

"Chronic periodontitis" prevalence estimates ranged from 19 to 83% depending on age and severity [Demmer et al., 2010]. Severe periodontitis with a mean prevalence of 11.2% is the sixth-most prevalent condition in the world [Kassebaum et al., 2014]. The typical patient is over 30 years of age, and the amount of bone destruction is consistent with the presence of plaque and calculus [Armitage, 2004]. In general, the disease progresses slowly but there may be bursts of destruction. In addition, the rate of disease progression can be modified by local factors, systemic diseases, and extrinsic factors such as smoking or emotional stress.

"Aggressive periodontitis" prevalence estimates range from 0.1% in Caucasians residing in north and mid Europe to 5% in African populations [Albandar, 2014]. The primary features are rapid attachment loss in otherwise healthy individuals and familial aggregation, usually affecting persons under 30 years of age [Albandar, 2014]. Some types of aggressive periodontitis seem to be inherited in a Mendelian manner, and both autosomal modes and X-linked transmission have been proposed [Meng et al., 2011]. However, review of pedigree analysis, linkage and linkage disequilibrium studies have so far been inconclusive [Meng et al., 2011].

There are several "monogenic syndromes with significant periodontitis" as part of the clinical phenotype. The majority of syndromes with severe periodontal destruction in childhood are inherited as autosomal recessive or X-linked traits and are associated with neutrophil dysfunction, for example, congenital and cyclic neutropenia (OMIM 202700), Chediak-Higashi syndrome (OMIM 214500), leukocyte

adhesion deficiencies types I, II, and III (OMIM 116920; OMIM 266265; OMIM 612840), WHIM (OMIM 193670), Cohen Syndrome (OMIM 216550), and agranulocytosis (OMIM 610738).

Conditions with more complex dermatological phenotypes include Kindler syndrome (hereditary acrokeratotic poikiloderma OMIM 173650), Papillon-Lefevre syndrome and cathepsin C associated phenotypes (\*602635), hypotrichosis osteolysis periodontitis (OMIM 607658). Multiple loci associated with an aggressive periodontitis have been reported (OMIM 170650) [Hart and Atkinson, 2007]. The Singleton-Merten syndrome (OMIM 182250) with periodontal destruction and aortic calcification has recently been shown to result from dominant mutations in the *IFIH1* gene [Rutsch et al., 2015].

Hypophosphatasia (OMIM 146300) is a highly variable autosomal dominant disorder associated with enamel hypoplasia, early loss of primary dentition, bowed long bones, and osteopenia. Odontohypophosphatasia is the least severe form of hypophosphatasia, characterized by premature exfoliation of primary and/or permanent teeth in the absence of skeletal system abnormalities. It is associated with a low circulating alkaline phosphatase.

## FUTURE RESEARCH AND GAPS

During the last decade, there has been an explosion of diverse but overlapping novel EDS or EDS-like phenotypes for which molecular defects have been identified in an array of new genes, as illustrated above. The genomic era promises to shed additional light on unsolved forms of Ehlers-Danlos syndrome. However, while our knowledge of the molecular basis of EDS has greatly progressed since the Villefranche Nosology, our understanding of the pathophysiological mechanisms underlying these conditions remains very limited, and elucidation of the genetic basis has not

translated to improved clinical management strategies.

***However, while our knowledge of the molecular basis of EDS has greatly progressed since the Villefranche Nosology, our understanding of the pathophysiological mechanisms underlying these conditions remains very limited, and elucidation of the genetic basis has not translated to improved clinical management strategies.***

Future research could focus on different aspects, including:

- Natural history studies
- Quality of life studies
- The creation of an international patient register, with prospective collection of detailed information regarding genotype and phenotype. This information should, among others, help address questions regarding:
  - genotype–phenotype correlations
  - prevalence
  - clinical variability (inter- and intrafamilial)
  - age-dependent organ-system involvement outcomes
  - risk related to pregnancy and delivery
- Development of surveillance, and management and care guidelines, including pain management
- Collection of tissue samples for the study of the pathogenetic basis of disease
- Development of animal models for study of pathogenetic mechanisms and for preclinical studies
- Identification of biomarkers to follow evolution of disease
- With the advent of next-generation sequencing techniques, many variants

of unknown significance are being identified. As such there is a need for the development of functional tests to study the pathogenic nature of these variants

- Identification of therapeutic targets

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