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Review

The Ehlers–Danlos syndrome, a disorder with many faces

De Paepe A, Malfait F. The Ehlers–Danlos syndrome, a disorder with many faces.

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The Ehlers–Danlos syndromes (EDSs) comprise a heterogeneous group of diseases, characterized by fragility of the soft connective tissues and widespread manifestations in skin, ligaments, joints, blood vessels and internal organs. The clinical spectrum varies from mild skin and joint hyperlaxity to severe physical disability and life-threatening vascular complications. The current Villefranche classification recognizes six subtypes, most of which are linked to mutations in genes encoding fibrillar collagens or enzymes involved in post-translational modification of these proteins. Mutations in type V and type III collagen cause classic or vascular EDS respectively, while mutations involving the processing of type I collagen are involved in the kyphoscoliosis, arthrochalasia and dermatosparaxis type of EDS. Establishing the correct EDS subtype has important implications for genetic counseling and management and is supported by specific biochemical and molecular investigations. Over the last years, several new EDS variants have been characterized which call for a refinement of the Villefranche classification. Moreover, the study of these diseases has brought new insights into the molecular pathogenesis of EDS by implicating genetic defects in the biosynthesis of other extracellular matrix (ECM) molecules, such as proteoglycans and tenascin-X, or genetic defects in molecules involved in intracellular trafficking, secretion and assembly of ECM proteins.

Conflict of interest

The authors declare no conflict of interest.

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Key words: arterial fragility – collagen – Ehlers – Danlos syndrome – joint hyperlaxity – molecular pathogenesis – natural history – wound healing

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EDS is a heritable collagen disorder

Definition

The Ehlers–Danlos syndrome (EDS) comprises a spectrum of monogenic conditions with multi-systemic and variable clinical manifestations affecting primarily the skin, ligaments and joints, blood vessels and internal organs. Most forms of EDS recognized to date result from mutations in one of the genes encoding fibrillar collagens or enzymes involved in the biosynthesis of these collagens. Like osteogenesis imperfecta, EDS represents a paradigm collagen disorder among the larger group of heritable connective tissue diseases.

Collagen proteins constitute a large family of structural extracellular matrix (ECM) proteins, among which the fibrillar collagens, represented by the collagen types

I, II, III, V and XI, are the principal components. They form fibrillar structures that provide strength and structure to the ECM of essentially all tissues and organs in the body. Fibrillar collagen proteins are trimeric molecules, which consist of three, either identical or genetically distinct polypeptide chains, designated as α -chains, which form characteristic triple helical structures. Each α -chain consists of a repetition of (Gly-Xaa-Yaa) triplets, in which the presence of glycine is an essential sterical requirement for correct helix formation and in which the Xaa position is frequently occupied by a proline and the Yaa position by hydroxyproline, formed by post-translational hydroxylation of prolines and involved in inter- and intramolecular crosslinking necessary for stabilization of the collagen molecules (1).

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The biosynthesis of fibrillar collagens is a complex process that starts with the intracellular synthesis of precursor 'procollagen' molecules which are extensively modified by specific hydroxylation and glycosylation events and processed to mature collagen molecules in the ECM after cleavage of the amino (N) and carboxy (C) propeptides by specific proteinases. Individual collagen molecules spontaneously assemble to form fibrils and fibers, which are stabilized by covalent crosslinking reactions which are catalyzed by lysyl oxidase (1).

Classification

Genetic defects affecting the biosynthesis and structure of collagen type I, III and V have been implicated in EDS and form the basis of the 1997 Villefranche classification of EDS, which recognizes six subtypes, based on phenotype, inheritance pattern and underlying biochemical and molecular defect(s) (2). The classic, hypermobility and vascular subtype of EDS are the most common, whereas the kyphoscoliosis, arthrochalasia and dermatosparaxis type constitute very rare conditions. For each of these subtypes, a set of major and minor diagnostic criteria has been defined. Over the last years, the clinical and molecular delineation of several new EDS variants has called for an expansion of the classification. From these new data it has become clear that, besides the collagens, genetic defects affecting the biosynthesis of other ECM components and processes as diverse as signaling pathways or intracellular trafficking can contribute to EDS pathogenesis.

General clinical manifestations of EDS

The main clinical characteristics listed below are present in varying degrees in each subtype of EDS. One of the most typical features is the skin hyperextensibility, which means that the skin stretches easily but snaps back after release (unlike cutis laxa). The skin is often smooth and velvety to the touch. In the vascular subtype, the skin is not hyperextensible but thin and transparent, with prominent venous pattern. The skin is fragile and splits easily after minor trauma especially over pressure points and exposed areas, which typically show widened and thin atrophic scars, often referred to as 'cigarette paper scars' (Fig. 1). Joint hypermobility is usually generalized and variable in severity and with age. It is assessed using the Beighton scale (Table 1). While often an innocent 'asset' in childhood and adolescence, it can become a serious burden over time, often complicated by repetitive (sub)luxations, sprains and chronic joint pain that is difficult to treat and may lead to devastating physical, social and emotional disability. Muscle hypotonia may cause delay in motor development, problems with ambulation and mild motor disturbance. Easy bruising is common, manifesting as spontaneous ecchymoses and hematomas that often recur and may cause unaesthetic discoloration of the skin due to hemosiderin deposition in exposed areas such as shins and knees. There is a tendency toward pronounced bleeding (e.g. following brushing of teeth)



Fig. 1. Widened, atrophic scars on the knees and shins of a patient with Ehlers-Danlos syndrome, classic type. Note the broad, flat feet with hallux valgus and hammer toes.

Table 1. The Beighton scale for joint hypermobility

Joint/finding	Negative	Unilateral	Bilateral
Passive dorsiflexion of the fifth finger $>90^\circ$	0	1	2
Passive flexion of thumbs to the forearm	0	1	2
Hyperextension of the elbows beyond 10°	0	1	2
Hyperextension of the knees beyond 10°	0	1	2
Forward flexion of the trunk with knees fully extended and palms resting on the floor	0	Present = 1	

despite a normal coagulation status. A range of clinical manifestations that result from a generalized weakness and fragility of the soft connective tissues are observed in patients with EDS including obstetrical and gynecological complications such as cervical insufficiency, premature rupture of membranes, vaginal tears and lacerations, surgical complications such as wound dehiscence and incisional hernia, tissue prolapses, umbilical or hiatal hernia.

Diagnosis and molecular pathogenesis of the classic subtype of EDS

The diagnosis of the autosomal dominant (AD) classic type of EDS requires the presence of skin hyperextensibility, widened atrophic scars and joint hypermobility, which constitute the three major diagnostic criteria,

- John, 20 years
- Born at 37 weeks of gestation due to premature rupture of the membranes
- ‘Floppy infant’ and loose joints noted in the first months of life
- At age 7 months: corrective surgery for severe strabismus, repeated 4 times during childhood
- ‘Spontaneous’ ecchymoses and bleedings, splitting of skin following minor trauma became apparent at the time he started to walk independently
- Joint hyperlaxity involving large and small joints became obvious in early childhood
- At age 5 years: suspicion of battered child syndrome because of confluent hematomas on the face (chin, forehead) and upper and lower limbs, for which he was referred to a clinical geneticist who established the diagnosis of Ehlers–Danlos syndrome. A type V collagen defect was identified
- Presently, at age 21 years, chronic pain in the back, shoulders and hands are the major subjective complaint and have led to temporary inability to perform his job

Fig. 2. A typical case history of classic Ehlers–Danlos syndrome.

next to a series of ‘minor’ diagnostic manifestations such as smooth, velvety skin, molluscoid pseudotumors (fleshy lesions over pressure points), subcutaneous spheroids (small, hard cyst-like nodules), easy bruising and bleeding, muscle hypotonia, delayed gross motor development and a range of manifestations of soft tissue weakness such as inguinal and umbilical hernia. Characteristic facial features include epicanthic folds, excess skin over the eyelids, presence of dilated scars on the forehead and a pale, somewhat prematurely aged appearance of the face (Fig. 2).

Ultrastructural examination of the skin in classic EDS shows irregular and loosely packed collagen fibrils and presence of typical ‘cauliflower’ fibrils which represent the histological hallmark of disturbed fibrillogenesis of the heterotypic collagen fibrils which consist of type I and V collagen. The molecular basis of classic EDS is a deficiency of type V collagen, a quantitatively minor fibrillar collagen that is widely distributed in tissues such as skin, bone, tendon, cornea, placenta and foetal membranes. It consists of three different α -chains encoded by the *COL5A1*, *COL5A2* and *COL5A3* genes, respectively. The most common isoform in vertebrate tissues is the $[\alpha 1(V)2\alpha 2(V)]$ heterotrimer. Collagen type V plays a key role in collagen fibrillogenesis via its huge N-propeptide domain that is the only part of the type V collagen molecule that emerges from the surface of the fibrils whereas the entire triple helix is buried within the fibril (3).

The relationship between type V collagen and classic EDS became apparent from studies in transgenic mice, showing that mice with a homozygous deletion of the *col5a2* gene presented clinical and ultrastructural features of classic EDS (4) and it was subsequently confirmed by the identification of a (9,X) translocation that disrupted the *COL5A1* gene in a patient presenting with classic EDS and hypomelanosis of Ito (5). The first mutations reported in classic EDS were respectively an exon skipping mutation (6) and a missense mutation substituting a highly conserved cysteine for a serine in the C-propeptide domain of the $\alpha 1(V)$ collagen

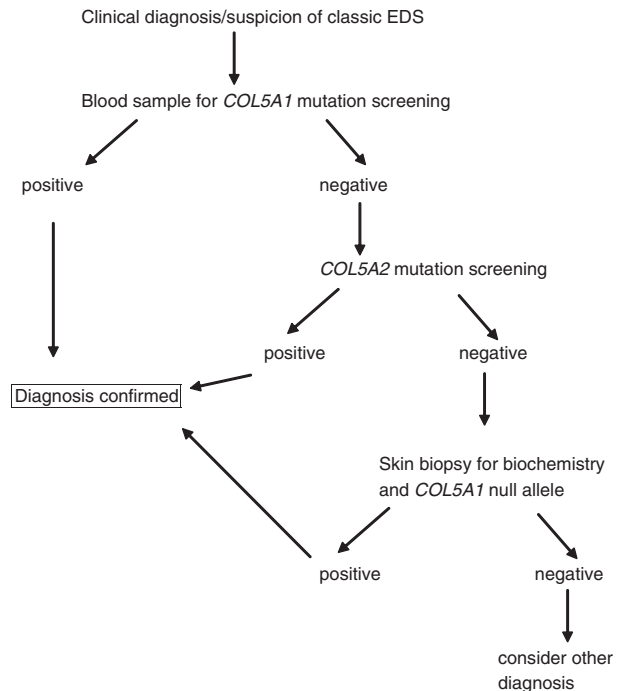


Fig. 3. Diagnostic flow chart for Ehlers–Danlos syndrome, classic type.

chain (7). This cysteine residue is essential for intra-chain disulphide bonding prior to chain assembly and initiation of trimerization. The mutation prevents incorporation of the mutant collagen chain into the molecule and thus causes a reduction of type V collagen, a mechanism that was subsequently confirmed to be central in the pathogenesis of classic EDS. Indeed, since then a growing number of mutations in type V collagen have been identified, including for the most part heterozygous nonsense, frameshift or splice-site mutations in *COL5A1* that abolish one *COL5A1* allele through the nonsense-mediated mRNA decay mechanism or impair normal molecular assembly of type V collagen (8–11). The current working hypothesis states that these mutations result in *COL5A1* haploinsufficiency and lead to the production of approximately half the normal amount of type V collagen. A minority of mutations consist of splice-site or missense mutations in either *COL5A1* or *COL5A2* that lead to the production of an abnormal polypeptide chain that is incorporated in the molecule and results in the production of structurally abnormal type V collagen molecules. Although to date the number of type V collagen mutations identified in classic EDS approaches ~150 different mutations (published and unpublished results), no particular phenotype–genotype correlations have emerged from these findings, except perhaps for those mutations residing in the highly conserved N-terminal propeptide domain of $\alpha 1(V)$ that cause atypical splicing outcome and have been associated with a more severe EDS phenotype (12). On the basis of the data gathered to date, it is fair to conclude that mutations in type V collagen account for the great majority of classic EDS cases (Symoens et al.,

submitted). Molecular testing of type V collagen is therefore helpful to confirm a clinical diagnosis of classic EDS. A helpful diagnostic flowchart is presented in Fig. 3.

Patients with EDS require a multi-disciplinary approach with cardiovascular work-up, physiotherapy, pain management, and psychological support. Special attention should be given to skin care, joint protection and pain management. Follow-up and monitoring of pregnancy are recommended. Clinical management guidelines for the classic EDS are reviewed in Ref. (13). These generally apply to most of the EDS phenotypes.

EDS variants caused by defects in type I collagen

Although clinical recognition of classic EDS is usually straightforward and based on the presence of the typical major clinical manifestations, some phenotypic overlap exists with other EDS subtypes, including some recently characterized, rarer EDS variants that are associated with mutations in type I collagen. One of those is a rare form of EDS referred to as the cardiac-valvular EDS, an autosomal recessive (AR) condition caused by total absence of the $\alpha 2(I)$ collagen chain which results in the production of $[\alpha 1(I)]_3$ homotrimers. This condition presents in childhood with mild skin – and joint hypermobility, osteopenia and muscular hypotonia and is complicated in adulthood by the development of severe cardiac valve insufficiency that may need cardiac valve replacement (14, 15).

A specific class of defects in type I collagen that cause a phenotype resembling classic EDS involve missense mutations in *COL1A1* that result in the substitution of an arginine (R) residue in the Xaa position of the Gly-Xaa-Yaa repeat by a cysteine (C) residue (16). These mutations lead to the production of $\alpha 1(I)$ dimers that are detectable by their abnormal electrophoretic mobility pattern on sodium dodecyl sulfate polyacrylamide gel electrophoresis of radiolabeled collagens obtained from cultured skin fibroblasts. Affected individuals present, in addition to skin and joint hypermobility, easy bruising and atrophic scarring, a propensity for arterial rupture in young adulthood (17). Some R-to-C substitutions, in either the Xaa or Yaa position of the Gly-Xaa-Yaa repeat, have been reported in association with rupture of medium-sized arteries in individuals that did not present overt skin manifestations of EDS (17) or were shown in others to cause an EDS/osteogenesis imperfecta (OI) overlap phenotype (18), or autosomal dominant Caffey disease (19).

Defects that interfere with the cleavage of the N-terminal propeptide of type I procollagen also result in particular EDS phenotypes. The autosomal dominant arthrochalasia type of EDS (previously EDS VIIA and B) is caused by heterozygous mutations that lead to loss of exon 6, or part of it, in the mRNA coding for the $\alpha 1$ - or $\alpha 2$ -chain of type I procollagen. These defects lead to loss of the N-terminal telopeptide, which links the N-propeptide to the main triple helical domain. This N-telopeptide contains the procollagen-I-N-proteinase cleavage site and a critical crosslinking lysyl residue.



Fig. 4. Extreme hyperlaxity of the finger joints in a patient with Ehlers–Danlos syndrome, arthrochalasia type.

Lack of this segment leads to a deficient processing of the N-propeptide of type I collagen. The clinical hallmark of this EDS variant is congenital bilateral hip dislocation. The phenotype comprises also severe generalized joint hypermobility (Fig. 4) with recurrent dislocations, hyperextensible, bruisable skin, poor wound healing with atrophic scars, muscular hypotonia, kyphoscoliosis and osteopenia. Biochemical confirmation of the diagnosis is based on electrophoretic demonstration of pN $\alpha 1(I)$ (EDS VIIA) or pN $\alpha 2(I)$ (EDSVIIB) chains of type I procollagen harvested from cultured skin fibroblasts.

Mutations residing within the N-terminal stretch of 85 amino acid residues in the triple helical domain of type I collagen result in a distinct EDS/OI overlap phenotype characterized by OI-like bone fragility and variable skin and joint hypermobility, reminiscent of that seen in EDS (20). This 85-amino acid region acts as a stabilizing ‘anchor’ for the N-terminal end of the type I collagen triple helix, and defects in this $\alpha 1(I)$ N-anchor region were shown to lead to a conformational change at the adjacent N-propeptide cleavage site, resulting in inefficient cleavage of the N-propeptide (21). So, although the cleavage site itself remains intact, inefficiently cleaved collagen molecules are incorporated in the fibrils, leading to EDS symptoms by a mechanism similar to EDS type VIIA/B.

Deficient activity of the procollagen-N-proteinase, the enzyme responsible for cleavage of the N-terminal propeptide in type I, II and III collagen and encoded by the *ADAMTS2* gene, causes the dermatosparaxis type of EDS, an AR condition characterized by pronounced skin fragility and a sagging, redundant appearance of the skin. Other distinctive features are delayed closure of the fontanels, characteristic facies with edema of the eyelids and blue sclera, umbilical hernia, short stature and short fingers (Fig. 5). Fragility of internal tissues, with spontaneous bladder rupture, has been reported (22). Whereas most of the initially reported patients showed a very severe phenotype, recognizable from birth, it is now clear that some patients present



Fig. 5. Patient with Ehlers–Danlos syndrome, dermatosparaxis type. Note the typical facial appearance with epicanthic folds, downslanting palpebral fissures, blue sclera, micrognathia, prominent lips and facial scars especially around the mouth. There is a large bruise on the thorax from minor trauma.

with a milder condition, which can delay the diagnosis. As a result of the deficient activity of the procollagen-N-proteinase, uncleaved pN-collagen molecules are incorporated into mature collagen fibrils, which leads to pathognomonic abnormalities of the dermal collagen fibril architecture, characterized by fibrils that have lost their normal cross-sectional circular aspect and have a hieroglyphic appearance (23). Biochemical analysis shows aberrant processing of type I procollagen with characteristic accumulation of type I pN-collagen (24).

EDS related to tenascin-X and overlap with collagen VI myopathies

AR EDS due to complete deficiency of tenascin-X is a condition resembling, but phenotypically distinct from classic EDS (25). Patients present with skin and joint hypermobility, and easy bruising, but they also suffer from generalized muscle weakness and distal contractures. Atrophic scarring is not observed. The diagnosis can be confirmed by the absence of tenascin-X in serum and mutation analysis of the *TNX-B* gene. Truncating mutations as well as large deletions in both alleles of the *TNX-B* gene have been reported. Tenascin-X is part of a family of ECM proteins with a complex multidomain structure that allows interaction with many other ECM components and is considered to be an important player in the organization of the ECM. It has

been shown to regulate the expression of type VI collagen (26) and to affect type I collagen fibril formation *in vitro* and *in vivo* together with type VI collagen (27). Recent studies in *col6al*-deficient mice have shown dysfunctional regulation of tendon collagen (28). Collagen type VI interacts with different other ECM components, including fibronectin, decorin and cell receptor molecules (integrins). Mutations in type VI collagen cause Ullrich congenital muscular dystrophy (UCMD) and Bethlem myopathy (BM), disorders typically presenting with moderate to severe muscle weakness, joint hypermobility and distal joint contractures (29). Ultrastructural findings of abnormal collagen fibril morphology in patients with type VI collagen defects overlap with those seen in EDS (30). Moreover, there is substantial clinical overlap between collagen VI-related myopathies and EDS due to *TNX* deficiency (31). In a recent protein interaction study aiming to explore the role of the conserved $\alpha 1(V)$ N-propeptide domain, we identified, among other novel interacting proteins, type VI collagen as a binding partner for the N-propeptide of type V collagen, indicating that the type V/type VI collagen protein complex may serve as a molecular bridge in the cell-matrix environment and may be essential in maintaining the architecture of the dermal matrix (32). Ongoing studies in our lab suggest abnormal immunohistochemical staining patterns for dermal collagen type VI in patients with a confirmed type V collagen mutation. These observations warrant further investigation on possible common pathogenetic mechanisms connecting the ‘classic EDS/tenascin-X-related EDS/UCMD-BM spectrum’.

EDS-HT: a clinical and molecular challenge!

The exact clinical definition and nosologic delineation of this form of EDS subtype is still a matter of debate and uncertainty, and, since its genetic basis is largely unknown, a precise biomarker or reliable diagnostic test for this EDS subtype is lacking. Moreover, joint hypermobility is a common manifestation in the general population, its phenotypic expression is variable even within families and suitable large families in which the phenotypic status of all relatives can be unequivocally established on clinical grounds are scarce. Therefore, this EDS subtype represents a real diagnostic challenge to the clinician! According to the Villefranche nosology, the major diagnostic criteria are generalized joint hypermobility and presence of typical skin manifestations such as hyperextensibility and smooth, velvety skin, although these are usually much more subtle than in the classic type of EDS. They are nevertheless helpful to differentiate this form of EDS from the more common ‘(familial) joint hypermobility syndrome (JHS), although at present, it is still a matter of debate whether the EDS hypermobility type (EDS-HT) and/or JHS share a common genetic basis. The presence and degree of hypermobility can be scored by the Beighton hypermobility score (Table 1) (33).

- Nicole, 25 years
- Was born at term after uneventful pregnancy and delivery
- She presented loose joints and slow wound healing since early childhood
- She was brilliant in gymnastic class but ruptured her Achilles tendon at age 9 years after relatively minor trauma
- After puberty, she started to suffer from recurring (sub)luxations of different joints, including fingers, wrists, shoulders, knees, hips, ankles
- Her family history was positive for joint hyperlaxity and pain, repetitive dislocations
- She had multiple surgeries for articular instability, started to complain increasingly from chronic fatigue, joint pain and episodes of depression, and was diagnosed with fibromyalgia
- She became ultimately wheelchair-bound at age 21 years because of severe joint instability and chronic pain. At that point she was referred to a clinical geneticist, who diagnosed Ehlers–Danlos syndrome in presence of soft skin, widened scars and easy bruising and generalized joint hyperlaxity

Fig. 6. A typical case history of Ehlers–Danlos syndrome, hypermobility type.

Although often considered as a ‘mild’ form of EDS, EDS-HT can present with severe and debilitating complications such as recurring dislocations and chronic articular pain, which represent a significant burden with respect to physical activities of affected individuals and which may lead to social isolation and emotional distress and depression (34). In practice, it is not uncommon that patients with the EDS-HT are diagnosed with fibromyalgia, chronic fatigue syndrome and/or depression (Fig. 6).

Over the last years, our group performed several studies, which aimed to document in a more precise way the functional musculoskeletal status and health in patients with EDS-HT. Severe joint hypermobility with recurrent joint dislocations and chronic moderate to severe pain were the most frequent and severe complaints, but also muscle cramps, tendinitis, headache and fatigue were frequently reported among EDS-HT subjects. Moreover, symptoms caused by autonomic dysfunction were reported in more than half of the EDS-HT subjects. These complaints were shown to have a considerable impact on the physical, social and emotional daily life of the EDS subjects (34). In a comparative study, physical impairment and impact of joint pain were shown to be substantially greater in EDS-HT compared to rheumatoid arthritis, and were comparable to the burden of disease observed in fibromyalgia (35).

Factors that have been shown to contribute to the joint instability include impaired proprioception, postural control and muscular strength. Our studies showed that EDS-HT patients have reduced knee joint proprioception (36), as well as a severely reduced quantitative muscle function and impaired physical functioning, compared to age and sex-matched controls. EDS-HT patients present lower extremity muscle weakness, which appears not to be caused by reduced muscle mass but rather by intrinsic muscular dysfunction, associated with muscle pain and fatigue (Rombaut et al., under review).

The genetic basis of EDS-HT remains, at present, largely unknown. The striking preponderance of affected women vs men in EDS-HT is also presently unexplained. Ultrastructural studies have shown that patients with EDS-HT show collagen fibril abnormalities with presence of collagen cauliflower-like aspect as seen in classic EDS (37). These findings suggest that somehow, collagen fibrillogenesis is impaired also in this EDS subtype, but so far, except for some anecdotal observations, molecular evidence for this is lacking and the major fibrillar collagens have all been excluded as candidates by linkage studies. Zweers et al. have showed that a subset of patients with EDS-HT or JHS presents haploinsufficiency for tenascin-X (38), an ECM molecule already linked to an AR form of EDS resembling classic EDS (see above). This interesting observation needs further study to evaluate the exact role of tenascin-X in EDS-HT.

Studies in transgenic mice have suggested that another class of ECM molecules, the ‘small leucine-rich proteoglycans’ (SLRPs) interact directly with fibrillar collagens and modulate fibril formation, growth and morphology. Mice deficient for SLRPs such as decorin, lumican or fibromodulin display clinical and ultrastructural features reminiscent of human EDS, but so far no human disorders have been identified that match these mouse models (39). The SLRPs nevertheless represent an interesting group of candidate molecules for the EDS-HT or other EDS variants.

Diagnostic and therapeutic issues in the vascular type of EDS

Of all EDS subtypes, the vascular subtype has the worst prognosis because of a propensity to rupture of arteries and hollow organs at young age. Unlike other EDS types, the skin is not hyperextensible, but rather thin and translucent, showing a visible venous pattern over the chest, abdomen and extremities. Excessive bruising is the most common sign and is often the presenting complaint, especially in children. Other early manifestations include premature rupture of the membranes, congenital clubfoot or congenital hip dislocation, inguinal hernia, recurrent joint dislocation or subluxation and precocious and severe varicosities. Patients with vascular EDS often display a characteristic facial appearance, with prominent eyes (due to lack of subcutaneous adipose tissue around the eyes), a thin, pinched nose and small lips, hollow cheeks and lobeless ears. Hypermobility is usually limited to the small joints of the hands. Excessive wrinkling and thinness of the skin over hands and feet may produce an old-looking appearance, referred to as ‘acrogeria’. The clinical appearance of patients with vascular EDS may, however, deviate from the typical picture, and especially the facial and cutaneous features may be very subtle or even absent. In the absence of a positive family history or a major vascular or intestinal complication, early clinical diagnosis is difficult.

The generalized vascular fragility largely dominates the clinical picture. Apart from excessive bruising

- Tom, 24 years
- Born with unilateral clubfoot, surgically corrected
- Multiple 'spontaneous' ecchymoses since early age, but no wound-healing problems, hyperlaxity confined to the small joints
- No specific medical problems up to age 24 years
- At age 24: hospitalisation after sudden collapse due to spontaneous bilateral rupture of two intercostal arteries
- Angiography reveals presence of multiple aneurysm of A. carotis and A. Renalis
→ genetic consultation: Diagnosis of Ehlers–Danlos syndrome, type III collagen mutation present

Fig. 7. A typical case history of vascular Ehlers–Danlos syndrome.

and bleeding, it may cause arterial rupture, potentially resulting in sudden death, usually in the third or the fourth decade of life (Fig. 7).

In a retrospective study, performed on 100 independent, molecularly proven vascular EDS probands, we found that 60% of them were referred for molecular analysis after one or more major complication(s), including arterial rupture or rupture of an internal organ (Malfait et al., in preparation). The majority of these had experienced already more than one major event before the diagnosis was established. At the time of study 22% were deceased, at a median age of 33 years, the major cause of death being arterial rupture. Only one death occurred after surgery for sigmoid colonic perforation. Forty percent was referred because of suspicious physical features, including excessive bruising, translucent skin, acrogeria and facial appearance, either with or without a family history of a major event or sudden death. The median age at diagnosis was 29 years, ranging between 4 and 74 years. Seven percent (7%) of the probands experienced a first major event by the age of 20 years, whereas up to 75% experienced a first major complication by age 40 years. The vast majority (82%) of all major complications were arterial. These mostly involved aneurysm, dissection or rupture of medium-sized abdominal vessels (mainly renal, iliac, femoral, mesenteric and hepatic arteries) and the abdominal aorta. Other frequent vascular lesions involved carotid, subclavian, ulnar, popliteal and tibial arteries. Coronary rupture, leading to acute myocardial infarction was a rare, but severe complication. Of note, ruptures were not always preceded by detectable aneurysmal dilatation. Presence of a carotid-cavernous fistula was reported in 6/100 probands. Gastrointestinal complications accounted for 15% of the complications, the vast majority of which were spontaneous ruptures of the sigmoid colon, whereas ruptures of the upper gastrointestinal tract were rare. Of interest, four probands experienced a spontaneous organ rupture, including spleen or liver. Pneumothorax was a frequent complication. Obstetrical complications were recorded for 34 pregnancies among which five were complicated by arterial, uterine or splenic rupture. Other reported complications included severe vaginal lacerations and hemorrhage, and severe rectal tearing.

Vascular EDS is caused by heterozygous mutations in the *COL3A1* gene, encoding type III collagen. To date more than 250 *COL3A1* mutations have been identified (40), the majority of which are point mutations

leading to substitutions for glycine in the triple helical region of the collagen molecule. Other types of mutations include splice-site mutations, partial gene deletions, and, rarely, null mutations resulting in *COL3A1* haploinsufficiency (41). Confirmation of a suspected diagnosis of vascular EDS is possible by biochemical demonstration of quantitative or qualitative type III collagen defects, which identifies more than 95% of affected individuals (Malfait et al., in preparation). Of note, *COL3A1* null mutations do not usually lead to a detectable alteration in electrophoretic mobility of type III collagen. If there is a strong clinical suspicion of vascular EDS, direct DNA analysis is therefore mandatory, even in the absence of an abnormal biochemical abnormality.

Genotype–phenotype correlations have been investigated extensively in vascular EDS. Missense mutations located at the extreme C-terminal end of the molecule usually cause the so-called acrogeric form of EDS, associated with severe vascular problems and early death. This relationship is however not absolute and severe clinical phenotypes have been reported with more N-terminal-located mutations as well. It has recently been suggested that patients with *COL3A1* null mutations may present a milder phenotype, with an extended life span, delay in age of first complication and complications that appear to be limited to vascular events (42).

Parental mosaicism for *COL3A1* mutations has been documented in vascular EDS (43) and may explain unexpected recurrences in families where a 'new' dominant mutation was identified. Prenatal and pre-implantation diagnosis based on direct demonstration of the mutation in embryonic tissues can be offered in at-risk pregnancies.

For the vascular type of EDS, some prophylactic measures are of special importance.

Invasive vascular procedures such as catheterization and arteriography should be avoided because of the risk of vascular ruptures, which cause significant morbidity and may have fatal outcome. They should rather be replaced by ultrasonography and subtraction angiography. Surgical interventions are generally discouraged because of increased vascular fragility and conservative therapy is recommended. When surgery is required for the treatment of arterial or bowel complications or other health problems, thorough investigation of platelet function and clotting is appropriate, as affected persons are already subject to bleeding from ruptured vessels or organs and an intrinsic clotting defect may complicate clinical outcome. Surgical exploration and intervention should be minimized and manipulation of vascular and other tissues should be done with extreme care.

Recently, a multicenter randomized trial showed that celiprolol, a longacting β_1 antagonist with partial β_2 -agonist properties, decreased the incidence of arterial rupture or dissection by three times in patients with the clinical diagnosis of vascular EDS (44). This study represents a substantial breakthrough in the evidence-based management of the syndrome.

Pregnancy for women with the vascular type of EDS is a high-risk venture. It is prudent to follow pregnant

women with the vascular type of EDS in a high-risk obstetrical program. It is not clear whether elective caesarean section is preferred to vaginal delivery.

Novel pathogenetic insights emerging from rare EDS subtypes

In the last decade, the molecular basis of several of the new EDS variants, not yet represented in the Villefranche nosology, have been identified. Together, these studies have expanded the phenotypic definition of the EDS, and brought new insights in its molecular pathogenesis by showing that besides the collagens, other ECM-related molecules are implicated in this complex connective tissue disorder.

The EDS kyphoscoliotic type or type VIA is an AR disorder characterized by early onset progressive kyphoscoliosis, severe neonatal muscular hypotonia with delayed gross motor development, generalized joint hyperlaxity, osteopenia, fragile, hyperextensible and bruisable skin, microcornea and in some patients scleral fragility with risk for rupture of the globe, or rupture of medium-sized arteries. It is caused by a deficient activity of the enzyme lysyl hydroxylase 1 (LH-1) (45) and historically is the first EDS subtype for which the molecular defect has been elucidated. LH-1 or *PLOD1* hydroxylates lysyl residues in the (Gly-Xaa-Lys) triplets of collagen type I to hydroxylysyl residues, which are involved in the formation of stable intermolecular crosslinks that provide tensile strength and mechanical stability to the collagen fibrils and serve as attachment sites for carbohydrate units known to modulate the lateral packing of collagen molecules into fibrils. The diagnosis can be confirmed by showing an increased ratio of lysylpyridinoline (LP) to hydroxylysylpyridinoline (HP) crosslinks in the urine, a decreased LH-1 activity in cultured skin fibroblasts or the presence of a homozygous or compound heterozygous mutation in the *PLOD1* gene. A homozygous multi-exon duplication accounts for ~20% of mutations reported so far, but missense, nonsense and small indel mutations leading to loss-of-function of *PLOD1* have also been identified (46). Interestingly, in the brittle cornea syndrome (BCS), a rare AR condition that shows significant phenotypic overlap with EDS type VIA, mutations have been found in *ZNF469*, a gene encoding a zinc finger protein of unknown function, belonging to the C2H2 zinc finger family and expressed in skin, muscle, cornea and sclera (47). In BCS, thin, brittle cornea and ocular fragility, blue sclera and keratoconus are prominent features but can be associated with skin and joint hypermobility and kyphoscoliosis. Histologic examination of the sclera shows a significantly decreased corneal thickness. Recently, a second gene, *PRDM5*, was identified for BCS (48). *PRDM5* encodes a widely expressed transcriptional regulator containing 16 C2H2 zinc fingers, and has been shown to modulate both canonical and non-canonical Wnt-signaling pathways in early zebrafish development (49). The phenotypic spectrum of BCS appears to be identical in patients with either

ZNF469 or *PRDM5* mutations, suggesting that the two genes act within the same developmental pathway.

It has long been known that some patients who clinically appear to fit within the EDS type VI phenotypic spectrum do present normal LP/HP ratios. These were referred to as EDS type VIB or EDS type VI with normal LH-1 ratios (50). While some of these patients were shown to harbor mutations in *ZNF469* (personal observation), we have recently shown that mutations in *CHST14*, encoding dermatan-4-sulfotransferase-1 (D4ST-1) cause an EDS phenotype that closely resembles and is probably identical to this EDS type VIB variant (51). Comprehensive phenotypic characterization of these patients has shown that they present a range of clinical features that overlap with EDS type VIA, such as the typical skin manifestations, joint hypermobility, kyphoscoliosis, clubfeet, muscular hypotonia and ocular features which include microcornea, blue sclera, myopia, retinal detachment and glaucoma. However, they also present clinical manifestations that are distinct from type VIA such as characteristic craniofacial abnormalities (Fig. 8), joint contractures, wrinkled palms, tapered fingers and gastrointestinal and genitourinary manifestations. Loss-of-function mutations in *CHST14* had previously been shown to cause a rare AR condition called 'adducted thumb clubfoot syndrome' (ATCS) (52). Patients with EDS type VIB show many of the clinical features of the ATCS including the craniofacial manifestations and joint contractures but differ in the presence and severity of the skin manifestations and the more severe kyphoscoliosis and ocular involvement. The Japanese EDS variant reported by Kosho et al. (53) and also shown to be associated with loss-of-function mutations in *CHST14* (54), falls within this EDSVIB-ATCS spectrum. In view of the prominent muscular hypotonia and typical contractures, we proposed to designate the *CHST14*-related EDS variant as the EDS, musculocontractural subtype (EDSVIB). Ultrastructural studies in this EDS subtype show small collagen fibrils with variable diameter and the presence of flower-like fibrils, which are characteristic for EDS. Unlike in EDS type VIA, biochemical collagen studies as well as LP/HP ratios are normal. So far the molecular spectrum of *CHST14* comprises homozygous or compound heterozygous missense, nonsense and frameshift mutations, as well as homozygous 20-bp duplication.

The identification of mutations in *CHST14* unequivocally links EDS pathogenesis to defects of proteoglycan metabolism. D4ST-1 is a key enzyme in the biosynthesis of dermatan sulfate (DS), where it catalyzes 4-O-sulfatation of N-acetyl-galactosamine residues and is one of three major sulfotransferases in the DS/CS (chondroitin sulfate) synthesis, which have different substrate specificities. Different epimerization and sulfatation reactions during DS/CS biosynthesis reflect a tightly controlled system that determines the ultimate structural variability and functional interactions of the DS/CS chain. Deficiency of the D4ST-1 enzyme perturbs normal DS/CS balance in DS proteoglycans (DSPG) (such as versican and thrombospondin) and SLRPs such as decorin and biglycan. As a

Table 2. Updated EDS classification

EDS subtype	Inheritance pattern	Protein	Gene
Classic	AD	Procollagen type V	<i>COL5A1/COL5A2</i>
		Procollagen type I	<i>COL1A1</i>
Cardiac-valvular	AR	Tenascin-X	<i>TNX-B</i>
		Deficiency of $\alpha 2(I)$ collagen chain	<i>COL1A2</i>
Hypermobility	AD	Unknown	?
		(Tenascin X)	<i>TNX-B</i>
Vascular	AD	Procollagen type III	<i>COL3A1</i>
Vascular-like	AD	Procollagen type I (R-to-C)	<i>COL1A1</i>
Kyphoscoliotic	AR	Lysyl hydroxylase-1	<i>PLOD1</i>
Musculocontractural	AR	Dermatan-4-sulfotransferase-1	<i>CHST14</i>
Spondylocheirodysplastic	AR	ZIP13	<i>SLC39A13</i>
Brittle cornea syndrome	AR	ZNF469	<i>ZNF469</i>
		PRDM5	<i>PRDM5</i>
Arthrochalasia	AD	Procollagen type I (deletion of N-propeptide cleavage site)	<i>COL1A1/COL1A2</i>
EDS/OI overlap	AD	Procollagen type I (delay in N-propeptide cleavage)	<i>COL1A1/COL1A2</i>
Dermatosparaxis	AR	Procollagen-I-N-proteinase	<i>ADAMTS2</i>

AD, autosomal dominant; AR, autosomal recessive; EDS, Ehlers–Danlos syndrome.



Fig. 8. Patient with Ehlers–Danlos syndrome, musculocontractural type, showing craniofacial dysmorphism with malar hypoplasia, downslanting palpebral fissures, blue sclera and microcornea, long philtrum with thin upper lip and protruding jaw with pointed chin.

consequence, the functional and structural integrity of these DSPG, which display a widespread tissue distribution and are important in many processes including organization of the ECM, wound repair, anticoagulant processes, and cell adhesion, may be compromised. In particular, loss of the normal hybrid DS/CS configuration in decorin may decrease its capacity to regulate the interfibrillar spacing of collagen fibrils and thus lead to disorganized collagen bundle organization (54).

Of note, a rare, AR progeroid EDS variant has been described, characterized by progeroid appearance with wrinkled face, curly fine hair and periodontitis in addition to typical features of EDS and caused by homozygous mutations in *B4GALT7*, the gene encoding

β -1,4-galactosyltransferase (55). This enzyme catalyzes the second glycosyltransfer reaction in the assembly of the DS chain.

The musculocontractural type of EDS also shows phenotypic resemblance with another recently identified novel EDS variant, the spondylocheirodysplastic form of EDS, which is characterized by hyperextensible, thin skin, easy bruising, hypermobility of the small joints with a tendency to contractures, prominent eyes with bluish sclera, wrinkled palms, atrophy of the thenar muscle and tapering fingers. In addition, patients show moderate short stature and a mild skeletal dysplasia characterized by platyspondyly, osteopenia and widened metaphyses. The total urinary pyridinolines are elevated with a LP/HP ratio of ~ 1 , which is higher than normal values (~ 0.2) but less than in EDSVIA (~ 6). A homozygous 9-bp deletion in *SLC39A13*, a zinc transporter involved in the intracellular trafficking of zinc, necessary for normal LH-1 function, has been shown to be causative (56).

In conclusion, a range of EDS phenotypes have been delineated over the last years, which all present with multi-systemic involvement and diverse, but overlapping clinical phenotypes. Recently identified new EDS variants call for an update of the EDS Nosology (Table 2). Studies on the molecular pathogenesis of EDS have shown links to defects in transcription factors, intracellular trafficking of ECM molecules, proteoglycan metabolism, and supramolecular organization of the ECM and cell-matrix interactions. An emerging phenotypic continuum between the EDS and neuromuscular disorders such as Ullrich and BM is corroborated by identification of common pathogenetic links. Future studies are needed to explore the role of processes such as endoplasmic reticulum stress and autophagy, the involvement of which have recently been implicated in other collagen disorders such as osteogenesis imperfecta and Ullrich muscular dystrophy. Ultrastructural intracellular characteristics observed in diverse EDS

subtypes indeed suggest the contribution of these processes to the molecular pathogenesis of EDS. These insights might provide new perspectives for therapeutic intervention.

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