The Ehlers-Danlos Syndrome

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Abstract

The Ehlers-Danlos Syndromes comprise a heterogeneous group of diseases, which are characterized by fragility of the soft connective tissues and widespread manifestations in skin, ligaments and 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 one of the genes encoding fibrillar collagen proteins or enzymes involved in post-translational modification of these proteins. Establishing the correct EDS subtype has important implications for genetic counselling and management and is supported by specific biochemical and molecular investigations. Over the last years, the characterisation of several new EDS variants has broadened insights into the molecular pathogenesis of EDS by implicating genetic defects in the biosynthesis of other extracellular matrix molecules, such as proteoglycans and tenascin-X, or genetic defects in molecules involved in intracellular trafficking, secretion and assembly of extracellular matrix proteins.

Keywords

Ehlers-Danlos syndrome (EDS) • Villefranche classification • Six subtypes • Non-functional *COL5A1* allele • *COL3A1* gene • Beighton hypermobility score • *COL1A1* and *COL1A2* mutations

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Abbreviations

ATCS	Adducted Thumb Clubfoot Syndrome
ECM	Extracellular Matrix
EDS	Ehlers-Danlos Syndrome
ER	Endoplasmic Reticulum
HP	Hydroxylysylpyridinoline
JHS	Joint Hypermobility Syndrome
LP	Lysylpyridinoline

9.1 Classification

The Ehlers-Danlos syndrome (EDS) comprises a spectrum of monogenic conditions with multisystemic and variable clinical manifestations affecting primarily the skin, ligaments and joints, blood vessels and internal organs. Like Osteogenesis Imperfecta, EDS represents a paradigm collagen disorder among the larger group of heritable connective tissue diseases. Genetic defects affecting the biosynthesis or structure of collagen type I, III and V have currently been implicated in EDS and form the basis of the 1997 Villefranche classification of EDS, which recognizes six subtypes, based on clinical phenotype, inheritance pattern and underlying biochemical and molecular defect(s) [1] (Table 9.1). The classic, hypermobility and vascular subtype of EDS are the most common, whereas the kyphoscoliosis, arthrochalasis and dermatosparaxis types 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 current classification and also demonstrated that, besides the collagens, genetic defects affecting either the biosynthesis of other extracellular matrix (ECM) components or processes as diverse as signalling pathways or intra-cellular trafficking can contribute to EDS pathogenesis.

Table 9.1 Updated EDS-classification

EDS-subtype	Inheritance pattern	Protein	Gene
Classic	AD	Procollagen type V	*COL5A1/COL5A2*
		Procollagen type I	*COL1A1*
	AR	Tenascin-X	*TNX-B*
Cardiac-valvular	AR	Deficiency of α2(I) collagen chain	*COL1A2*
Hypermobility	AD	?	*?*
		Tenascin-X	*TNX-B*
Vascular	AD	Procollagen type III	*COL3A1*
Vascular-like	AD	Procollagen type I (R-to-C)	*COL1A1*
Kyphoscoliotic	AR	Lysyl hydroxylase-1	*PLOD1*
Musculocontractural	AR	Dermatan-4-sulfotransferase-1	*CHST14*
Progeroid	AR	Galactosyltransferase 1	*B4GALT7*
Spondylocheirodysplastic	AR	ZIP13	*SLC39A13*
FKBP14	AR	FKBP14	*FKBP14*
Brittle cornea syndrome	AR	ZNF469	*ZNF469*
		PRDM5	*PRDM5*
Arthrochalasis	AD	Procollagen type I (deletion of N-propeptide cleavage site)	*COL1A1/COL1A2*
EDS/OI overlap	AD	Procollagen type I (delay in N-propeptide cleavage)	*COL1A1/COL1A2*
Dermatosparaxis	AR	Procollagen-I-N-proteinase	*ADAMTS2*

AD autosomal dominant, *AR* autosomal recessive

9.2 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 lax skin in "cutis laxa" syndromes). The skin is often smooth and velvety to the touch. In the vascular subtype, the skin is not hyperextensible but thin and transparent, showing a 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***, referred to as 'cigarette paper scars'. ***Joint hypermobility*** is usually generalised and variable in severity and with age. It is assessed using the Beighton scale (Table 9.2). 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, debilitating 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 deposits in exposed areas such as shins and knees. There is a tendency towards pronounced ***bleeding*** (e.g. following brushing of teeth) despite a normal coagulation status. A range of clinical manifestations that result from ***generalized weakness and fragility of the soft connective tissues*** are observed in patients with EDS including obstetrical and gynaecological 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.

9.3 EDS-Subtypes

9.3.1 Ehlers-Danlos Syndrome, Classic Type

The diagnosis of the classic type of EDS requires the presence of skin hyperextensibility, widened atrophic scars and joint hypermobility, which constitute the three major diagnostic criteria, in association with a varying set of 'minor' manifestations such as smooth, velvety skin, molluscoid pseudotumours (fleshy lesions over pressure points), subcutaneous spheroids (small, hard cyst-like nodules), easy bruising and bleeding, muscle hypotonia, delayed gross motor development and inguinal and/or umbilical hernia. Characteristic facial features include epicanthic folds, excess skin over the eyelids, presence of one or more dilated scars on the forehead and a pale, somewhat prematurely aged appearance of the face.

Ultrastructural examination of the skin in classic EDS shows irregular and loosely packed collagen fibrils and typical "cauliflower" fibrils which represent the histological hallmark of disturbed fibrillogenesis of the heterotypic type I/V collagen fibrils. The molecular basis of classic EDS is a deficiency of ***type V collagen***, a

Table 9.2 The Beighton scale for joint hypermobility. A total score of at least five defines hypermobility

Joint/finding	Negative	Unilateral	Bilateral
Passive dorsiflexion of the 5th finger >90°	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	

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* gene respectively. The most common isoform in vertebrate tissues is the [α1(V)2α2(V)] heterotrimer. Collagen type V is thought to play 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 domain is buried within the fibril [2].

A causal role for type V collagen in classic EDS first became apparent from studies in transgenic mice which showed that mice with a homozygous deletion of the *col5a2* gene present clinical and ultrastructural features of classic EDS [3]. 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 [4]. The first mutations reported in classic EDS were respectively an exon skipping mutation [5] and a missense mutation substituting a highly conserved cysteine for a serine in the C-propeptide domain of the α1(V) collagen chain [6]. This cysteine residue is essential for intra-chain disulphide bonding prior to chain assembly and initiation of trimerisation. 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. Since then a growing number of mutations in type V collagen have been identified by different groups, including for the most part heterozygous *COL5A1* nonsense, frameshift or splice site mutations that abolish one *COL5A1* allele through the nonsense-mediated mRNA decay mechanism or impair normal molecular assembly of type V collagen [7–10]. 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 *CO L5A1* 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, approximately 150 different type V collagen mutations have been identified in classic EDS, 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 α1(V) that cause atypical splicing outcome and (have been associated with) a more severe EDS phenotype [11]. Based on the data gathered to date, it is now clear that mutations in type V collagen account for approximately 90 % of classic EDS cases [12].

9.3.2 Ehlers-Danlos Syndrome, Hypermobility Type (EDS-HT)

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 with EDS-HT 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 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 EDS subtype from the more common '(familial) joint hypermobility syndrome' (JHS). The presence and degree of hypermobility can be scored with the Beighton hypermobility score (Table 9.1) which assesses hypermobility at hands, elbows, knees and spine through different manoeuvres which each are scored with one point to a maximum of nine points [13].

Although often considered in the literature as a 'mild' form of EDS, the hypermobility type of EDS can present with severe and debilitating complications such as recurring dislocations and subluxations and chronic articular pain, which represent a significant burden in daily life of affected individuals and may lead to social isolation, emotional distress and depression [14]. In practice, it is not uncommon to see patients with the EDS-HT diagnosed with fibromyalgia, chronic fatigue syndrome and/or depression.

Over the last years different studies have aimed to document in a more precise way the functional musculoskeletal status and health in patients with EDS-HT. Musculoskeletal symptoms and complaints were shown to be frequently present to a significant degree in EDS-HT patients. Severe joint hypermobility with recurrent joint dislocations and chronic moderate to severe pain were the most severe complaints, but also muscle cramps, tendinitis, headache and fatigue were frequently reported among EDS-HT subjects. 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 [14]. In a comparative study, physical impairment and impact of joint pain in EDS-HT were shown to be substantially greater in EDS-HT versus other chronic rheumatologic disorders such as rheumatoid arthritis, but rather comparable to the significant disease observed in fibromyalgia [15].

Factors that have been shown to contribute to joint instability include impaired proprioception, postural control and muscular strength. Our studies showed that EDS-HT patients have reduced knee joint proprioception [16], as well as a severely reduced quantitative muscle function and impaired in 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 [17].

The striking preponderance of affected women versus men in EDS-HT is presently unexplained. Ultrastructural studies have shown that some patients with EDS-HT show collagen fibril abnormalities with cauliflower-like aspect as seen in classic EDS [18]. These findings suggest that somehow, collagen fibrillogenesis is impaired also in this EDS subtype, but so far, besides 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 demonstrated that a small subset of patients with EDS-HT or JHS shows haploinsufficiency for tenascin-X [19], encoded by the *TNX-B* gene. Tenascin-X is part of a family of ECM proteins with a complex multidomain structure that allows interaction with many other ECM components. It is considered to be a very important player in the organisation of the ECM. An autosomal recessive form of EDS, resembling to but phenotypically distinct from classic EDS [20], had previously been shown to be caused by complete deficiency of **tenascin-X**, caused by truncating mutations or large deletions in both *TNX-B* alleles. Patients with this condition present with joint hypermobility, skin hyperextensibility and easy bruising, but they also suffer from generalized muscle weakness and distal contractures. Atrophic scarring has not been observed. Further discussion of EDS-HT and other less common forms of EDS is also presented in Chap. 10 by Miyake et al.

9.3.3 Ehlers-Danlos Syndrome, Vascular Type

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 can be difficult.

Generalised vascular fragility largely dominates the clinical picture. Apart from excessive bruising and bleeding, it may cause arterial ruptures, potentially resulting in sudden death, usually in the third or the fourth decade of life. Other life-threatening complications include gastrointestinal rupture, rupture of the uterus and internal organ rupture.

A retrospective study, performed on 100 independent, molecularly proven vascular EDS-probands, showed that 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/or 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 % of the probands. Gastro-intestinal 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. Four probands experienced a spontaneous organ rupture, including spleen or liver. Pneumothorax was a frequent complication. The median age of death was 33 years, and the major cause of death was arterial rupture. Obstetrical history was recorded for 34 pregnancies among which five were complicated by arterial, uterine or splenic rupture. Other pregnancy-or delivery-related complications included severe vaginal lacerations and haemorrhage, and severe rectal tearing. Sixty percent of all probands was referred for molecular analysis only after the occurrence of a major internal complication, such as an arterial or internal organ rupture, whereas the remaining 40 % was referred because of typical clinical manifestations, including excessive bruising, translucent skin, acrogeria and facial appearance, either with or without having a family history of a major event or sudden death. The median age at diagnosis was 29 years, but ranged widely between 4 and 74 years (Malfait and de Paepe, personal observation).

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 [21], the majority of which are missense mutations leading to substitutions for glycine in the triple helical region of the collagen molecule. Other mutations include splice site mutations, partial gene deletions, and, rarely, null- mutations resulting in *COL3A1* haplo-insufficiency [22].

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 premature death. This relationship is however not absolute and severe clinical phenotypes have been reported for more N-terminal-located mutations. It was suggested that patients with *COL3A1* null mutations may present a milder phenotype, that is associated with a longer life span, later age of first complication (by 15 years), and risk of complication limited to vascular events [23].

Parental mosaicism for *COL3A1* mutations has been documented in vascular EDS [24–27] and may explain unexpected recurrences in families in which a 'new' dominant mutation has been identified.

9.3.4 Ehlers-Danlos Syndrome, Kyphoscoliotic Type and Related Phenotypes

The ***EDS kyphoscoliotic type or type VIA*** is an autosomal recessive 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 occurrence of life-threatening rupture of medium-sized arteries. This form of EDS is caused by a deficient activity of the enzyme lysyl hydroxylase 1 (LH-1) [28] and is historically 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 intermolecular cross links (pyridinolines) that provide tensile strength and stability to the collagen fibrils and serve as attachment sites for carbohydrate units which modulate the lateral packing of collagen molecules into fibrils. The diagnosis can be confirmed by demonstrating an increased ratio of lysylpyridinoline (LP) to hydroxylysylpyridinoline (HP) cross-links in the urine, decreased LH-1 activity in cultured skin fibroblasts or a homozygous or compound heterozygous mutation in the *PLOD1* gene. A homozygous multi-exon duplication accounts for ~20 % of mutations reported so far [29], but missense, nonsense and small indel mutations leading to loss-of-function of *PLOD1* have also been identified [30]. Interestingly, in **the *Brittle Cornea Syndrome (BCS)*,** a rare autosomal recessive condition that shows significant phenotypic overlap with EDS type VI A, 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 [31]. 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 sclerae shows a significantly decreased corneal thickness. Recently, *PRDM5*, a second gene for BCS was identified [32]. *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 [33]. 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 [32].

A subset of patients who clinically appear to fit within the EDS type VI phenotypic spectrum do present with normal LP/HP ratios and have been referred to as 'EDS type VIB' or 'EDS type VI with normal LH-1 ratios' [34]. We have recently shown that mutations in ***CHST14***, encoding dermatan-4-sulfotransferase 1 (D4ST-1), and previously associated with an autosomal recessive condition called "adducted thumb clubfoot syndrome" (ATCS) (59), underly this EDS type VIB variant [35]. Affected individuals present a range of clinical features that overlap with EDS type VIA, such as the typical skin and joint manifestations, kyphoscoliosis, congenital clubfeet, muscular hypotonia and ocular abnormalities which include microcornea, blue sclera, myopia, retinal detachment and glaucoma. However they also present characteristic clinical manifestations that are distinct from type VIA. These include craniofacial abnormalities, joint contractures, wrinkled palms, tapered fingers and gastro-intestinal and genitourinary manifestations. The craniofacial manifestations and joint contractures are similar to those in ATCS but differ in the severity of the skin manifestations and the more severe kyphoscoliosis and ocular involvement. A Japanese EDS variant reported by Kosho et al. [36] was also shown to be associated with loss-of-function mutations in *CHST14* [37], and falls within this EDSVIB-ATCS spectrum. In view of the prominent muscular hypotonia and typical contractures we have proposed to designate the *CHST14* related EDS variant as the ***EDS, musculocontractural subtype (EDSVIB)***. Morphological and ultrastructural studies in this EDS subtype shows 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. The molecular spectrum of *CHST14* mutations is varied and comprises homozygous or compound heterozygous missense, nonsense and frameshift mutations, as well as homozygous 20-bp duplication [38].

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 catalyses 4-O-sulfatation of N-acetyl-galactosamine (GalNAc) residues. It is one of three major sulfotransferases in the DS/CS (chondroitin sulfate) synthesis, which display different substrate specificities. The different epimerisation and sulfatation reactions during DS/CS biosynthesis reflect a tightly controlled system that determines the 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 trombomodulin and small leucine-rich proteoglycans, such as decorin and biglycan. This may compromise 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. 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 disorganisation of the collagen network [37].

Of note, a rare, autosomal recessive condition, **progeroid EDS,** has also been associated with PG biosynthesis. This EDS variant is 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 beta-1, 4 galactosyltransferase or galactosyltransferase I [39]. This enzyme catalyzes the transfer of the first galactose residue on the O-linked xylose in the tetrasacchardie linker region that initiates the biosynthesis of glycosaminoglycans. Very recently, biallelic mutations were identified in *B3GALT6*, which encodes galactosltransferase II, responsible for the transfer of the second galactose residue in this tetrasaccharide linker region, in patients with a pleiotropic EDS-like disorders, presenting also bone fragility, muscle hypotonia, severe kyphoscoliosis and progressive contractures [40, 41] (see also Chap. 10).

These conditions shows phenotypic resemblance with two other recently identified novel autosomal recessive EDS variants. One is 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 and 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 [42]. The other novel EDS variant is characterized by severe progressive kyphoscoliosis, muscle hypotonia at birth, myopathy, joint hypermobility, hyperelastic skin, sensorineural hearing impairment and normal pyridinoline excretion in the urine. This condition is caused by mutations in *FKBP14*, encoding an endoplasmic reticulum (ER)-resident protein belonging to the family of FK506-binding peptidyl-prolyl cis-trans isomerases. ER-resident FKBPs have been suggested to act as folding catalysts by accelerating cis-trans isomerisation of peptidyl-prolyl bonds and to act occasionally as chaperones. The wide connective tissue involvement in the affected patients is attributed to a disturbance of protein folding in the ER affecting one or more components of the ECM [43].

9.3.5 Ehlers-Danlos Syndrome Subtypes That Result from Aberrant Processing of the Procollagen Type I-N-Propeptide

Defects that interfere with the cleavage of the N-terminal propeptide of type I procollagen result in the *arthrochalasis or dermatosparaxis type of EDS* respectively. The autosomal dominant *arthrochalasis type of EDS* (previously EDS VII A & B) is caused by heterozygous mutations that lead to skipping of exon 6, or part of it, in the mRNA coding for the α1 or α2-chain of type I procollagen. These exon-skipping mutations lead to loss of the N-terminal telopeptide, which links the N- propeptide to the main triple-helical domain and contains the procollagen-I-N-proteinase cleavage site as well as a critical cross-linking lysyl residue. The clinical hallmark of this EDS-variant is congenital bilateral hip dislocation, in addition to severe generalized joint hypermobility with recurrent joint dislocations, variable cutaneous involvement with 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 pNα1(I) (EDS VIIA) or pNα2(I) (EDSVIIB) procollagen chains in cultured skin fibroblasts.

Mutations residing within the N-terminal stretch of 85 amino acid (AA) residues in the triple helical domain of type I collagen result in an EDS/OI overlap phenotype characterised by OI-like bone fragility and variable skin- and joint hypermobility, reminiscent of that seen in EDS [44, 45]. This 85 AA region acts as a stabilizing "anchor" for the N-terminal end of the type I collagen triple helix, and defects in this α1(I) N-anchor region were shown to lead to a conformational change in the adjacent N-propeptide cleavage site, resulting in inefficient cleavage of the N-propeptide [46]. So, although the cleavage site itself remains intact, inefficiently cleaved collagen molecules are incorporated in the collagen 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 which is encoded by the *ADAMTS2* gene, causes the *dermatosparaxis type of EDS*, an autosomal recessive 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 and short stature. Fragility of internal tissues, with spontaneous bladder and diaphragmatic rupture, has been reported [47]. Whereas most of the initially reported patients showed a very severe phenotype, recognizable from birth, it is now clear that some patients present 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. This causes pathognomonic abnormalities of the dermal collagen fibril architecture, characterised by fibrils that have lost their normal cross-sectional circular aspect and have a hieroglyphic appearance [48]. Biochemical analysis shows aberrant processing of type I procollagen with characteristic accumulation of type I pN-collagen [49].

9.3.6 Other EDS-Variants Caused by Mutations in the Type I Collagen-Encoding Genes

Over the last years, a number of atypical mutations have been identified in the genes encoding type I collagen (*COL1A1* and *COL1A2*), which have been shown to result in EDS-phenotypes that show overlap with the classic type of EDS. One of those is a rare autosomal recessive form of EDS referred to as the *cardiac-valvular EDS*, caused by total absence of the α2(I) collagen chain which results in the production of $[\alpha1(I)]_3$ homotrimers. This condition presents in childhood with mild skin - and joint hypermobility, mild osteopenia and muscular hypotonia but is complicated in adulthood by the development of severe cardiac valve insufficiency that may need cardiac valve replacement [50, 51].

Another type of type I collagens defects linked to EDS are missense mutations in *COL1A1* that result in the substitution of an arginine (R) residue by a cysteine (C) residue in the Xaa or Yaa position of the triple-helical Gly-Xaa-Yaa repeat. These mutations lead to the production of α1(I)-dimers that are detectable on SDS-PAGE of radiolabelled collagens obtained from cultured skin fibroblasts. One R-to-C substitution in pro 1(I) chain of type I collagen (p. (R312C)) has been identified in a series of patients with classic EDS-like phenotype, showing skin hyperextensibility, easy bruising, atrophic scarring and joint hypermobility, and propensity for arterial rupture at adult age [52, 53]. Two other pro-α1(I) R-to-C substitutions (p.(R574C) and p.(R1093C)) have also been associated with rupture of medium-sized arteries, but affected individuals did not present EDS-like skin features [53]. Furthermore a p.(R1036C) and a p.(R1066C) were reported in families with an EDS/OI overlap phenotype, without signs of vascular fragility [54, 55]. Intriguingly one specific α1(I)_p.(R1014C) substitution was reported in a number of families with autosomal dominant infantile cortical hyperostosis or Caffey disease, a benign and self-limiting disorder of early childhood, characterized by systemic inflammation and subperiosteal new bone formation [56].

9.4 Diagnosis

Comprehensive clinical evaluation is the first and pivotal step in the diagnostic assessment for EDS and may be sufficient to establish a correct clinical diagnosis. For certain EDS-subtypes, ultrastructural, biochemical and molecular analysis are helpful to diagnose the correct EDS subtype.

9.4.1 Ultrastructural Examination of the Skin

Ultrastructural examination of the skin, performed by electron microscopy, usually reveals abnormalities of collagen fibrils. These include irregular, disrupted collagen fibrils ("collagen flowers"), and variability within the diameter of the collagen fibrils. However, these abnormalities are common to several EDS variants and usually not specific enough to discriminate between individual EDS subtypes.

A pathognomonic ultrastructural aspect of the collagen fibril architecture is observed only in the dermatosparaxis subtype of EDS. Collagen fibrils in this EDS subtype lose their normal cross-sectional circular aspect and display an irregular, branched, "hieroglyphic" appearance instead (2).

9.4.2 Skin Biopsy for Fibroblast Culture and Biochemical Analysis

The first step in the laboratory diagnosis of EDS is to establish a fibroblast culture from a skin biopsy. Protein-based analysis of the collagens type I, III and V by means of SDS-polyacrylamide gel electrophoresis allows to detect qualitative or quantitative abnormalities of these collagen proteins.

Biochemical analysis of type III collagen is highly sensitive to confirm a diagnosis of vascular EDS as it identifies structural alterations of the type III collagen proteins in 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. Therefore, direct DNA analysis of the *COL3A1* gene is still indicated in clinically suspect cases for vascular EDS, even in the absence of a detectable type III collagen protein defect.

Biochemical analysis is also helpful to confirm the diagnosis of the arthrochalasis, kyphoscoliosis, and dermatosparaxis subtypes of EDS. Rare, unclassified variants of EDS with distinct fibrillar collagen protein abnormalities, such as R-to-C substitutions in the α1(I)-chain of type I collagen, may also be picked up by biochemical collagen analysis. These findings may further guide molecular analysis of the specific collagen gene(s) involved.

In contrast, for the majority of patients with classic EDS, biochemical analysis of type V

collagen is an ineffective method for routine diagnostic evaluation (3).

In the hypermobility type of EDS, as well as in the benign joint hypermobility syndrome (BJHS), biochemical analysis of the fibrillar collagens shows no abnormalities (4).

9.4.3 Urine Analysis

A highly specific and sensitive urinary assay is available to confirm the diagnosis of the kyphoscoliotic type of EDS. In this EDS subtype, deficiency of LH-1 results in a deficiency in hydroxylysine-based pyridinoline cross-links in types I and III collagen. As a result, cross-linked peptides are excreted in the urine as by-products of collagen turn-over. The diagnosis relies upon the demonstration of an increased ratio of lysylpyridinoline (LP) to hydroxylysylpyridinoline (HP) crosslinks in the urine measured by HPLC, which is a highly sensitive and specific test. In the kyphoscoliotic type of EDS, the LP/HP ratio is ~6, whereas normal ratios are ~0.2 [57]. In EDS spondylocheirodysplastic subtype, the LP/HP is slightly elevated to ~1 [42]. In the other EDS-subtypes, LP/HP ratio is are normal.

9.4.4 Molecular Genetic Testing

Molecular analyses usually start from genomic DNA (gDNA) and mRNA extracted from cultured dermal fibroblasts. For the classic subtype of EDS, the majority of mutations result in a non-functional *COL5A1* allele. As a first step therefore, a *COL5A1* "null-"allele test can be performed to determine if the individual is heterozygous for one of several *COL5A1* polymorphic exonic markers in the genomic DNA and to establish at the cDNA level whether or not both alleles are expressed. Sequence analysis of the genes involved in the various subtypes of EDS can be performed either on gDNA or cDNA. Once a mutation is identified in the patient, its presence or absence can easily be verified on gDNA obtained from other family members.

Prenatal diagnosis in at risk pregnancies is possible by analysis of DNA extracted from fetal cells obtained by amniocentesis usually performed at about 15–18 weeks' gestation or chorionic villus sampling (CVS) at about 10–12 weeks' gestation. The disease-causing allele of an affected family member must be identified before prenatal testing can be performed. Requests for prenatal testing for conditions like classic EDS which do not affect intellect or life span are not common. Differences in perspective may exist among medical professionals and within families regarding the use of prenatal testing, particularly if the testing is being considered for the purpose of pregnancy termination rather than early diagnosis. Although most centers would consider decisions about prenatal testing to be the choice of the parents, discussion of these issues is appropriate.

Preimplantation genetic diagnosis (PGD) may be available for families in which the disease-causing mutation has been identified in an affected family member.

9.5 Management

In view of the multisystemic involvement, different medical specialists need to be involved in the management of EDS patients, depending on the extent and severity of the disease manifestations. Each patient presents with a particular set of clinical problems, requiring a treatment plan that is tailored to his or her (specific) needs. Coordination of the multi-disciplinary approach to ensure that all areas of the syndrome are covered is preferably done by a clinical geneticist, who will also provide genetic counselling. No causal therapy is available for EDS; however, a series of 'preventive' guidelines are applicable to all forms of EDS. The key aspects of management include cardiovascular work-up, physiotherapy, pain management, and psychological follow-up.

A baseline echocardiogram should be performed in all patients with EDS in order to evaluate aortic root diameter, as adjusted for age and body surface area, and to evaluate cardiac valvular abnormalities. Because longitudinal data on

progression of aortic dilation are not available, specific recommendations for follow-up in individuals with a normal aortic diameter do not exist. However, if no abnormalities are found on echocardiogram in an adult, a follow-up echocardiogram is probably not necessary. For children and adolescents, it is reasonable to repeat the echocardiogram, approximately every 3 years until adulthood. Annual echocardiograms are warranted only if an abnormality such as aortic dilatation or mitral valve prolapse is present.

Children with pronounced skin fragility should wear protective pads or bandages over the forehead, knees and shins in order to avoid skin lacerations. Dermal wounds should be closed without tension, preferably in two layers. Deep stitches should be applied generously. Cutaneous stitches should be left in place twice as long as usual, and additional fixation of adjacent skin with adhesive tape can help to prevent stretching of the scar.

Patients with pronounced bruising are advised to avoid contact sports and heavy exercise. Protective pads and bandages can be useful in the prevention of bruises and haematomas. Drugs that interfere with platelet function and prolong the bleeding time should be avoided whenever possible. These include for example aspirin (acetylsalicylic acid, ASA), dipyridamole, clopidogrel, and non-steroidal anti-inflammatory drugs (NSAIDs) such as ibuprofen and diclofenac. Paracetamol (acetaminophen) and COX-1 sparing NSAIDS, such as celecoxib, do not influence haemostasis and can be considered safe. By definition, anticoagulant drugs will increase the bleeding tendency, and should also be avoided, especially in the vascular subtype of EDS. Examples are the oral vitamin K antagonists (coumadins) such as acenocoumarol, fenprocoumon and warfarin, heparin and low molecular weight heparins (LMWH), and more recent oral thrombin inhibitors and pentasaccharides. Supplementation with ascorbic acid, a cofactor for cross-linking of collagen fibrils, can ameliorate the tendency towards bruising in some patients. The vasopressin analogue DDAVP (desmopressin acetate, 1-Desamino-8-D-Arginine Vasopressin) has been reported to reduce a bleeding tendency temporarily in subjects undergoing a dental or surgical procedure [14]. A case report describes the successful use of recombinant factor VIIa in a patient with the vascular type of EDS, in whom continued bleeding was successfully stopped after intravenous administration of recombinant factor VIIa [58].

In patients with hypotonia and delayed motor development, a physiotherapeutic program is important. Non-weight-bearing muscular exercise, such as swimming, is useful to promote muscular development and coordination. In contrast, sports with heavy joint strain, such as contact sports, should be discouraged. Besides prescription of physiotherapy, the rheumatologist and/or physical therapist may also provide assisting devices such as braces, ring splints, soft collar necks, while the occupational therapist may provide tools that make the living and working environment more comfortable for the patient. Many patients will be subjected to one or more orthopedic procedures prior to the diagnosis of EDS, such as arthroplasty and capsulorraphy, although with variable (or limited) success. The degree of joint stabilization, pain reduction, and duration of improvement is usually far less successful in patients with EDS than in those without this disorder. It is often preferable to delay surgery in EDS patients in favour of physical therapy and bracing.

Pain management is also very important. Pain medication should be tailored to the individual's subjective symptoms. Cognitive behavioural therapy can be beneficial in patients with hypermobility and chronic pain. Finally, psychological follow-up designed to explore coping strategies and to recognize and treat depression is of utmost importance.

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

Invasive vascular procedures such as arteriography and catheterization should be avoided because of the risk of vascular ruptures which cause significant morbidity and mortality [59, 60]. They should rather be replaced by ultrasonography and/or subtraction angiography. Surgical interventions are generally discouraged because of increased vascular fragility and a conservative approach is recommended in this condition.

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 additional 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 [61]. Recently a multicentre randomised 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. This study represents a substantial breakthrough in the evidence-based management of the syndrome [16].

Pregnancy for women with the vascular type of EDS is a high-risk venture. The risk of maternal death is as high as 12 % from uterine rupture or peripartum arterial rupture [62]. On the other hand, some women with vascular EDS have had one or several successful pregnancies, even prior to recognition of their underlying condition. 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.

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