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The Ehlers–Danlos syndromes

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Abstract | The Ehlers–Danlos syndromes (EDS) are a heterogeneous group of hereditary disorders of connective tissue, with common features including joint hypermobility, soft and hyperextensible skin, abnormal wound healing and easy bruising. Fourteen different types of EDS are recognized, of which the molecular cause is known for 13 types. These types are caused by variants in 20 different genes, the majority of which encode the fibrillar collagen types I, III and V, modifying or processing enzymes for those proteins, and enzymes that can modify glycosaminoglycan chains of proteoglycans. For the hypermobile type of EDS, the molecular underpinnings remain unknown. As connective tissue is ubiquitously distributed throughout the body, manifestations of the different types of EDS are present, to varying degrees, in virtually every organ system. This can make these disorders particularly challenging to diagnose and manage. Management consists of a care team responsible for surveillance of major and organ-specific complications (for example, arterial aneurysm and dissection), integrated physical medicine and rehabilitation. No specific medical or genetic therapies are available for any type of EDS.

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The Ehlers–Danlos syndromes (EDS) comprise a genetically heterogeneous group of heritable conditions that share several clinical features, such as soft and hyperextensible skin, abnormal wound healing, easy bruising and joint hypermobility. Additional clinical features that differ among EDS subtypes include fragility of soft tissues, vessels and hollow organs, and involvement of the musculoskeletal system, all of which can result in chronic and severe disability and/or early mortality, and may affect the quality of life (QOL) of patients and their families.

Edvard Ehlers and Henri-Alexandre Danlos were dermatologists who, in the early twentieth century, described patients with joint hypermobility, excessive skin extensibility, easy bruising and abnormal scar formation after injury[1,2]. Several years before their description, patients with similar manifestations were described by Chernogubow in Russia, where his name is still used to describe what we refer to as classical EDS (cEDS)[3]. Frederick Park-Weber suggested that the condition be called 'Ehlers–Danlos syndrome'[4]. Since then, individuals with the shared clinical features discussed above and with additional clinical findings have been classified into the different EDS types; however, the classification has changed over time with the discovery of the genetic basis of these conditions. The 1986 'Berlin Nosology' recognized 11 types of EDS, which were defined by Roman numerals and which were based on clinical findings, mode of inheritance and biochemical alterations[5]. After the elucidation of the biochemical and/or molecular basis of several of these types, a revised classification, the 'Villefranche Nosology' was published in 1998, which recognized six EDS types, denominated by a descriptive name[6]. The most recent classification, the revised EDS classification in 2017 (TABLE 1) identified 13 distinct clinical EDS types that are caused by alterations in 19 genes[7]. Of note, research published after the 2017 classification has described another genetically distinct EDS type, provisionally classified as classical-like EDS type 2 (clEDS2), bringing the total number of EDS-associated genes to 20 (REF.[8]). The extended 2017 classification (which includes clEDS2) guides the clinical diagnosis, genetic confirmation, management and genetic counselling of EDS.

Most EDS types that have a known genetic cause result from pathogenetic variants in genes encoding fibrillar collagens types I, III and V, modifying or processing enzymes for these collagens, or enzymes that have key roles in the biosynthesis of the glycosaminoglycan (GAG) chains of proteoglycans. These molecules contribute to the physical properties of the extracellular matrix (ECM) in essentially all tissues and organs. Despite the advances in gene identification, some patients have clinical features that are compatible with EDS but do not fit within a currently defined type and have no pathogenetic variants in the known EDS causative genes, which indicates that the genetic heterogeneity of EDS has not been completely resolved.

Next-generation sequencing analysis and the ability to sequence all relevant genes at once has facilitated timely and cost-effective genetic diagnosis of EDS, and has refined the phenotypic spectra associated with pathogenetic variants in these genes. Definitive diagnosis of EDS relies on genetic confirmation, with the

Table 1 | **The 2017 international Ehlers–Danlos syndrome classification**

EDS type (abbreviation)	Gene (encoded protein)	Major clinical criteria	Minor clinical criteria
Disorders of collagen primary structure and collagen processing			
Classical (cEDS)[a]	*COL5A1* (α1(V) procollagen chain) *COL5A2* (α2(V) procollagen chain) *COL1A1* (α1(I) procollagen chain: p.Arg312Cys (rare))	Skin hyperextensibility with atrophic scarring and generalized joint hypermobility	Easy bruising, soft doughy skin, skin fragility (or traumatic splitting), molluscoid pseudotumours (bluish-grey, spongy nodules, which are herniations of subcutaneous fat, seen over easily traumatized areas), subcutaneous spheroids, hernia (or history thereof), epicanthal folds, complications of joint hypermobility (such as sprain, (sub)luxation, pain, flexible flatfoot), family history of a first-degree relative who meets criteria
Vascular (vEDS)[a]	*COL3A1* (α1(III) procollagen chain) *COL1A1* (α1(I) procollagen chain: p.Arg312Cys (rare), p.Arg574Cys (rare), p.Arg1093Cys (rare))	Family history of vEDS with documented pathogenetic variant in *COL3A1*, arterial rupture at young age, spontaneous sigmoid colon perforation in the absence of known colon disease, uterine rupture during third trimester of pregnancy, carotid-cavernous sinus fistula (in the absence of trauma)	Bruising unrelated to identified trauma and/or in unusual sites such as cheeks and back, thin, translucent skin with increased venous visibility, characteristic facial features (large eyes, periorbital pigmentation, small chin, sunken cheeks, thin nose and lips and lobeless ears), spontaneous pneumothorax, acrogeria, talipes equinovarus, congenital hip dislocation, small joint hypermobility, tendon and muscle rupture, gingival recession and gingival fragility, early-onset varicose veins
Arthrochalasia (aEDS)[a]	*COL1A1* (α1(I) procollagen chain) *COL1A2* (α2(I) procollagen chain)	Congenital bilateral hip dislocation, severe generalized joint hypermobility with multiple dislocations, skin hyperextensibility	Muscle hypotonia, kyphoscoliosis, radiologically mild osteopenia, tissue fragility including atrophic scars, easy bruising
Dermatosparaxis (dEDS)[b]	*ADAMTS2* (ADAMTS2, N-proteinase)	Extreme skin fragility with congenital or postnatal tears, craniofacial features (large fontanel, puffy eyelids, excessive peri-orbital skin, downslanting palpebral fissures, blue sclerae, hypoplastic chin), progressively redundant, almost lax skin with excessive skin folds at wrists and ankles, increased palmar wrinkling, severe bruisability with risk of subcutaneous haematoma, umbilical hernia, postnatal growth retardation with short limbs, perinatal complications related to tissue fragility	Soft and doughy skin texture, skin hyperextensibility, atrophic scars, generalized joint hypermobility, complications of visceral fragility (e.g. rectal prolapse, bladder or diaphragm rupture), delayed motor development, osteopenia, hirsutism, tooth abnormalities, refractive errors, strabismus
Cardiac valvular (cvEDS)[b]	*COL1A2* (α2(I) procollagen chain (total absence))	Severe progressive cardiac valvular insufficiency, skin involvement, joint hypermobility (generalized or restricted to small joints)	Inguinal hernia, pectus deformity, joint dislocations, foot deformities (pes planus, pes planovalgus and hallux valgus)
Disorders of collagen folding and collagen crosslinking			
Kyphoscoliotic (kEDS-*PLOD1* or kEDS-*FKBP14* depending on the causative mutation)[b]	*PLOD1* (lysyl hydroxylase 1) *FKBP14* (FKBP22)	Congenital muscle hypotonia, congenital or early-onset kyphoscoliosis, generalized joint hypermobility with (sub)luxations	For both genetic causes: skin hyperextensibility, easy bruising, rupture/aneurysm of medium-sized artery, osteopenia/osteoporosis, blue sclerae, umbilical or inguinal hernia, pectus deformity, marfanoid habitus, talipes equinovarus, refractive errors. For *PLOD1* mutations: skin fragility, microcornea, characteristic craniofacial features. For *FKBP14* mutations: congenital hearing impairment, muscle atrophy, bladder diverticula
Disorders of structure and function of the myomatrix			
Classical-like (clEDS)[b]	*TNXB* (tenascin-X)	Skin hyperextensibility with velvety skin texture and absence of atrophic scarring, generalized joint hypermobility, easily bruisable skin/spontaneous ecchymoses	Foot deformities, oedema in legs in absence of cardiac failure, mild proximal and distal muscle weakness, axonal polyneuropathy, atrophy of muscle in hands and feet, acrogeric hands, mallet fingers, clino- or brachydactyly, vaginal, uterine or rectal prolapse

Table 1 (cont.) | **The 2017 international Ehlers–Danlos syndrome classification**

EDS type (abbreviation)	Gene (encoded protein)	Major clinical criteria	Minor clinical criteria
Disorders of structure and function of the myomatrix (cont.)			
Myopathic (mEDS)[c]	*COL12A1* (α1(XII) procollagen chain)	Congenital muscle hypotonia and/or muscle atrophy, proximal joint contractures, hypermobility of distal joints	Soft, doughy skin, atrophic scarring, motor developmental delay, myopathy on muscle biopsy
Disorders of glycosaminoglycan biosynthesis			
Musculocontractural (mcEDS-*CHST14* or mcEDS-*DES* depending on the causative mutation)[b]	*CHST14* (dermatan-4-*O*-sulfotransferase 1) *DSE* (dermatan sulfate epimerase 1)	Congenital multiple contractures (typically adduction/flexion contractures and talipes equinovarus), characteristic craniofacial features (large fontanelle, short downslanting palpebral fissures, blue sclerae, hypertelorism, short nose with hypoplastic columella, low-set and rotated ears, long philtrum with thin upper lip vermillion, small mouth and hypoplastic chin), characteristic cutaneous features (skin hyperextensibility, easy bruising, skin fragility with atrophic scars, increased palmar wrinkling)	Recurrent/chronic dislocations, pectus deformities, spinal deformities, peculiar fingers, progressive talipes deformities, large subcutaneous haematomas, chronic constipation, colonic diverticulae, pneumo(haemo)thorax, nephrolithiasis/cystolithiasis, hydronephrosis, cryptorchidism in males, strabismus, refractive errors, glaucoma
Spondylo-dysplastic (spEDS-*B4GALT7* or spEDS-*B3GALT6* depending on the causative mutation)[b]	*B4GALT7* (galactosyl-transferase I) *B3GALT6* (galactosyl-transferase II)	Short stature (progressive in childhood), muscle hypotonia (ranging from severe congenital to mild later-onset), bowing of limbs	For both genetic causes: skin hyperextensibility, soft and doughy, thin and translucent skin, pes planus, delayed motor development, osteopenia, delayed cognitive impairment. For *B4GALT7* mutations: radioulnar synostosis, bilateral elbow contractures, single transverse palmar crease, characteristic craniofacial features, characteristic X-ray findings of skeletal dysplasia, clouded cornea. For *B3GALT6* mutations: kyphoscoliosis (congenital or early-onset), joint hypermobility (generalized or restricted to distal joints), joint contractures (congenital or progressive), peculiar fingers, characteristic craniofacial features, tooth discoloration, dysplastic teeth, characteristic X-ray findings of skeletal dysplasia, osteoporosis with spontaneous fractures, aortic aneurysm, lung hypoplasia, restrictive lung disease
Disorders of intracellular processes			
Spondylo-dysplastic (spEDS)[b]	*SLC39A13* (ZIP13)	Short stature (progressive in childhood), muscle hypotonia (ranging from severe congenital to mild later-onset), bowing of limbs	Skin hyperextensibility, soft and doughy, thin and translucent skin, pes planus, delayed motor development, osteopenia, delayed cognitive impairment, protuberant eyes with bluish sclerae, hands with finely wrinkled palms, skeletal dysplasia, atrophy of thenar muscles and tapering fingers, hypermobility of distal joints, characteristic X-ray findings of skeletal dysplasia
Brittle cornea syndrome (BCS)[b]	*ZNF469* (ZNF469) *PRDM5* (PRDM5)	Thin cornea with/without rupture, early-onset progressive keratoconus and/or keratoglobus, blue sclerae	Enucleation or corneal scarring as a result of previous rupture, progressive loss of corneal stromal depth, high myopia, retinal detachment, deafness (often mixed conductive and sensorineural), hypercompliant tympanic membranes, developmental dysplasia of hip, hypotonia in infancy (usually mild), scoliosis, arachnodactyly, hypermobility of distal joints, pes planus, hallux valgus, mild finger contractures, soft, velvety and/or translucent skin
Disorders of complement pathway			
Periodontal (pEDS)[a]	*C1R* (C1r) *C1S* (C1s)	Severe and intractable early-onset periodontitis, lack of attached gingiva, pretibial plaques, family history of first-degree relative who meets clinical criteria	Easy bruising, joint hypermobility (mostly distal), skin hyperextensibility and fragility, wide or atrophic scarring, increased infection rate, hernias, marfanoid facial features, acrogeria, prominent vasculature

Table 1 (cont.) | **The 2017 international Ehlers–Danlos syndrome classification**

EDS type (abbreviation)	Gene (encoded protein)	Major clinical criteria	Minor clinical criteria
Molecularly unresolved forms of EDS			
Hypermobile (hEDS)[a]	Unknown	In summary: generalized joint hypermobility and at least 2 of the following: 1) systemic manifestations of generalized connective tissue disorder, 2) positive family history and 3) musculoskeletal complaints. Exclusion of other EDS types and other causes of generalized joint hypermobility	–
Additional EDS variants			
Classical-like type 2 (provisional) (clEDS2)[b]	*AEBP1* (ACLP)	Skin hyperextensibility with atrophic scarring, generalized joint hypermobility, foot deformities, early-onset osteopenia	–

ACLP, aortic carboxypeptidase-like protein; EDS, Ehlers–Danlos syndromes. [a]Denotes autosomal dominant inheritance. [b]Denotes autosomal recessive inheritance. [c]Denotes autosomal dominant or autosomal recessive inheritance. Adapted with permission from REF.[7], Wiley.

exception of the hypermobile EDS (hEDS) type for which the genetic cause has not been identified. Although the autosomal recessive types of EDS are generally characterized by congenital abnormalities, the diagnosis of EDS for the more common types (that is, cEDS, hEDS and vascular EDS (vEDS)) may be missed in childhood. Indeed, although signs of connective tissue fragility are already present in childhood for most patients with these types of EDS, they are often viewed as within the normal range for age and so not perceived as leading to a distinct diagnosis. In addition, joint hypermobility is common in childhood, making the distinction between a pathological and physiological pattern of joint hypermobility difficult. In newborn babies and infants, skin hyperextensibility can be masked by abundant subcutaneous tissue, and bruising and skin-splitting tendencies do not usually manifest until the child starts to walk and fall. Severe, life-threatening complications of some EDS types, such as the arterial and gastrointestinal ruptures in those with vEDS, are uncommon before adolescence or adulthood but, when they occur, unexplained bruising is often a feature in childhood. In elderly people, joint hypermobility may have diminished, and skin manifestations may change as the connective tissue ages.

This Primer uses the extended 2017 classification of EDS to provide an overview of the clinical presentations, epidemiology and genetics of EDS types, and insights into their pathophysiology, diagnosis and management.

Epidemiology

Accurate data on the incidence and prevalence of the different types of EDS are not available. An incidence of at least ~1 in 5,000 individuals for all forms of EDS was proposed in 2002 and reported no predisposition according to ethnicity[9], but the basis of this estimate is not clear. The diagnosis of hEDS is more frequent in women than in men, but whether this finding is due to an increased prevalence or more severe manifestations in women is unknown. Of note, the prevalence of other types of EDS is similar in males and females[10–12].

The incidence of cEDS and vEDS have each been estimated at ~1:20,000 and 1:50,000–1:200,000 individuals born per year globally. For vEDS, the incidence estimates are derived from the known number of pathogenetic variants identified in diagnostic laboratories in the USA and adjusted for the under-representation of some pathogenetic variants that are associated with milder presentations (such as null variants and substitutions of glycine by alanine in the triple helical domain of the gene product of *COL3A1*), and adjusted for estimates of the likely ascertainment proportion. A similar approach with data from a single Belgian laboratory for pathogenetic variants in *COL5A1* and *COL5A2* have been used to estimate the prevalence of cEDS[9,11,13]. Although these estimates were obtained in the USA and Europe, it is expected that the incidence in other regions is similar given that the pathogenetic variants are spread throughout the target genes (that is, *COL3A1*, *COL5A1* and *COL5A2*), there are few common sites, and few ethnic-specific alleles are known. For the other types of EDS for which causative variants have been identified, no prevalence estimates have been determined, but the number of people who have been reported worldwide with these disorders ranges between ~5 and ~100 individuals per EDS type[12]. As cEDS and vEDS are considered the most common EDS types that have a known molecular basis, it is assumed that the other EDS types with a known cause have a prevalence comparable to or below the cut-off of a rare disease (which in Europe is defined as a condition that affects <5 individuals in 10,000, and in the USA as a condition that affects <200,000 people)[14].

The perceived prevalence of EDS as a whole has changed over the past two decades. Shortly after the publication of the Villefranche classification in 1998 (in which clinical criteria for hEDS were proposed for the first time)[6], it seemed that the clinical boundaries of hEDS overlapped with those of joint hypermobility syndrome (JHS), a condition associated with joint hypermobility and musculoskeletal and systemic symptoms as defined by the revised Brighton criteria[15]. In 2009, several years after the publication of the Villefranche criteria for hEDS and the revised Brighton criteria for JHS, a group of experienced rheumatologists and geneticists stated that hEDS and JHS were sufficiently similar that they should be considered as the same condition until genetic studies could determine otherwise[16]. JHS is presumably a common phenotype (the diagnostic criteria include joint hypermobility and persistent pain for >3 months in four or more joints not due to an inflammatory condition at any point in life), although it is difficult

to measure the prevalence of JHS given these criteria, and systematic epidemiological studies have not been carried out. On the basis of survey data without clinical confirmation, some studies have suggested a frequency of 0.75–2% in white individuals; these estimates combine prevalence data of generalized joint hypermobility in various populations and assume that ~10% of individuals with hypermobility will develop related musculoskeletal problems during their lives[17]. On the basis of the overlap in manifestations of hEDS and JHS, some clinicians proposed that the prevalence of hEDS should be comparable with that of JHS[16]. However, the revised 2017 EDS classification emphasizes that, among individuals with otherwise unclassified joint hypermobility, the term hEDS should be limited to individuals with features indicative of a systemic and/or Mendelian connective tissue disorder[7]. In this context[18], hEDS is likely a rare or, perhaps, uncommon disorder. Individuals with symptomatic joint hypermobility who do not fulfil the 2017 diagnostic criteria for hEDS (and who do not have signs and symptoms of other joint hypermobility-associated conditions) are now considered to fall into the group of 'hypermobility spectrum disorders' (HSD)[19], which is likely a common phenotype (BOX 1). Accordingly, the term JHS has been discontinued and most individuals with a previous diagnosis of JHS who do not meet the 2017 criteria for hEDS are now reclassified as having a diagnosis of HSD.

Box 1 | **Hypermobility spectrum disorders**

Hypermobility spectrum disorders

Hypermobile Ehlers–Danlos syndrome (hEDS) is in a phenotypic continuum with non-syndromic joint hypermobility, which encompasses patients with various clinical forms of non-syndromic joint hypermobility and a variety of musculoskeletal complaints or complications (defined as 'hypermobility spectrum disorders' or HSD)[19]. The diagnosis of HSD is, therefore, attributed to patients with symptomatic joint hypermobility who do not fulfil the current hEDS diagnostic criteria and who do not show elements indicative of a pleiotropic syndrome (such as intellectual disability, internal organ malformations or major facial dysmorphism)[227]. HSD are clinically variable and aetiologically heterogeneous disorders, but knowledge of HSD is still in its infancy. Data are missing concerning the cause or causes of HSD; however, indirect evidence and single-centre observations suggest an oligogenic or multifactorial aetiology. In general, three different clinical forms of joint hypermobility are considered to represent separate entities: generalized (joint hypermobility affecting small and large joints of the four limbs, as well as the axial joints), peripheral (joint hypermobility limited to the small joints of hands and feet) and localized joint hypermobility (limited to a single or a few joints with a predominant involvement of large joints). Some individuals report that they have been hypermobile in childhood but not now (that is, historical joint hypermobility), so there is likely to be an influence of time on clinical presentation. HSD are currently classified by mirroring the recognized clinical forms of joint hypermobility.

Joint hypermobility-associated comorbidities

Both hEDS and HSD can associate with a multitude of functional, extra-musculoskeletal manifestations, including chronic fatigue, pelvic floor problems, bladder dysfunction, various dysautonomic features (orthostatic decompensation, unstable cardiac rhythms and rates, postural orthostatic tachycardia syndrome and gastrointestinal dysfunction, including gastroparesis), alterations of the immune system (including mast cell activation syndrome), behavioural disturbances (such as 'brain fog') and psychological distress. These concurring manifestations are now recognized, with different levels of evidence, as joint hypermobility-associated comorbidities. The prevalence of these comorbidities in other, molecularly defined types of EDS has not been documented to date, and the biological connection to tissue alterations is not clear, but it is being investigated. These comorbidities are therefore not implemented in the diagnostic criteria for hEDS (or any other type of EDS), and their concurrence does not affect the primary diagnosis. Nonetheless, recognizing and characterizing these comorbidities in individuals with hEDS is crucial for management as they are considered health-related quality of life determinants. Why hEDS and HSD are more common in females remains unexplained.

Mechanisms/pathophysiology

The genes, and thus the expected pathways to phenotype, differ among the types of EDS. We describe them separately and indicate where the pathways converge in the hope that this type of consideration will lead to an integrated mechanistic view of these conditions.

Fibrillar collagen structure and processing

The first EDS types for which the biochemical and/or molecular underpinnings were identified all result from defects in the primary structure, processing or modification of the fibrillar procollagen types I, III and V[6] (FIGS 1, 2). These procollagens are trimeric molecules that consist of three identical ('homotrimer') or genetically distinct ('heterotrimer') polypeptide chains, which are referred to as proα-chains and which form typical triple helix structures characterized by the Gly-Xaa-Yaa triple repeat, comprising glycine and two other amino acids (FIG. 1). Procollagens are cleaved to form mature collagen molecules by ADAMTS and bone morphogenetic protein 1 (BMP1)/tolloid-like proteinases. This cleavage initiates collagen fibril formation and the fibrils are stabilized by intermolecular crosslink formation (FIG. 2).

Type V collagen forms the initial scaffold on which type I collagen molecules assemble in the dermis, tendon and bone to form heterotypic fibrils of type I and type V collagen. Type V collagen comprises only 2–5% of the total collagen content in most tissues and is present mainly as heterotrimers of two α1(V) chains (encoded by *COL5A1*) and one α2(V) chain (encoded by *COL5A2*)[20,21]. Homotrimers of three α1(V) chains or heterotrimers comprised of an α1(V), α2(V) and α3(V) chain also exist (with the α3(V) chain encoded by *COL5A3*)[22], but their physiological function remains largely unclear. Type V collagen is a major regulator of collagen fibrillogenesis and has a critical role during the early process of collagen fibril nucleation[23–25]. Accordingly, the complete absence of collagen V in mice (owing to homozygous knockout of *Col5a1*) results in the absence of fibril formation and embryonic lethality[26]. By contrast, a reduction of collagen V expression results in fewer collagen I fibrils with increased diameters and irregular boundaries[19], similar to fibrils in individuals with *COL5A1* haploinsufficiency. Collectively, these studies indicate that fibril formation and integrity have a key role in the physical properties of skin and other tissues, but the exact pathway remains to be determined.

Type III collagen is a homotrimer of three α1(III) chains (encoded by *COL3A1*) and, similar to type V collagen, co-assembles with type I collagen to form heterotypic fibrils. Type III collagen is most abundant in tissues that have compliant properties, including the dermis, blood vessel wall, gastrointestinal tract, uterus, lungs, liver and spleen, in which it constitutes 10–30% of the total collagen content[27,28]. The function of type III collagen in the organization and biological properties of the ECM

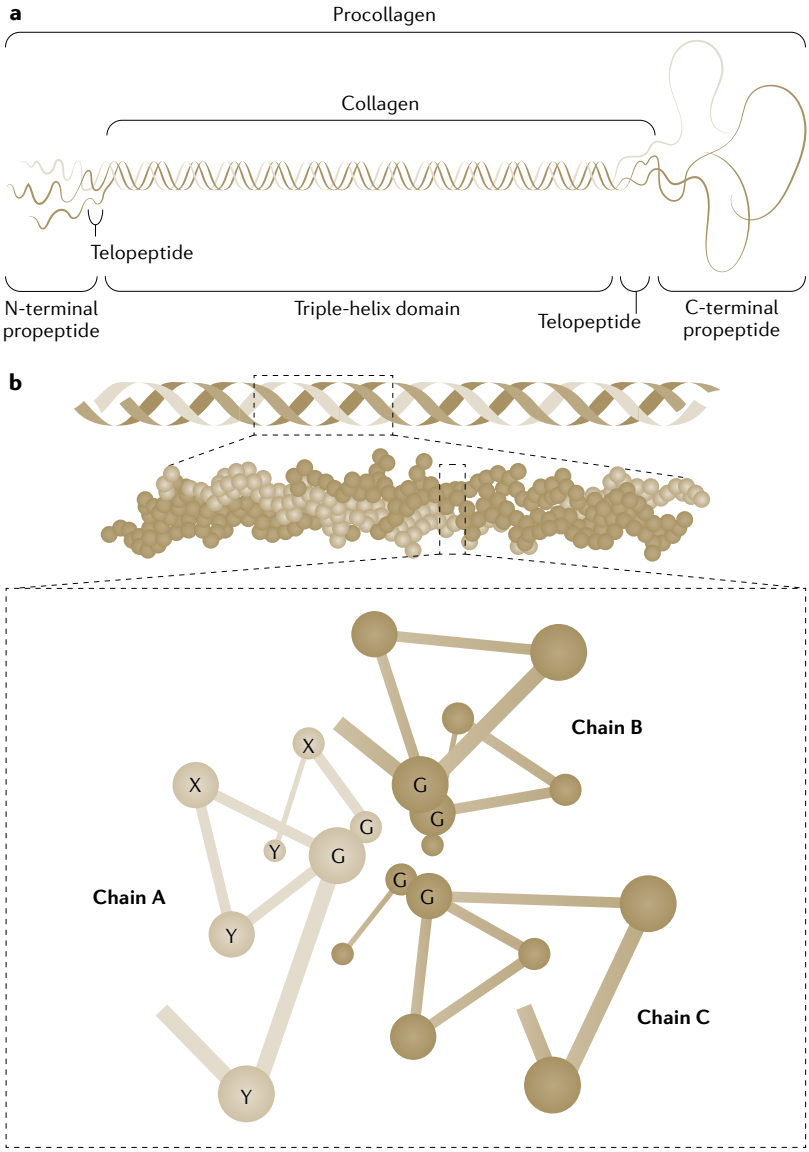


Fig. 1 | **General structure of fibrillar type I collagen. a** | The general structure of type I collagen is depicted, but types III and V fibrillar collagens have a similar structure. These collagens are composed of three α-chains that are assembled into a right-handed triple helix. The collagen chains are synthesized as procollagens that contain globular amino-terminal (N-terminal) and carboxy-terminal (C-terminal) propeptide sequences, which are proteolytically cleaved by specific proteases to produce the mature collagen molecules. **b** | The sequence of each collagen chain is characterized by Gly-Xaa-Yaa repeats that extend for >1,000 residues. The presence of glycine (which has no side chain) in every third position permits the formation of the helical structure. The Xaa and Yaa can be any amino acid but are often proline and hydroxyproline (in the Y position). Hydroxylation of prolyl residues in the Yaa-positions stabilizes the helical structure.

has been studied less than type V collagen, but type III collagen is also presumed to be a regulator of collagen fibril assembly and diameter based on the observation that heterotypic collagen fibrils (consisting of collagen types I and III) reduce in diameter with increasing ratios of type III collagen to type I collagen[29,30]. In support of a role for type III collagen in regulation of collagen fibril diameter, *Col3a1*-knockout or transgenic mice (the latter expressing mutant type III collagen containing a helical glycine substitution (p.Gly182Ser)) have a reduced number of collagen fibrils with a higher variation in fibril diameter in tissues that usually have the most type III collagen[31,32].

Type I collagen is the major protein component of the ECM in many tissues such as the bone, dermis, blood vessel walls and tendon. Type I collagen is a heterotrimer consisting of two α(I) chains (encoded by *COL1A1*) and one α2(I) chain (encoded by *COL1A2*); most pathogenetic variants in these genes cause osteogenesis imperfecta, but a smaller number of recurrent alterations can cause rare forms of EDS (arthrochalasia EDS (aEDS), cardiac valvular EDS (cvEDS) and a form of EDS that overlaps with cEDS and vEDS)[33].

Defects in type V procollagen

Classical EDS. cEDS is caused by heterozygous pathogenetic variants in *COL5A1* or *COL5A2*. Approximately 75% of identified pathogenetic variants are in *COL5A1* and lead to haploinsufficiency (in which one copy of the gene is inactivated or deleted and the remaining functional copy of the gene does not compensate for the reduced protein production)[11,34–37]. This haploinsufficiency can result from nonsense-mediated mRNA decay caused by nonsense variants, small out-of-frame genomic duplications or deletions, splicing errors, or from the deletion of one allele. As type V procollagen molecules cannot accommodate more than a single proα2(V) chain, the reduction of available proα1(V) chains results in production of about half the normal amount of type V collagen[38]. By contrast, proα1(V) chains can form functional homotrimers[39]; of note, no *COL5A2*-null variants have been identified[40]. Other *COL5A1* variants (such as those that prevent the association of proα1(V) chains at the C-terminal propeptide) can also lead to reduced secretion of type V procollagen[41–43]. The remaining identified pathogenetic variants in *COL5A1* and *COL5A2* are splice site variants that lead to single or multiple in-frame exon skips and missense variants that cause substitutions for glycine residues within the triple helical domain. These variants probably have a dual effect on type V procollagen function (altered secretory efficiency and inefficient incorporation into heterotypic fibrils), although the exact mode of action has not been completely elucidated[11,37].

Decreased type V collagen in the ECM is a key factor in the pathogenesis of cEDS[11], but the molecular consequences contributing to the pathogenesis of this disorder remain poorly characterized. In vitro studies using fibroblasts from patients with cEDS have revealed a disorganization of ECM components (for example, type III collagen, type V collagen, fibronectin and fibrillin) and of collagen-specific and fibronectin-specific integrin receptors[44], and a reduced migration capability[45]. Transcriptome profiling of fibroblasts from patients with cEDS also demonstrated disturbances in ECM modelling and wound healing in addition to dysregulated expression of genes involved in endoplasmic reticulum (ER) homeostasis and autophagy[46]. Further studies are needed to better elucidate the contribution of these processes to the molecular pathogenesis of cEDS[47].

Although no clear genotype–phenotype correlations have emerged, *COL5A2* missense and exon-skipping

variants are believed to result in a more severe phenotype of cEDS than the known *COL5A1* variants, but the observations are too few to draw any firm conclusions[11,37]. Approximately 5–10% of individuals with clinical cEDS have no identified alterations in type V collagen[11], suggesting that their cEDS is caused by variants in genes that have not yet been identified as causative, and/or due to technical failure to find, for example, deep intronic variants that affect splice outcomes or (intragenic) genomic rearrangements not detectable by standard procedures. So far, no pathogenetic variants in *COL5A3* have been identified. Ultrastructural studies on skin from individuals with type V collagen defects have demonstrated typical 'collagen flowers' that result from abnormal organization of the collagen fibrils comprising types I and V collagen[48]. However, collagen flowers are not unique to cEDS as they have been observed in other EDS types and other unrelated disorders (such as Ullrich congenital muscular dystrophy)[49,50], so their presence can support, but not confirm, a diagnosis of cEDS[51].

Defects in type III procollagen

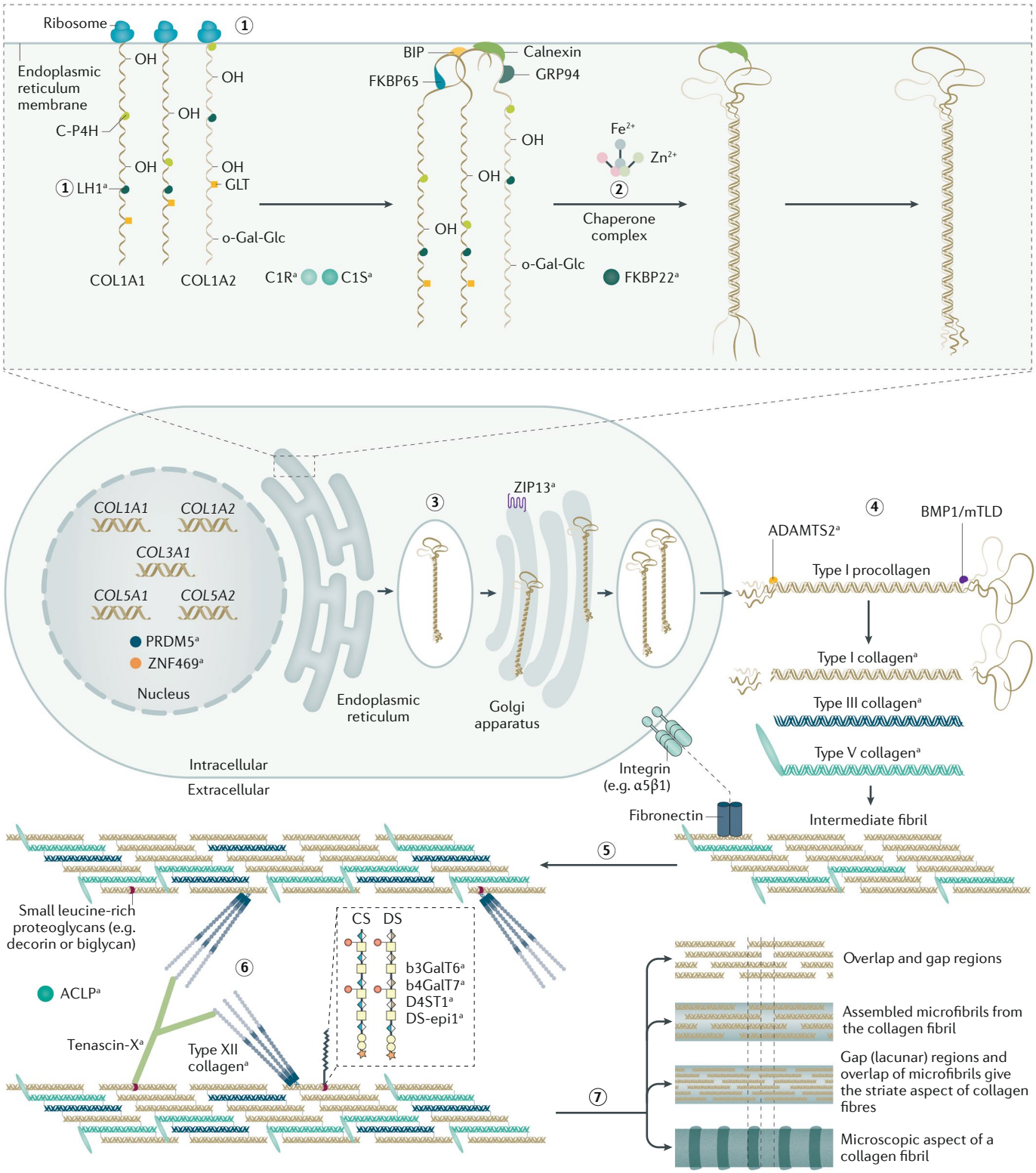
Vascular EDS. In most cases, vEDS is caused by heterozygous pathogenetic variants in *COL3A1*. About two-thirds of the identified variants result in substitutions of glycine residues in the canonical triplet repeats of the triple helical domain (Gly-Xaa-Yaa), whereas splice site variants that result in in-frame exon skipping constitute a quarter of known variants, and a small number of variants result in short in-frame deletions or insertions[13]. All of these variants have a dominant negative action in that, although half the proα1(III) chains are affected, seven-eighths of the homotrimers are abnormal[9]. Some variants (most commonly substitutions for glycine residues in the canonical triplets (Gly-Xaa-Yaa) of the triple helical domain and exon-skipping variants at the carboxy-terminal end of the triple helix) have been shown to result in an almost complete failure of type III procollagen secretion from fibroblasts, with accumulation of type III collagen in the rough ER observed in cultured fibroblasts and in skin biopsies from individuals with these variants[9,52]. However, the mechanisms by which these molecules are retained in the rough ER are unclear.

COL3A1 haploinsufficiency, which accounts for <5% of identified vEDS causative variants, delays the onset of complications by almost two decades on average compared with the more severe forms[10,53]. Missense variants in the region of *COL3A1* encoding the C-terminal propeptide of the proα1(III) chain can alter the association of chains and create a protein-based 'haploinsufficiency' with only half the chains capable of assembling into trimers. Substitutions in the Xaa and Yaa positions in the triple helical domain can be associated with mild vEDS and arterial fragility[54]. Among the latter group, substitutions of glutamic acid with lysine seem to be associated with skin hyperextensibility that is similar to that seen in cEDS and classical-like EDS (clEDS), combined with gastrointestinal and vascular fragility[55]. A few individuals with biallelic *COL3A1* variants have been identified, and these variants lead to a severe vEDS phenotype that is associated with neuronal postmigrational disorder (polymicrogyria)[56–59]. Finally, some individuals with Loeys–Dietz syndrome have clinical presentations that overlap with vEDS, although this syndrome is not considered a type of EDS and has distinct genetic causes[60].

In terms of genotype–phenotype correlations, individuals with in-frame exon-skipping splice site variants in *COL3A1* have the lowest median survival, followed by individuals with substitutions for glycine by a bulky residue (arginine, aspartic acid, glutamic acid or valine) within the α1(III) triple helical domain, whereas individuals with small residue substitutions for glycine (alanine, serine or cysteine) have milder phenotypes[10]. These effects on phenotype and survival could reflect the complex effects of the assembly of abnormal molecules, altered secretion and of intracellular accumulation on cell function and signalling, and alterations in the ECM itself. Transcriptome data from fibroblasts from three independent patients with vEDS (either harbouring a substitution for glycine in the α1(III) triple helical domain or an in-frame skip of exon 14 of *COL3A1*) have demonstrated differential expression of genes that encode ECM molecules and of genes involved in ER homeostasis (including *FKBP14*), the latter of which could contribute to ER retention of misfolded type III collagen molecules[61]. Ultrastructural studies of skin from individuals with vEDS have demonstrated thinning of the dermis[49,62], variable degrees of ER dilatation, and alterations in the size and distribution of major collagen fibrils and elastic fibres[62,63]. However, these findings cannot substitute for DNA sequence studies for diagnosis as they are not specific for vEDS and can be observed in other types of EDS and other heritable connective tissue disorders[7].

Defects in type I procollagen

Arthrochalasia EDS and dermatosparaxis EDS. Heterozygous acceptor site and donor site alterations adjacent to exon 6 of *COL1A1* or *COL1A2* lead to deletion of part or all of the amino acids encoded by these exons and cause aEDS[64]. Exon 6 in these genes encodes the N-terminal propeptide cleavage site, the telopeptide crosslink lysine residue, a cleavage site for proteinases such as pepsin and α-chymotrypsin, and the first triplet of the major triple helical Gly-Xaa-Yaa domain[3]. These splice site alterations can lead to loss of the entire exon or, when a cryptic exonic acceptor site is used, loss only of the N-proteinase cleavage site[9,65]. Of note, the observation that the latter alterations lead to the full phenotype of aEDS suggests that persistence of the N-terminal propeptide is sufficient to create the clinical picture. These causative variants lead to a partial processing of type I procollagen to form an intermediate form known as pN-collagen (in which the collagen is mature but has the N-terminal propeptide attached), which disturbs collagen fibrillogenesis[64]. In aEDS, the alterations in either the proα1(I) chain or the proα2(I) chain are always heterozygous and still lead to the production of normal collagen molecules; 25% of molecules are normal if the proα1(I) chain is affected, whereas 50% of molecules are normal if the proα2(I) chain is affected[3]. Incorporation of these pN-collagen molecules into the collagen fibrils leads to fibrils with irregular contours and smaller diameters, with the greatest effect observed with *COL1A1* variants[64].



Biallelic loss-of-function variants in *ADAMTS2* (which encodes ADAMTS2, the procollagen I N-proteinase) cause dermatosparaxis EDS (dEDS)[66]. A founder pathogenetic variant (c.673C>T, p.Gln225Ter) that originated in western Poland has been reported in Ashkenazi individuals[67]. In contrast to aEDS (in which heterozygous variants in *COL1A1* or *COL1A2* lead to the production of some normal collagen molecules), variants in *ADAMTS2* render the enzyme non-functional, meaning that both the proα1(I) and proα2(I) chains are not cleaved into mature chains unless there is some residual enzyme function and/or some cleavage by other enzymes[68–70]. In dEDS, all type I collagen molecules have the pN structure and fibrils are completely distorted, leading to a pattern resembling hieroglyphs when viewed in cross-section by electron microscopy[71,72].

◀ Fig. 2 | **Collagen fibrillogenesis in the context of Ehlers–Danlos syndromes.** Nascent collagen proα-chains undergo extensive post-translational modification by prolyl and lysyl hydroxylases, including LH1 (REF.[9]), which allow hydroxylysyl glycosylation, and the priming of α-chain sites that will participate in the formation of intermolecular crosslinks in the extracellular matrix (ECM), to stabilize the fibril structure (step 1). The C-terminal propeptide domains of three proα-chains associate into a trimer and initiate triple helix formation at the carboxy-terminal end that folds in a zipper-like fashion towards the amino-terminal end in the endoplasmic reticulum (step 2). After folding, post-translational modification stops, and the procollagen molecules are transported from the endoplasmic reticulum to the Golgi apparatus (step 3). The procollagen molecules begin to aggregate laterally during transport to form secretory vesicles and are eventually directed to the extracellular environment. During transport and/or in the ECM, the N-terminal propeptides and C-terminal propeptides are cleaved by ADAMTS and BMP1/tolloid-like proteinases (BMP1/mTLD), respectively (step 4). Once cleavage of the N-terminal propeptides and C-terminal propeptides is complete, the resulting mature collagen molecules assemble into striated fibrils. This process requires several assisting proteins, categorized into organizers, nucleators and regulators[223] (step 5). At the plasma membrane, fibronectin and integrins serve as organizers of fibril assembly. Nucleators, such as type V collagen, initiate immature fibril assembly at the cell surface. Collagen type V imbeds with its triple helical domain in the collagen type I fibril, whereas its N-terminal propeptide (which is only partially cleaved) protrudes at the fibril surface and controls fibrillogenesis by sterically hindering the addition of other collagen monomers[23]. The intermediate fibrils are then deposited into the ECM. Stabilization of these fibrils involves interaction with 'regulators' such as decorin, tenascin-X and collagen type XII, which influence the rate of assembly, size and structure of the collagen fibrils (step 6). The resulting intermediate collagen fibrils form increasing numbers of covalent crosslinks that stabilize the mature fibrils. These fibrils are arranged in a quarter-staggered array with a characteristic 67-nm axial periodicity (D-periodicity)[224] (step 7). This periodic structure arises from the regular staggering of the triple helical molecules in which gap and overlap zones are distinguished. The gap-zone is present between the N-termini and C-termini of adjacent molecules, whereas complete molecular overlap is observed in the overlap zone. This gives rise to a characteristic alternating light and dark banded pattern observed on electron microscopy, which is consistent with the D-periodicity. The resulting fibrils are indeterminate in length and, depending on the developmental stage and tissue, range in diameter from 12 to >500 nm (REF.[225]). CS, chondroitin sulfate; DS, dermatan sulfate. [a]Pathogenetic variants in the genes encoding these proteins are involved in the pathogenesis of Ehlers–Danlos syndromes.

The hallmarks of dEDS are extremely fragile skin, severe bruising and progressive redundancy of the skin, whereas aEDS is characterized by severe generalized joint hypermobility and bilateral congenital hip dislocation (TABLE 1). The relatively mild skin phenotype in aEDS compared with dEDS might be explained by procollagen N-proteinase activity of other enzymes in the dermis, such as ADAMTS3 and ADAMTS14 (REFS[69,70,73]), whereas some of the features in dEDS might be explained by the N-proteinase activity of ADAMTS2 on types II, III and V procollagen[68,74].

Cardiac valvular EDS. Total absence of proα2(I) chains, due to biallelic loss-of-function splice site or nonsense variants in *COL1A2* that result in unstable *COL1A2* mRNA and nonsense-mediated mRNA decay, leads to formation of α1(I) homotrimers (as opposed to the normal structure comprised of two α(I) chains and one α2(I) chain) and results in cvEDS, which is characterized by severe polyvalvular cardiac involvement[75–79]. By contrast, biallelic loss-of-function *COL1A2* variants that lead to stable mRNA, but unstable proteins, lead to a mild-to-moderate form of osteogenesis imperfecta[80,81]. This observation supports the concept that, in the latter case, the production of unstable mutant protein triggers an unfolded protein response, which contributes to the osteogenesis imperfecta phenotype, whereas for cvEDS, *COL1A2* mRNA instability and absence of proα2(I) chains reflects what seems to be a more limited response in the ECM[82]. The exact pathogenetic mechanisms by which α1(I) homotrimers affect ECM structure and homeostasis and result in these phenotypes are unknown.

Arginine-to-cysteine substitutions in triple helical domain of the proα1(I) chain. Heterozygous variants in *COL1A1* that result in substitutions of arginine by cysteine at positions 312 (134 in the triple helical domain), 574 (396) and 1,093 (915) of proα1(I) chains have been reported in individuals with a vEDS-like propensity for rupture of median-sized arteries[83,84]. The triple helical domain begins at position 179 of the protein and extends for 1,014 residues, and so ends at position 1,193 of the chain. The precise consequences of these arginine-to-cysteine substitutions on the structure and secretion of type I collagen are not well understood. Introduction of a cysteine residue in the triple helix of the α1(I) chains, a domain from which cysteine is excluded, leads to the production of disulfide bonded α1(I) dimers in molecules that have two altered chains and free sulfhydryls in those that have one[83,85]. Although the free reactive sulfhydryl groups in some molecules could lead to disulfide bonding with other proteins, either intracellularly (during transport through and from the rough ER) or in the ECM, no partners have yet been identified. Loss of the arginine residue could contribute to local destabilization of the type I collagen molecules[83,85].

In addition to a susceptibility to arterial rupture, some individuals with the p.Arg312Cys substitution also present with skin hyperextensibility, atrophic scarring and joint involvement (similar to that observed in cEDS), sometimes without signs of vascular fragility[37,86–89]. These individuals may meet clinical diagnostic criteria for cEDS, but variants in *COL5A1* or *COL5A2* would not be identified on genetic testing, suggesting that these individuals should undergo genetic testing of *COL1A1*. Other proα1(I) arginine-to-cysteine substitutions at positions 1,036 (858) and 1,066 (888) have been reported in families with joint hypermobility and mild bone fragility without signs of vascular fragility; these individuals were labelled as having an EDS/osteogenesis imperfecta overlap phenotype[85,90].

Defects in collagen crosslinking and folding

***Kyphoscoliotic EDS*-PLOD1.** Kyphoscoliotic EDS (kEDS)-*PLOD1* is a recessively inherited condition caused by biallelic pathogenetic variants in *PLOD1* that result in deficiency of its gene product, the collagen-modifying enzyme lysyl hydroxylase 1 (LH1). kEDS-*PLOD1* was the first EDS type to be characterized at the biochemical level[91]. Approximately 30% of reported pathogenetic *PLOD1* variants comprise a 7-exon duplication that includes exon 10 to exon 16 and Alu–Alu elements (these are common repeat elements of about 300 bp that are distributed throughout the human genome) at each end that mediate the recombination event[12,92,93]. Other pathogenetic *PLOD1* variants include nonsense, missense and splice site alterations[12]. LH1 is an ascorbate-dependent enzyme that catalyses the co-translational and post-translational hydroxylation of some lysyl residues in

Gly-Xaa-Lys sequences to form hydroxylysyl residues (FIG. 2). Hydroxylysyl residues can be glycosylated with either galactose or glucosyl-galactose. Two triple helical hydroxylysyl residues (positions 87 and 933 of the triple helical domain in proα1(I) chains) are essential for the formation of intermolecular collagen crosslinks in the ECM, which provide tensile strength to most soft tissues and bone. LH1 deficiency owing to *PLOD1* variants results in impaired crosslink formation, which leads to mechanical instability of the affected tissues, difficult to treat scoliosis, and arterial fragility in patients with kEDS-*PLOD1* (REF.[94]).

***Kyphoscoliotic EDS*-FKBP14.** In 2012, a rare recessively inherited form of EDS with clinical signs that overlap those of kEDS-*PLOD1* (TABLE 1) was shown to result from biallelic pathogenetic variants in *FKBP14* (REF.[95]). Reported *FKBP14* pathogenetic variants include a founder c.362dupC, p.Glu122ArgfsTer7, which accounts for ~70% of identified kEDS-*FKBP14* causative alleles, disrupts the reading frame and leads to mRNA instability, in addition to a few splice site variants that result in frameshifts, and one missense variant, p.Met48Lys[95–97]. The c.326dupC variant has been linked to the same haplotype in all investigated individuals thus far[98], suggesting the existence of a founder effect.

FKBP14 encodes the ER-resident mixed-function protein FKBP22, which shows preferential binding to types III, VI and X procollagen[99]. As a molecular chaperone, FKBP22 acts as a quality control on the folded triple helix of type III collagen, and has peptidylprolyl isomerase activity that accelerates triple helical formation of type III collagen[100,101]. Thus, deficiency of FKBP22 may lead to premature interaction and accumulation of collagen molecules in the ER, which likely explains the enlargement of the ER cisterns that has been observed in fibroblasts from patients with kEDS-*FKBP14* (REF.[95]). Consequently, the correct deposition of the collagen fibrils in the ECM of connective tissues might be affected. A common mechanism that bridges kEDS-*PLOD1* and kEDS-*FKBP14* remains elusive. A similar pairing of osteogenesis imperfecta phenotypes that resulted from alterations in *PLOD2* and *FKBP10* could be explained by the role of FKBP65 (encoded by *FKBP10*) in LH2 (encoded by *PLOD2*) dimerization, which is essential for LH1 function[102].

Box 2 | **The contiguous gene syndrome CAH-X**

Tenascin-X (TNX) deficiency was initially reported in a single patient with combined signs of congenital adrenal hyperplasia (CAH) and Ehlers–Danlos syndrome (EDS)[103]. CAH is caused by 21-hydroxylase deficiency owing to pathogenetic variants in its encoding gene, *CYP21A2*. *TNXB* partially overlaps with *CYP21A2* (REF.[228]). In rare cases, patients with severe, salt-wasting CAH have deletions in *CYP21A2* (often a 30-kb deletion) that extend into *TNXB*, resulting in a contiguous gene syndrome of CAH and signs of EDS, termed CAH-X. In a prospective observational study, 7% of 193 consecutive unrelated patients with CAH were heterozygous for a *TNXB* deletion and were more likely to have joint hypermobility, chronic joint pain, multiple joint dislocations and a structural cardiac valve abnormality than age-matched and sex-matched patients with CAH and wild-type *TNXB*[228].

Defects in ECM bridging molecules

Classical-like EDS. Some types of EDS are caused by pathogenetic variants in genes that do not encode fibrillar collagens or their modifying or processing enzymes. Indeed, tenascin-X (TNX), encoded by *TNXB*, was the first non-collagenous molecule to be implicated in EDS pathogenesis. Biallelic loss-of-function variants in *TNXB* result in a recessively inherited EDS type (later named as clEDS), which is characterized by skin hyperextensibility without atrophic scarring, significant bruising, joint hypermobility and sometimes the presence of muscle weakness and distal contractures[103,104] (TABLE 1). The first patient characterized had a homozygous deletion that had end points in a repeated section of the last 12 exons that was downstream from the functional *TNXB* gene by some 10 kb (REF.[103]). Subsequently, most affected individuals were found to have biallelic pathogenetic variants that led to loss of function[12]. The deletion in the original individuals included *CYP21A2* (encoding 21-hydroxylase), pathogenetic variants in which can cause congenital adrenal hyperplasia[105]. This finding gave rise to the hypothesis that deletions such as this could account for some salt losing in heterozygotes, and perhaps for the hEDS phenotype (BOX 2). Initial investigation of 20 obligate heterozygous family members (of which 14 were female and six male) of patients with complete TNX deficiency showed reduced TNX serum levels in all individuals. These heterozygous individuals did not have clEDS, but 9 out of the 14 women (and none of the six men) had generalized joint hypermobility[106]. Reduced TNX serum levels were subsequently reported in ~5% of patients diagnosed with hEDS[106], but wider genetic screening of *TNXB* revealed that only ~2.5% of patients with hEDS carry heterozygous deleterious *TNXB* variants[107]. The effect of missense variants in *TNXB* has been very difficult to assess.

Myopathic EDS. Heterozygous and biallelic variants in *COL12A1* (which encodes type XII collagen) have been found in individuals who presented with a phenotype that couples signs of EDS with myopathy (resembling the collagen VI-associated Bethlem myopathy), which is now referred to as myopathic EDS (mEDS)[108–110]. These variants include heterozygous missense and in-frame exon skipping variants, and one homozygous splice site variant leading to a frameshift and the introduction of a premature termination codon[108–110]. Patients with biallelic *COL12A1* variants seem to have a more severe, congenital disease, whereas children with heterozygous variants have a subtler presentation[110].

Type XII collagen is a fibril-associated collagen with interrupted triple helices (FACIT) and which strongly binds to TNX[111]. Both molecules interact with fibrillar collagens either directly, or indirectly through the small leucine-rich proteoglycans (SLRPs) such as decorin and fibromodulin[112–115]. Collagen XII, TNX and their binding partners can form flexible bridges between collagen fibrils and other non-collagenous ECM molecules to regulate the organization and mechanical properties

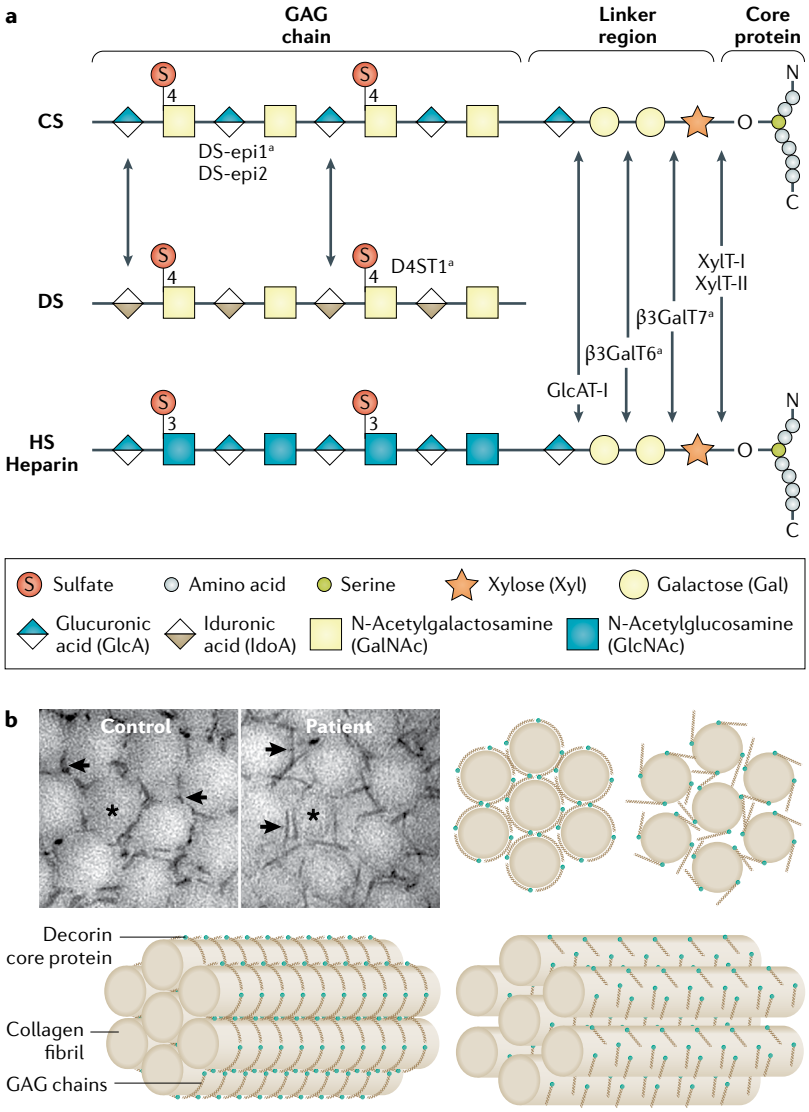


Fig. 3 | **Biosynthetic pathway of CS/DS and HS/heparin proteoglycans. a** | Proteoglycan core proteins are synthesized in the endoplasmic reticulum and undergo further modification in the Golgi apparatus. First, a tetrasaccharide linker region (Xyl-Gal-Gal-GlcA) is synthesized. The biosynthesis of this linker region is a stepwise process that commences with the addition of xylose residue onto a serine residue of the proteoglycan core protein, a process that is catalysed by xylosyltransferases I/II (XylT-I/II). After this step, two galactose residues are added to the saccharide linker region by the enzymes galactosyltransferase I and II, respectively, following which the addition of one glucuronic acid by glucuronosyltransferase I completes the formation of the tetrasaccharide linker region. The addition of N-acetylglucosamine or N-galactosylglucosamine to the terminal GlcA residue of the linker region leads to formation of heparan sulfate (HS) or chondroitin/dermatan sulfate (CS/DS) proteoglycans, respectively. The glycosaminoglycan (GAG) chains are further modified by epimerization and sulfation reactions. DS epimerases (DS-epi1 and DS-epi2) catalyse epimerization of GlcA residues to L-iduronic acid (IdoA), and subsequent 4-*O*-sulfation by D4ST1 of GalNAc adjacent to IdoA prevents back-epimerization of IdoA to GlcA, and generates DS. In musculocontractural Ehlers–Danlos syndrome (mcEDS) due to D4ST1 deficiency, IdoA is back-epimerized to GlcA, leading to the formation of CS and depletion of DS. **b** | Transmission electron microscopy-based cupromeronic blue staining and schematic figures of collagen fibrils and GAG chains. GAG chains are curved and are in close contact with attached collagen fibrils in normal skin (control), but in skin of a patient with mcEDS-*CHST14* (patient) they are linear, stretching from the outer surface of collagen fibrils to adjacent fibrils[136]. Decorin core protein binds to D bands of collagen fibrils in normal skin and in skin from a patient with mcEDS-*CHST14*. In normal skin, GAG chains comprised of DS adhere to collagen fibrils along D bands, starting from the core protein, whereas in patients with mcEDS-*CHST14* GAG chains composed of CS extend linearly and perpendicularly to collagen fibrils from the core protein[136]. [a]Pathogenetic variants in the genes encoding these enzymes are involved in the pathogenesis of EDS. Panel **b** adapted with permission from REF.[136], Elsevier.

of collagen fibrils in several tissues (FIG. 2). Qualitative and/or quantitative alterations in one of these molecules interferes with the normal organization of collagen fibrils in the ECM. Indeed, ultrastructural analysis of the dermis of individuals with clEDS or mEDS shows increased interfibrillar distance[110,116]. However, the precise structural and physiologic consequences of these alterations on the ECM and their translation to phenotype are not well understood.

Defects in glycosaminoglycan biosynthesis

Proteoglycans are abundant in the ECM and on the surface of all animal cells and are involved in a wide range of functions such as cell–cell communication, cell–matrix interactions, cell growth and differentiation, and interact with many ECM components, including collagens. Proteoglycans consist of a core protein and one or more GAG side chains such as heparan sulfate (HS), chondroitin sulfate (CS) and/or dermatan sulfate (DS). Biosynthesis of proteoglycans starts with the formation of a tetrasaccharide linker (comprising xylosyl, galactosyl, galactosyl and glucuronic acid) on the core protein that can then accept the sugars of the GAG (FIG. 3).

Spondylodysplastic EDS. A progeroid type of EDS, which was first documented in the 1980s, combined features of EDS (joint hypermobility and skin hyperextensibility) and early ageing and seemed to be caused by defective GAG addition to several proteoglycan core proteins[117–121]. This phenotype was later shown to result from biallelic variants (including missense, nonsense and frameshift variants) in *B4GALT7*, which encodes galactosyltransferase I, the enzyme that adds the first galactosyl residue to xylosyl during the biosynthesis of the tetrasaccharide linker region[122] (FIG. 3). In addition, biallelic variants (including missense, nonsense and frameshift variants and small deletions and insertions) in *B3GALT6*, which encodes galactosyltransferase II that adds the second galactosyl residue to the tetrasaccharide linker (FIG. 3), have been found in a series of patients with a complex pleiotropic connective tissue disorder that combines signs of EDS (that is, joint hypermobility and skin hyperextensibility) with spondyloepimetaphyseal dysplasia, bone fragility, progressive contractures and muscle hypotonia[123,124]. The revised 2017 EDS classification merged both

conditions into 'spondylodysplastic EDS' (spEDS), together with EDS caused by variants in *SLC39A13* (see Defects in other intracellular molecules below)[7].

Studies on cultured fibroblasts from patients with either spEDS-*B4GALT7* or spEDS-*B3GALT6* have demonstrated variably reduced galactosyltransferase I and galactosyltransferase II enzyme activities, respectively, with markedly reduced and/or shorter HS and CS GAG chains, and partial or complete lack of DS on decorin[123–126]. The severe pleiotropic phenotypes associated with these enzyme defects presumably result from an abnormal GAG configuration in decorin and other SLRPs that are involved in the regulation of interfibrillar spacing of collagen fibrils, and abnormal cell signalling during development caused by altered interactions of affected HS and CS/DS proteoglycans with growth factors and other ligands[123,127].

Musculocontractural EDS. Further downstream in the biosynthetic pathway of GAGs, biallelic missense, frameshift or nonsense variants in *CHST14* (which encodes dermatan 4-*O*-sulfotransferase 1 (D4ST1)) and in *DSE* (which encodes DS-epimerase 1 (DSepi1)) result in musculocontractural EDS (mcEDS). This condition is inherited in an autosomal recessive manner and is characterized by multiple congenital anomalies (such as craniofacial features, multiple congenital contractures, ocular and visceral malformations) and progressive tissue fragility-related findings (such as skin fragility, joint hypermobility, large subcutaneous haematoma)[128–132] (TABLE 1). Pathogenetic variants in *CHST14* and *DSE* that lead to reduced activity of the encoded enzyme result in depletion of DS and replacement by CS[130,133]. This depletion of DS has been demonstrated both with skin fibroblasts and in urine of patients harbouring pathogenetic *CHST14* variants[130,133]. Decorin, a major SLRP that consists of a core protein and a single GAG chain, which consists mainly of DS moieties, has an important role in assembly of collagen fibrils in the skin[134]. In the skin of patients with pathogenetic *CHST14* variants the DS in decorin is completely replaced by CS, whereas some DS moieties remain in patients with pathogenetic *DSE* variants[131]. The phenotype in patients with *DSE* variants appears to be somewhat milder than that observed for *CHST14* pathogenetic variants[131,135]. Collagen fibrils in the papillary and reticular dermis of individuals with mcEDS-*CHST14* are not regularly and tightly assembled compared with normal skin[130,136], and the decorin GAG chains appear linear, stretching from the outer surface of collagen fibrils to adjacent fibrils, whereas in normal skin GAG chains are curved and maintain close contact with attached collagen fibrils[136] (FIG. 3). These structural alterations of GAG chains could cause spatial disorganization of collagen networks[130,137].

Defects in other intracellular molecules

Another rare autosomal recessive type of EDS, initially called spondylocheirodysplastic EDS, but now merged with spEDS (spEDS-*SLC39A13*), is caused by biallelic variants in *SLC39A13* (which encodes the zinc importer protein ZIP13)[138]. Only three homozygous pathogenetic variants have been identified in *SLC39A13*, including one 9-bp deletion, one missense variant and one nonsense variant[138–140]. ZIP13 is a homodimeric transmembrane Zrt/irt-like protein, which regulates the influx of Zn^{2+} into the cytosol[141]. Variants in *SLC39A13* lead to generalized underhydroxylation of lysyl and prolyl residues of collagen, and abnormal crosslinking of collagen in the ECM[138]. Several mechanisms underlying these abnormalities have been suggested and include Zn^{2+} overload in the ER and competition with Fe^{2+} for binding to lysyl hydroxylase and prolyl 4-hydroxylase[138]; trapping of Zn^{2+} in cytosolic vesicular stores ('zincosomes'), leading to the reduced availability of Zn^{2+} in the ER and other cellular components and induction of ER stress[142]; and alterations in the activation of BMP/TGFβ signalling via regulation of the intracellular localization of SMAD proteins in connective tissue-forming cells[139]. Of note, findings in *Drosophila melanogaster* have revealed that the fruitfly homologue of human ZIP13 is an Fe^{2+} exporter on the ER/Golgi membrane and, therefore, its absence might result in Fe^{2+} depletion in the ER/Golgi compartment, which could lead to underhydroxylation of lysyl and prolyl residues in collagen[143].

Brittle cornea syndrome. Brittle cornea syndrome (BCS) is a rare recessive generalized heritable connective tissue disorder that is clinically characterized by thin and fragile corneas that are at increased risk for spontaneous perforation. BCS was initially considered to be a form of kEDS[144]; however, cells from individuals with BCS were found to have normal LH1 activity, distinguishing the condition from kEDS[145,146]. Linkage studies identified *ZNF469* and later also *PRDM5* as the genetic causes of BCS[147,148]. ZNF469 is a zinc finger protein of unknown function, and the mechanism by which pathogenetic variants influence corneal development and structural tissue integrity remain largely unknown. By contrast, *PRDM5* encodes a widely expressed transcriptional regulator belonging to the PR/SET protein family that modulates many aspects of tissue development and maintenance in vertebrates via mechanisms that include Wnt signalling[149,150]. Transcript analysis of fibroblasts from patients with *ZNF469* or *PRDM5* variants showed dysregulation of several genes involved in the development and maintenance of the ECM, such as downregulation of *COL4A1*, *COL11A1* and *HAPLN1* (the last of which encodes the hyaluronan and proteoglycan link protein 1)[148]. Moreover, immunofluorescence staining demonstrated the altered deposition of type I collagen, type III collagen, fibronectin and their receptor α2β1 and α5β1 integrins in *PRDM5* and *ZNF469* in the ECM of fibroblasts from patients[148,151]. Together, these data point towards a regulatory role for ZNF469 and PRDM5 in the organization of the ECM in the eye and other connective tissues.

Defects in the complement pathway

Periodontal EDS. Periodontal EDS (pEDS) is characterized by aggressive periodontal disease and often premature tooth loss, with mild joint hypermobility and pretibial plaques, and was first described in 1977 (REF.[152]). Linkage analysis in three families mapped the phenotype to a 5-Mb region at chromosome 12p13 (REF.[153]), and exome analysis identified heterozygous missense variants

or in-frame insertion/deletions in *C1R* and *C1S*, contiguous genes in the previously reported linked region[154]. These genes encode subunits C1r and C1s of the first component of the classical complement pathway that form a heterotetramer (comprising two chains each of C1r and C1s) that combines with six C1q subunits[155–158] to form the complete C1 molecule. Binding occurs between the amino-terminal collagenous domain of C1q and the CUB domains of C1r and C1s[159]; CUB domains are evolutionary conserved protein–protein interaction domains that occur in several ECM proteins, including BMP1, the C-proteinase for type I procollagen, and its enhancer PCPE1 (REF.[160]). PCPE1, BMP1 and C1s can bind through their CUB domains to the triple helix of collagen and/or propeptides[161,162]. These findings suggest that some features of pEDS may be due to abnormal interaction between C1r and C1s with ECM molecules[154]. Pathogenetic variants in *C1R* have a gain-of-function effect, with constitutive intracellular activation of C1s and C1r serine proteases, which could result in cleavage of C4 and local complement cascade activation[163]. The discovery that defects in components of the complement pathway lead to a form of EDS has opened possibilities to understand the interplay between the immune system and connective tissues.

ACLP defects

Classical-like EDS type 2. After the publication of the 2017 EDS classification, an autosomal recessive type of EDS was identified from whole-exome sequencing studies, which is caused by biallelic variants in *AEBP1* (missense variants, nonsense variants and frameshift variants) and which is clinically hallmarked by skin hyperextensibility with atrophic scarring, generalized joint hypermobility, foot deformities and early-onset osteopenia[8]. *AEBP1* encodes the ECM-associated adipocyte enhancer-binding protein 1 (AEBP1; also known as aortic carboxypeptidase-like protein (ACLP)), which is abundantly present in tissues with a high collagen content[164,165]. This protein binds to fibrillar types I, III and V collagen, and assists in type I collagen polymerization[8]. In addition, AEBP1 is also involved in fibroblast-to-myofibroblast transition through activation of TGFβ receptors[166,167], and in bone development and homeostasis through frizzled 8-mediated and LRP6-mediated activation of the canonical Wnt signalling pathway[168]. The exact mechanisms by which these defects lead to an EDS phenotype are unknown. On the basis of the clinical resemblance to classical EDS, this condition is provisionally designated as classical-like EDS type 2 (clEDS2)[169].

Diagnosis, screening and prevention

Diagnosis

Clinical assessment. A clinical diagnosis of EDS is often suspected on the basis of (generalized) joint hypermobility, abnormal wound healing, unexplained bruising and/or other signs of vascular or tissue fragility. Nonetheless, a clinical diagnosis is often not straightforward as many features of EDS occur in the general population and some characteristics of EDS are found in other genetic conditions; as a result, there may be a long delay to diagnosis.

Both the Villefranche Nosology (1998) and the extended 2017 EDS classification defined the major and minor clinical criteria for EDS types[6,7] (TABLE 1). A major criterion is expected to have high diagnostic specificity as it is present in most individuals with that type of EDS and is absent or rare in the general population. In addition, the major criteria are considered characteristic for the specific type of EDS and may allow differentiation from other EDS types and/or other partially overlapping hereditary connective tissue disorders. By contrast, a minor criterion conveys less diagnostic specificity, but its presence supports the diagnosis and often the combination of several minor criteria are more suggestive of the specific EDS diagnosis. Before the introduction of genetic testing, these criteria often were the critical factors that established a specific diagnosis. This practice emerged from a clinical need to facilitate diagnosis to allow counselling about prognosis and recurrence risks, and to identify specific management strategies. In the past few decades it has facilitated grouping of individuals for the purposes of genetic studies to identify genes that harbour causal variants that have led to the recognition of genetic heterogeneity and allelic diversity, and has characterized pathways that may help to re-group individuals into 'mechanisms-based' groups. Although the link between phenotype and genotype has become easier to identify, clinicians and patients generally start from clinical signs and symptoms to get to a fundamental diagnosis, following which diagnostic gene panel testing should occur. However, because the genetic basis of hEDS is still unknown, the diagnosis of this type rests on clinical findings alone, as delineated in the revised criteria for hEDS[7,18] (BOX 3).

Most EDS types are pleiotropic conditions (that is, they affect many tissues and systems throughout the body), so initial screening should assess all systems (FIG. 4). In addition, the family history will often assist in diagnosis and provide clues to complications to be expected in the affected individuals. Differential diagnosis includes (depending on the signs and symptoms) cutis laxa syndromes, Marfan syndrome, Loeys–Dietz syndrome and other heritable thoracic aortic aneurysm syndromes, osteogenesis imperfecta, Stickler syndrome, Larsen syndrome and other skeletal dysplasias, and Bethlem myopathy. Useful investigations to rule out these differential diagnoses at first examination may include echocardiography, ophthalmological examination with slit-lamp for anterior chamber and lens anomalies, audiometry, skeletal X-ray, standard bone densitometry, and baseline bone metabolism serum and urine analyses. A case-to-case decision whether and which investigations are relevant should be made based on clinical findings and family history.

Assessment of skin hyperextensibility, skin texture and scarring. The characteristics of skin that are suggestive of EDS include hyperextensibility, doughy, velvety and/or unusually soft texture and translucency. Skin hyperextensibility can be assessed by pinching and lifting the cutaneous and subcutaneous layers of the skin with the tip of the thumb and the index finger in specific regions (such as the volar surface at the middle of the non-dominant

Box 3 | **Revised diagnostic criteria for hEDS**

Criterion 1

Presence of generalized joint hypermobility

- Beighton score
  - ≥6 for prepubertal children and adolescents
  - ≥5 for pubertal men and women ≤50 years of age
  - ≥4 for men and women >50 years of age
- If the Beighton score is 1 point below the age-specific and sex-specific cut-off and at least two of the following items (five-point questionnaire) are present, then a diagnosis of generalized joint hypermobility can be made
  - Can you now (or could you ever) place your hands flat on the floor without bending your knees?
  - Can you now (or could you ever) bend your thumb to touch your forearm?
  - As a child, did you amuse your friends by contorting your body into strange shapes or could you do the splits?
  - As a child or teenager, did your shoulder or kneecap dislocate on more than one occasion?
  - Do you consider yourself 'double-jointed'?
  - A 'yes' answer to ≥2 questions suggests joint hypermobility with 80–85% sensitivity and 80–90% specificity[229]

Criterion 2

At least two of the following features must be present

- At least five of the following systemic manifestations of a more generalized connective tissue disorder
  - Unusually soft or velvety skin
  - Mild skin hyperextensibility
  - Unexplained striae, for example, striae distensiae or striae rubrae at the back, groins, thighs, breasts, abdomen in adolescents, men and prepubertal women without history of significant gain or loss of body fat
  - Bilateral piezogenic papules of the heel
  - Recurrent or multiple abdominal hernias
  - Atrophic scarring involving at least two sites
  - Pelvic floor, rectal and/or uterine prolapse in children, men or nulliparous women without history of morbid obesity or other predisposing conditions
  - Dental crowding and high or narrow palate
  - Arachnodactyly, as defined in one or both of the following: 1) positive wrist sign (Steinberg sign) on both sides; 2) positive thumb sign (Walker sign) on both sides
  - Arm span to height ratio ≥1.05
  - Mitral valve prolapse[a]
  - Aortic root dilatation with z score >+2 (important note: the presence of aortic root dilatation should always prompt the exclusion of familial thoracic aortic aneurysm disorders, for example, Marfan syndrome and Loeys–Dietz syndrome)
- Positive family history of hypermobile Ehlers–Danlos syndrome (hEDS) with at least one first-degree relative independently meeting hEDS criteria
- At least one of the following musculoskeletal manifestations
  - Musculoskeletal pain in two or more limbs, recurring daily for at least 3 months
  - Chronic widespread pain for at least 3 months
  - Recurrent joint dislocations[b] or frank joint instability in the absence of trauma: three or more atraumatic dislocations at the same joint, or two or more atraumatic dislocations at different joints, at different times, or medical confirmation of joint instability at two or more sites not related to trauma

Criterion 3

Exclusion of other conditions

- Other EDS types
- Other heritable/acquired connective tissue disorders
- Alternative diagnoses

Criteria according to the 2017 international EDS classification. [a]Some studies show no increase in the frequency of clinically significant mitral valve prolaspe[230–232], others show an mitral valve prolapse frequency of 28–67% among hEDS patients[233,234]. This feature is included in the diagnostic criteria because it can be a marker of connective tissue laxity but is usually not clinically significant in patients with hEDS. [b]Dislocation is defined as displacement of a bone out of the joint socket (or out of normal position in the case of sesamoid bones such as the patella) sufficiently severe to limit motion of the joint and requiring manual reduction. Data from REF.[7].

forearm and the dorsum of hands and feet). Of note, sites that are naturally prone to skin stretching should be avoided (such as the elbows and knees). Skin is usually considered hyperextensible if it can be stretched >1.5 cm at the distal part of the forearms and the dorsum of the hand, and 3 cm for neck, elbows and knees[7]; however, these measures have not been validated or standardized. Skin is usually not hyperextensible or significantly doughy, velvety or soft in individuals with vEDS, but it can be thin and translucent with visible superficial veins, particularly on the trunk, arms and legs. Widened and atrophic scars can be observed in several EDS types, such as cEDS, aEDS, dEDS, cvEDS, kEDS, spEDS, mcEDS and clEDS2. Especially in cEDS, atrophic scarring may be widespread with marked widening of the scars, which are covered by a very thin and inelastic skin (that is, papyraceous scars) (FIG. 5).

Assessment of joint mobility and musculoskeletal system. As previously mentioned, one of the key manifestations of EDS is joint hypermobility. Joint mobility is a continuous trait in the general population, and can be modified by age, sex, ethnicity and environmental factors such as exercise[19]. Of note, based on how it is currently measured, joint mobility does not have a Gaussian distribution but is skewed to the low end of the range[170]. The reported prevalence of generalized joint hypermobility in the general population is 6–57% in females and 2–35% in males[171]. An easy-to-use scoring system to measure joint mobility (known as the Beighton score; FIG. 6) is currently the most widely used approach to assess the presence of generalized joint hypermobility[172], but it has major limitations. These limitations are corrections for factors that can modify joint mobility have not been developed; only a limited number of joints are assessed and may not include common problem areas; and standard cut-offs for normal values have not been clearly assigned. Given these concerns, better approaches to measure joint mobility in the clinical setting that incorporate standards that define 'normal' ranges in an extended set of joints is needed both for clinical use and research settings.

Using the Beighton score, hypermobility is generally defined by a score ≥6 for prepubertal children and adolescents, by a score ≥5 for pubertal men and women ≤50 years of age, and by a score ≥4 for men and women >50 years of age[7]. The Beighton score measures a small set of joints, which raises concerns; new measures would do well to consider a more extended set and to address the issue of how different combination of joint hypermobility could help define subsets of people prone to different complications.

Other skeletal features, such as congenital bilateral hip dislocation, spine deformities (scoliosis or kyphosis), pectus deformities (pectus carinatum or pectus excavatum), club feet, distal or proximal contractures, and deformities of the elbows, hands, knees and feet help to classify EDS or to identify other genetic conditions. Joint laxity and muscular hypotonia may cause floppy infant syndrome and/or delayed motor development and point to a limited group of EDS types, such as aEDS, kEDS or mEDS.

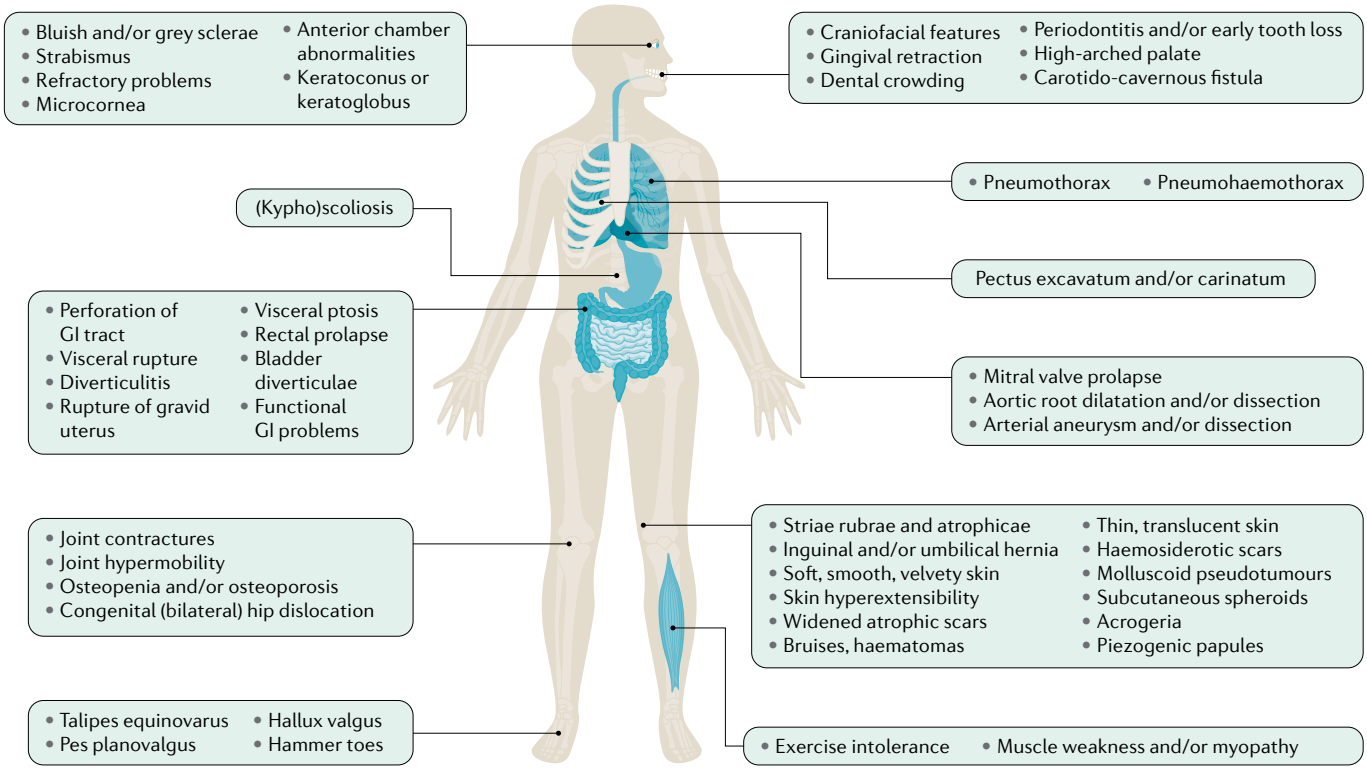


Fig. 4 | **Clinical presentations of Ehlers–Danlos syndromes.** The clinical presentation of Ehlers–Danlos syndromes (EDS) is variable between EDS types and between patients with the same type, and can encompass dysfunction of virtually any organ or tissue. Broadly speaking, EDS can affect the integumentary, musculoskeletal, cardiovascular, respiratory, gastrointestinal, genitourinary, craniofacial and ophthalmologic systems, and, rarely, the auditory system. Common and type-specific signs and symptoms are reflected in major and minor clinical criteria as outlined in TABLE 1. GI, gastrointestinal.

Molecular diagnosis. Genetic studies to identify causative variants in the candidate gene and to confirm or establish the diagnosis should be performed in all individuals who fulfil clinical criteria for an EDS diagnosis or have sufficient findings to warrant concern (as defined in the extended 2017 classification). Even among those who fulfil hEDS criteria there may still be concern about other types, particularly vEDS. As there is substantial allelic diversity among all forms of EDS, the sequence analysis provides a key to genotype–phenotype correlation, improved management of risk of complications (such as surveillance and treatment), identification of other affected family members, presymptomatic diagnosis, and represents the transformation in care from strictly clinical assessment to gene-based diagnosis and personalized medicine. Of note, the genetic diagnostic approaches are becoming well developed in technologically adapted medical systems, and costs for genetic testing have markedly decreased over the past few years. In other settings, such as low-income or middle-income countries, diagnosis is based on clinical assessment, and molecular confirmation is limited to a few individuals who have access to genetic testing.

The pathway to genetic diagnosis depends on several factors. If an EDS causative variant has been previously identified within the family, targeted analysis is appropriate. Most diagnostic studies are carried out using highly parallel sequence analysis (next-generation sequencing), in which a panel of known genes is sequenced and analysed simultaneously. Multi-gene next-generation sequencing panels that include the 20 EDS-related genes, and genes associated with the other overlapping connective tissue disorders, are the preferred diagnostic approach in those with complex phenotypes or individuals with no family history of EDS, as these panels are more time-effective and cost-effective and can identify large and small genomic deletions in the covered regions. DNA sequencing of *TNXB* is complicated by the presence of a pseudogene, *TNXA*, which is >97% identical to the 3′ end (exons 32–44) of *TNXB*, but effective strategies have been identified[7]. If no causative variant in any of the 20 EDS-related genes is identified, RNA sequencing, whole-exome sequencing and/or whole-genome sequencing can be considered to extend the range of candidates.

If variants of uncertain significance are identified, additional studies may help to interpret the pathogenicity of the variant. These include studies to determine whether variants segregate with the phenotype in the family, and ultrastructural, biochemical and/or functional protein assays. Gel electrophoretic analysis of collagen types I and III, produced by cultured fibroblasts from skin biopsies, can support DNA analysis in interpreting the consequence of specific variants, such as those that affect splicing. In addition, data using an immunoassay have revealed reductions in serum TNX in individuals with clEDS with biallelic pathogenetic *TNXB* variants[104]; however, this assay is not generally offered

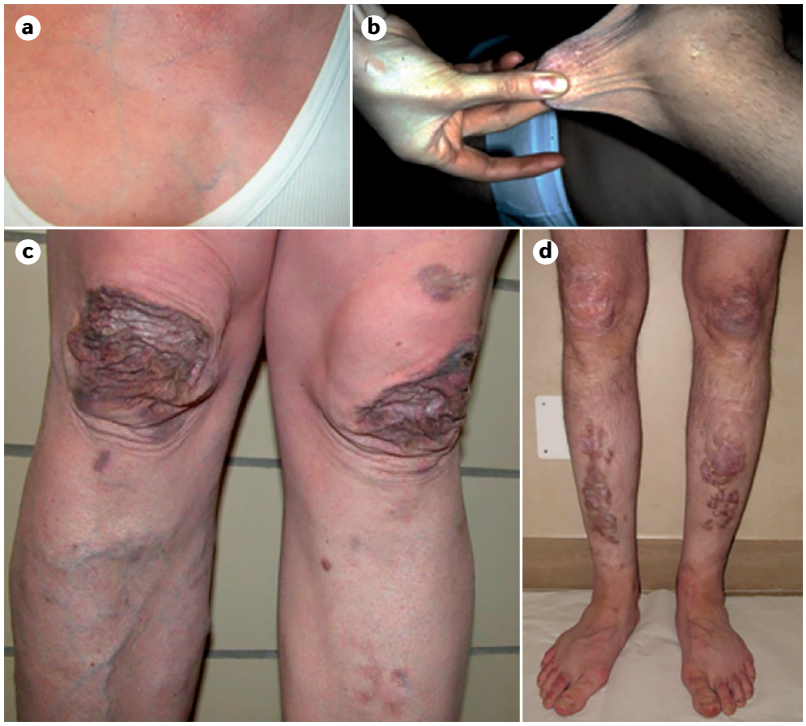


Fig. 5 | **Clinical skin features associated with Ehlers–Danlos syndromes. a** | Thin, translucent skin. **b** | Skin hyperextensibility. **c** | Widened atrophic scarring. **d** | Haemosiderotic scarring. Panels **a** and **c** adapted with permission from REF.[226], Oxford University Press.

as a clinical test owing to few laboratories having the required capability. Other useful studies include the quantification of deoxypyridinoline (DPyr) and pyridinoline (Pyr) crosslinks in urine using high-performance liquid chromatography to identify defects in lysyl hydroxylation due to *PLOD1* variants[173,174] and is also an efficient and cost-effective first diagnostic step towards the diagnosis of kEDS-*PLOD1* (REF.[7]). Milder increases in the DPyr to Pyr ratio (~1) are also observed in individuals who harbour pathogenetic *SLC39A13* variants[138]. However, in general, biochemical studies are more expensive and less informative than genetic testing and should not be performed as a first diagnostic step, but only to study pathogenicity of variants of uncertain significance. Ultrastructural analysis of the dermal ECM may show patterns of abnormal collagen fibrillogenesis in some EDS types (for example, a hieroglyphic pattern in dEDS and collagen flowers in cEDS); however, they are usually not specific and do not confirm a diagnosis.

Carrier screening and family planning

The identification of the EDS causative variant by DNA testing confirms the inheritance pattern of the disorder, which can allow family studies and reproductive genetic testing. Many EDS types have an autosomal dominant transmission (TABLE 1) with variable expressivity but nearly complete, although sometimes age-dependent, penetrance[12,13,40]. For dominantly inherited disorders, extended family testing allows diagnostic confirmation or exclusion in other relatives. EDS types with an autosomal dominant inheritance pattern are likely caused by de novo mutations if it is absent in both parents of the proband; however, parental germline mosaicism has been reported and, therefore, the recurrence risk to siblings of a proband with a presumably de novo variant is slightly increased (1–5%)[175]. The risk can be modified by studying the parental germ cell DNA if the mosaic parent is the father.

For the rare types of EDS that have an autosomal recessive pattern (TABLE 1), the heterozygous parents are usually healthy. The status of at-risk relatives can be determined by tiered testing. Carrier testing in the healthy partner of a heterozygous relative can confirm the predicted low rate of carrier status and provide confidence about a low risk of occurrence in their offspring. In these circumstances, consanguinity and founder effect should be assessed in the setting of genetic counselling to determine whether partner testing is appropriate. Finally, the identification of the genetic cause of EDS allows prenatal diagnosis as well as preimplantation diagnosis in couples at increased risk (that is, couples with a family member who has an EDS variant with dominant inherence, and couples in which both members carrier an autosomal recessive variant).

Management

EDS cannot be cured, and management is generally EDS-type specific. Diagnosis should lead to integration of the patient into a multidisciplinary care team (comprising the primary physician, geneticist and appropriate medical and surgical and allied health professionals; BOX 4), and a patient advocacy community (if available) that has experience with education, information sharing and social support, as it appears to substantially improve QOL. Evidence-based literature regarding clinical guidelines for EDS is limited, and there is little published research into management strategies and interventions. Accordingly, clinical decision-making is mostly based on clinical experience, and there is no consensus on the best practice for medical surveillance, management and surgical intervention for people with most EDS types[176]. Management strategies primarily rely on prevention and supportive treatment of symptoms and depend on the underlying EDS type and observed clinical manifestations.

Skin and mucosae manifestations

In patients with severe skin fragility (particularly those with cEDS or dEDS), the prevention of soft tissue traumas by the use of protections (such as helmets and protections for shins, knees and elbows) should be considered, especially in children and during sport activities. Education of children with these conditions in self-assessment and basic support after unexpected traumas and introduction of lifestyle habits to avoid soft tissue traumas is encouraged. Deep or severe skin wounds in patients with any type of EDS should be expertly closed via sutures without tension, and stitches should be applied generously in layers and should be left in place for twice as long as usual (or longer, depending on patient experience) to prevent wound opening and stretching of the scar. Tape over the repaired tissue can help prevent stretching of the scar, but needs careful removal to prevent tissue damage. A few anecdotal

observations have suggested improved wound healing and reduced scarring with application of silicone sheets over wounds, although there are no controlled studies to support these observations.

Although propensity to bleeding (such as after surgery) is common in EDS, it is often minor and does not convey a risk of serious complications except for EDS types for which a propensity to arterial ruptures is one of the manifestations (such as vEDS and kEDS) and all individuals with EDS who have a history of major bleeding events. Large subcutaneous haematomas are serious complications in individuals with mcEDS, and typically occur after minor trauma and progress acutely[177]. In these individuals, the off-label use of 1-deamino-8-D-arginine vasopressin (DDAVP) at standard doses can be considered to normalize bleeding time and can also be used before minor surgery[178,179].

Joint hypermobility, instability and musculoskeletal pain

Physiotherapy and rehabilitation therapy are recognized as indispensable components in the management of musculoskeletal alterations, and should be tailored to the functional impairments in each patient. Low-resistance exercises, such as walking, cycling and swimming, to increase muscle tone and strength of the core and the extremities may improve joint stability[180,181]. In addition, strength training is beneficial for joint stabilization and hypotonia[182]. The duration, frequency or repetition of the exercise should be increased over time, although resistance should not increase. Even in this context, measurable progress may only be observed after months or years of exercise[181]. 'Showing off' hypermobility in addition to competitive activities, such as gymnastics, repetitive heavy lifting and other sports that cause important joint stress, should be avoided[181].

If active and passive physical therapy is not sufficient or easily applicable to improve joint stability and reduce the associated complaints in patients with EDS, devices, including orthotic, braces and splints, can be used to provide extra support to unstable joints. In addition, wide-grip writing utensils may reduce strain on hypermobile finger and hand joints[181], and a wheelchair or scooter may help to offload stress on lower extremity joints.

In general, the non-operative treatment of musculoskeletal manifestations of EDS is preferable to surgery. However, surgical procedures, such as specific joint stabilization and nerve decompression, are an option for carefully selected patients, although there is very little supporting evidence. Examples include craniocervical instability and Arnold–Chiari malformation, unstable thumb carpometacarpal joints, recurrent shoulder or patellar dislocations, or compression neuropathies[183]. In these cases, multidisciplinary evaluation and careful planning of the surgical procedure and postsurgical recovery are warranted.

Pain is a major symptom in patients with EDS, particularly in those with hEDS[184,185], although the specific causes and mechanisms underlying pain in EDS are poorly understood. Several factors may contribute to pain development and maintenance. These include nociceptive pain directly related to structural changes in affected joints, muscle and connective tissue, neuropathic pain due to nerve damage, impaired proprioception (that is, perception and awareness of body movements and position) and muscle weakness, and central sensitization[184–186]. In addition, the pain phenotype may be further influenced by the presence of anxiety and depression[185]. Management of chronic

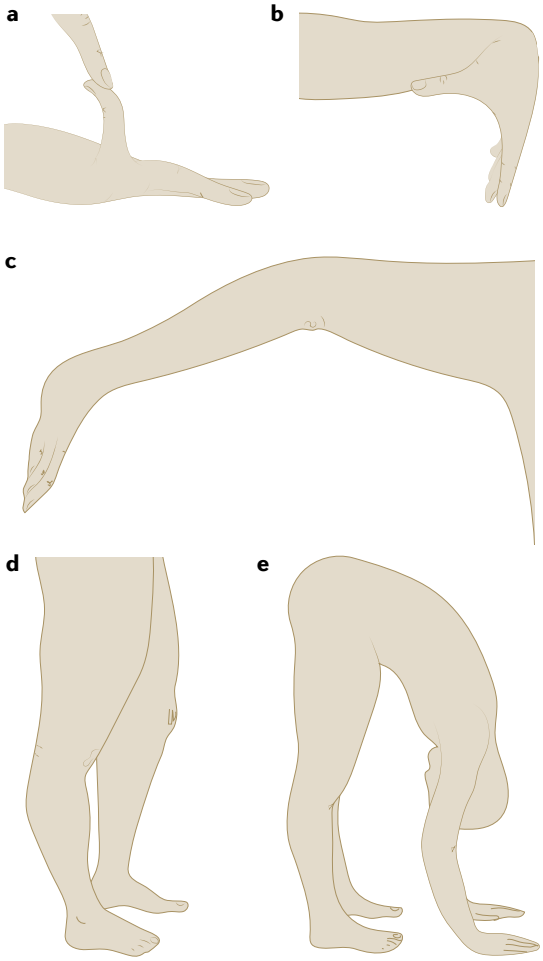


Fig. 6 | **The Beighton scale.** The Beighton scale is used to assess joint hypermobility in clinical practice. Five joints are assessed on each side of the body. The total possible score is 9; joint hypermobility is indicated by a score ≥6 for prepubertal children and adolescents, ≥5 for pubertal men and women ≤50 years of age, and by a score ≥4 for men and women >50 years of age[7]. **a** | Passive dorsiflexion and hyperextension of the fifth metacarpal phalangeal joint >90° with the palm of the hand and forearm resting on a flat surface with the elbow flexed at 90° scores 1 point for each side of the body. **b** | Passive apposition of the thumb to the flexor aspect of the forearm with arms outstretched forward but hand pronated scores 1 point for each side of the body. **c** | Passive hyperextension of the elbow >10° with the arms outstretched to the side of the body and hand supine scores 1 point for each side. **d** | Passive hyperextension of the knee >10° whilst standing upright with the knees locked in genu recurvatum scores 1 point for each side of the body. **e** | Active forward flexion of the trunk with the knees fully extended so that the palms of the hands rest flat on the floor scores 1 point. Image courtesy of B. Juul-Kristensen, University of Southern Denmark, Denmark.

Box 4 | **Special considerations in the care of individuals with vascular Ehlers–Danlos syndrome**

Referral to a centre with expertise and creation of a care team

Given the rarity of vascular Ehlers–Danlos syndrome (vEDS), referral to expertise centres (such as centres of excellence, which have been established in a few European countries) is of vital importance. A clear protocol for emergency room evaluation in the case of major complications (for example, bowel ruptures, arterial dissection or arterial rupture) should be established, and the patient and family members should know the protocol for contact. Individuals with vEDS should carry documentation of their genetic diagnosis, such as a MedicAlert, emergency letter or vEDS 'passport'. An organized care team, responsible for the organization of ordinary and extraordinary care, should be established that includes a primary care physician, cardiologist, vascular surgeon and general surgeon, and a geneticist who can aid in integration of care, genetic counselling and cascade testing (systematic identification and testing of members of the family of the proband). The psychosocial impact of the diagnosis of vEDS often requires psychological care.

Circumstances to avoid

- Trauma: individuals with vEDS are advised to avoid collision sports, such as boxing, ice hockey, American football and soccer, and isometric activities, such as weight training with extreme lifting. Mild to moderate recreational exercise is recommended.
- Arteriography: conventional arterial angiography (with contrast injection) is to be avoided as it is associated with an increased risk for complications, such as arterial tears and dissections at the site of entry of the catheter, and arterial aneurysm formation due to the injection pressure[235].
- Routine colonoscopy: due to increased fragility of the gastrointestinal wall, patients with vEDS have an increased risk for colonoscopy-associated bowel perforation. Virtual colonoscopy may also convey an increased risk of bowel perforation as this procedure also involves insufflation. Use of capsular cameras may provide sufficient data in individuals at increased risk for colon cancer or other gastrointestinal disease.
- Elective surgery: in general, surgical procedures are to be avoided in patients with vEDS in favour of more conservative management strategies, unless the benefit is expected to be profound.
- Anticoagulant or antiplatelet therapy: prescription of anticoagulant or antiplatelet therapy should be discussed on case-by-case basis. As they are associated with a risk of bleeding complications they should be limited to a short period of time. The use of NSAIDs should also be limited and, when necessary, used only on an infrequent basis and for a short period of time.

Treatment of major complications

Surgical intervention can be lifesaving in those with arterial and aortic rupture, gastrointestinal tract perforations, or organ ruptures, such as rupture of the gravid uterus. In general, surgical procedures are more likely to be successful when the involved health-care professionals are informed of the diagnosis of vEDS and its associated vascular and tissue fragility[236]. A targeted approach, with minimal surgical exploration, is recommended when surgery is necessary owing to the risk of unintentional damage to other tissues[188]. In addition, an approach of 'permissive hypotension' may help to prevent the recognized cycle of complications.

pain in patients with EDS is hindered by a lack of evidence-based studies that clearly demonstrate any effectiveness of different modalities. Multi-disciplinary management of chronic pain should be offered after a thorough diagnostic evaluation to identify the best analgesic strategy, which usually includes a combination of pharmacological treatment, physiotherapy and cognitive behavioural therapy[181,184]. As treating chronic pain in individuals with EDS is challenging and an effective strategy is lacking, the outcome of the selected management programmes should be monitored on a regular basis (such as monthly) to optimize symptom relief and minimize adverse effects.

Cardiovascular manifestations

Although no cardiac surveillance guidelines are available for EDS, periodic (with 3–5-year intervals, or more frequently if an abnormality is found) monitoring of the cardiac valves and aortic diameters, preferably using non-invasive procedures (ultrasonography and/or heart MRI), is generally advised. The vast majority of patients with hEDS do not have signs of aortic disease, and follow-up using echocardiography should be limited to patients with hEDS who have a family history of aortic aneurysm and those with abnormal auscultatory examination[187]. A precise cut-off value of the aortic root diameter for elective surgery for aortic aneurysm has not been defined for any type of EDS.

For EDS types that are associated with arterial aneurysm formation or dissection/rupture (such as vEDS and kEDS), no evidence-based guidelines for surveillance or management have been developed. Consequently, surveillance programmes range from routine interim evaluations and directed physical examination, with perhaps some imaging of the aorta, to annual assessment of the arterial tree by head-to-pelvis magnetic resonance angiography or CT angiography[13]. In addition, criteria for the treatment of arterial aneurysms have not been well established. Elective surgical repair is the only available treatment strategy for arterial aneurysms, but the use of endovascular stenting compared with open surgical replacement of arterial segments remains uncertain in people with EDS in terms of efficacy and safety[188,189]. Of note, one multicentre cross-sectional retrospective study of aortic and arterial pathology in individuals with vEDS showed that embolization and stenting of medium-sized arteries, as well as open repair of abdominal aortic aneurysm are well-tolerated procedures[189]. In any case, the best setting for vascular surgery is a planned repair of (dissecting) aneurysms[13].

Effective treatment to reduce the risk of spontaneous arterial rupture in vEDS or other types of EDS is

not available. Only two published studies have assessed the benefit of a pharmacological treatment to prevent arterial rupture in patients with vEDS, one multicentre, international, randomized, open-label clinical trial (the BBEST study)[190], and a French observational cohort study of 144 patients with molecularly confirmed vEDS[191]. Both studies suggested that treatment with celiprolol (a selective β1 receptor antagonist with a β2 receptor partial agonist activity, and weak α2 receptor antagonist) might reduce the frequency of arterial dissection or ruptures in patients with vEDS, and is a safe and well-tolerated drug[190,191]. However, both studies had limitations; in the BBEST trial, sequence analysis identified *COL3A1* pathogenetic variants in only approximately two-thirds of the participants who were not equally represented in the study arms, and the observational study lacked an adequate control group. Thus, these studies concluded that any changes in arterial event rates could not be attributed solely to celiprolol. However, until further evidence is available, it is deemed safe for individuals with vEDS using celiprolol to continue this medication. Whether the suggested beneficial effect of celiprolol in vEDS can be extrapolated to other β-blockers and other blood-pressure-reducing drugs, such as angiotensin-receptor blockers, remains unknown. Since the publication of the BBEST trial, the only ongoing interventional clinical trial in patients with vEDS is the ARCADE trial[192] (NCT02597361), a randomized, double-blind, placebo-controlled multicentre trial comparing the effect of adding the angiotensin II receptor antagonist irbesartan or placebo[108–110] to celiprolol over a 2-year period; enrolment in this study will continue into 2020.

Venous insufficiency is presumably at an increased rate in EDS. Treatment of venous insufficiency in patients with EDS follows standard care guidelines, which include regular exercise, avoidance of prolonged sitting and lying down, and the use of compression stockings or other compression garments. There are no specific recommendations regarding surgery for venous insufficiency in patients with EDS.

Gastrointestinal manifestations

Perforation of the gastrointestinal tract occurs in ~15% of individuals with vEDS. Although no management guidelines for perforation in patients with vEDS have been developed, immediate surgical intervention of bowel rupture is usually essential to limit the extent of infection. The most common site of perforation is the sigmoid colon and the usual treatment has been creation of a colostomy with repair after 6 months. One systematic review suggested that following subtotal colostomy those with vEDS are at high risk for colonic reperforation and a high rate of anastomotic leak[193]; these data represent a review of studies carried out over almost a 30-year period with few complete studies from single institutions. The experience, outcome and recommendations range from complete colectomy at the time of first perforation to re-anastomosis, careful dietary management and a wait-and-see approach, and are highly variable. A French retrospective study of patients with vEDS has suggested that colonic perforations are more severe in males with vEDS and found that there was a high risk of reperforation after a Hartmann procedure (resection of the rectosigmoid colon). They suggested that subtotal colectomy might reduce morbidity, particularly in males[194]. Discussion about alternatives between the family and the clinicians becomes a critical part of the decision-making in this context. Diverticular perforation has also been observed in individuals with mcEDS-*CHST14* (REF.[195]).

Some studies have provided evidence for an increased prevalence of functional gastrointestinal disorders (such as irritable bowel syndrome and functional dyspepsia) and pelvic floor problems (such as functional defaecation disorder, incomplete bowel or urine evacuation) in several types of EDS[196,197]. However, the potential role of dysfunctional connective tissue and its effect on mechanical and motility characteristics of the gastrointestinal tract and pelvic floor remains unknown. Pro-kinetic drugs, such as low-dose erythromycin, metoclopramide or domperidone, may help with gastrointestinal dysmotility. The risk–benefit profile of these drugs needs to be carefully considered, as adverse drug reactions are not rare[198]. Physical therapy for pelvic floor dysfunction is a first-line strategy, and surgical approaches should be undertaken only in those with severe symptoms after failure of physical therapy.

Skeletal involvement

Individuals with EDS can have fractures but a true increase in the risk of fractures seems limited to those with aEDS and spEDS-*B3GALT6*. In addition, some patients present with mixed features of EDS and osteogenesis imperfecta owing to heterozygous variants in *COL1A1* and *COL1A2* (REFS[199,200]), and these patients can present with multiple fractures. In these individuals, pharmacological treatment of reduced bone density should follow available strategies for osteogenesis imperfecta[201]. In particular, oral vitamin D and calcium supplements are indicated in both children and adults with this condition. Bisphosphonate therapy may be considered in some patients, especially children with moderate-to-severe skeletal manifestations. In these cases, oral vitamin D and calcium supplements should also be added to avoid transient hypocalcaemia.

Pregnancy

Before pregnancy, women with EDS should consult an obstetrician and a geneticist, if possible. The associated risks in pregnancy are EDS-type specific and depend on the mode of inheritance. No systematic studies of pregnancies in most EDS types have been carried out, although some studies have reported increased risk of complications in some forms of EDS. Infants with cEDS have an increased risk of premature birth regardless of whether the pathogenetic variant is de novo or inherited from either parent[40]. In addition, pregnancy in women with vEDS is associated with increased risk of death (maximum probably about 5/100 pregnancies), especially due to arterial and less often to uterine rupture[202]. The risk of prematurity in infants affected by vEDS is increased, as is the risk of complications of vaginal delivery including vaginal and cervical tears that may

be debilitating[202]; accordingly, these pregnancies should be managed at a perinatal centre for high-risk pregnancies. Whether delivery via Caesarean section improves outcome in this EDS type is uncertain.

The maternal issues during pregnancy for the other forms of EDS are uncertain, but anecdotal complications have been reported for several EDS types, including internal organ prolapse (for clEDS and mcEDS-*DSE*), premature rupture of the membranes and premature birth (dEDS, kEDS-*PLOD1* and spEDS-*B3GALT6*) and arterial rupture (kEDS-*PLOD1*)[12].

Quality of life

Studies addressing health-related QOL in adults with EDS are scarce and mostly focus on adults with hEDS. In one study, self-reported QOL in 280 Swedish adults with different types of EDS was significantly lower than the general population, and higher levels of anxiety and depression were detected[203]. A number of studies have shown that physical pain, fatigue, psychological discomfort and functional disability may be quite severe and have a serious negative effect on QOL in individuals with hEDS[204–206]. Moreover, the presence and/or severity of functional gastrointestinal disorders and pelvic floor problems have also been shown to influence QOL in patients with EDS[196,207]. In the paediatric population, a few studies in children with JHS or hEDS have showed that pain, fatigue, sleep disorders and functional disability have a considerable effect on QOL[208–211]. In one study[208], QOL reports were strongly correlated between parents of children with JHS and their affected offspring; however, this study was carried out before the 2017 diagnostic criteria for hEDS were established, and another study has not been published since. The effects on parents or caregivers of having a child with EDS have not been reported and are deserving of study. The effect on family life can be profound, based on clinical observation.

One study[205] suggested that there are several important elements related to physical therapy that promoted a higher QOL in patients with EDS. These included early initiation of physiotherapy, patient-centred care and a holistic approach by a therapist who is knowledgeable about the management of EDS and joint hypermobility. Support for patients and their families through patient organizations, such as the international Ehlers–Danlos Society and local patient organizations, can be extremely helpful in reducing the sense of isolation that often comes with coping with a rare disorder. In addition, these organizations provide practical advice for patients and their families, such as resources for navigating the complex medical system, information on obtaining assistance with school and workplace issues and access to providers knowledgeable in the management of EDS.

Outlook

Pathogenetic mechanisms

For some of the EDS types the scope of both genetic and allelic heterogeneity is well appreciated and phenotype–genotype correlations are beginning to emerge. Nonetheless, the pathways from a single (or more) nucleotide change to the phenotype remain elusive. However, it is clear that the small genetic alterations lead to a series of cellular and signalling responses that vary depending on the genetic status of individuals and very likely also on environmental influences, explaining individual variation in outcomes even for the same pathogenetic variant. To truly appreciate how to influence the broad effect of single nucleotide changes, strategies are needed to assess these pathways and the nodes for modification. These include the establishment of in vitro models and carrying out transcriptome and proteome studies, among other approaches (see, for example, the preliminary transcriptomics studies in cEDS and vEDS[46,61]). Even in individuals with suspected EDS and the absence of a known genetic defect, identifying altered transcripts from in vitro transcriptomics may highlight key pathways that have a role in the disorder and may help to identify the underlying genetic defects, which will hopefully be seen soon from whole-genome sequencing projects involving patients without a genetic diagnosis. The first development in this direction is represented by transcriptomics analysis of hEDS[212]. In addition to transcriptomic studies, the assessment of changes to signalling owing to aberrant production or processing of proteins implicated in EDS will also be important. Moreover, although several cellular and animal models that mirror the genetic defects and pathophysiological mechanisms of patients with different EDS types are available, the use of animal models to study EDS is still in its early stages, and creation of additional models (both in mice and in additional model organisms) that reflect different genetic defects and different types of genetic variants are needed. These models can yield new insights into disease mechanisms, identify biomarkers and clinically targetable signalling pathways or cellular processes for the development of personalized therapies.

Clinical and molecular diagnosis

In the medical community, 'EDS' is generally considered to refer to a group of people with joint hypermobility, often generalized pain, and some associated common complaints such as orthostatic instability. As the genetic basis of this condition (or more likely 'these conditions') is still unknown, and, therefore, there are no specific genetic tests to allow for diagnosis, frustration is high in both the medical community and among patients looking for definitive diagnosis and appropriate treatment. This focus also means that the medical community is less familiar with the other types of EDS and accurate and timely diagnosis for these types remains a challenge. This lack of awareness can lead to diagnostic odysseys associated with far-reaching financial and emotional consequences and to diagnostic delays which, in the case of vEDS, can be life-threatening. Collaborative networks such as the International EDS Consortium and the European Reference Networks for Rare Diseases (see Related links), in conjunction with patient advocacy organizations such as the Ehlers–Danlos Society and national and local patient organizations, are now investing in the development of educational programmes for clinicians and educators to increase awareness and knowledge about these conditions and develop clinical and diagnostic pathways.

For individuals with the genetically defined types of EDS, genetic testing is readily available in many countries. The challenge remains to provide similar pathways for hEDS. Despite multiple attempts, no definitive molecular explanation has been found for most people with this disorder. Several factors, including lack of clarity on inclusion criteria for the diagnosis, locus heterogeneity (clinically identical disorders caused by different genes), and improper application of the diagnosis in the current clinical climate, contribute to this apparent failure. Large-scale international studies of phenotypic traits, combined with whole-exome and whole-genome genetic projects, are under way to identify the genetic aetiologies of hEDS and genotype–phenotype relationships.

Natural history data are not well developed for most types of EDS. As a consequence, the available data are generally insufficient to counsel patients regarding the prognosis of their disease. International patient registries that couple longitudinal phenotypic follow-up information with genetic data are currently in development for many EDS types, and clinical studies that address prevalence and patterns of features that affect QOL among the different EDS types are also under way.

Classification

Classification is a dynamic process, usually for two reasons: it functions for clinical definition of conditions when clinical assessment is the only tool, and it creates cohorts of individuals for genetic testing to identify the bases of the conditions. The dynamic nature of classification of EDS is apparent by comparing the studies from 1967 (REF.[213]), 1970 (REF.[214]), 1988 (REF.[5]), 1997 (REF.[6]) and 2017 (REF.[7]). These schemes provide ways to identify the minimal criteria for diagnosis to be included in this condition, herald the genetic variation, and lead to the identification of new conditions that emerge as a consequence of sophisticated testing, such as genetic sequence analysis. It would not be surprising, for example, if in a subsequent iteration of the EDS classification the conditions characterized by defects in production of the GAG linker regions might become 'linkeropathies' rather than types of EDS, to reflect both the underlying defects and clinical findings well beyond those seen as a minimum in EDS.

Surveillance and management

Evidence-based recommendations for the treatment of EDS are needed to optimize medical care and improve health status. The International EDS Consortium together with the European Reference Networks for Rare Diseases are working to develop such recommendations. In addition, studies to identify clinically reliable biomarkers that could help clinicians in the early identification of disease progression (for example, development of chronic pain), or anticipation of life-threatening complications (such as arterial dissection), are needed. One study identified changes in pro-inflammatory biomarkers and leptin levels in a cohort of vEDS patients, representing the first evidence for a pre-inflammatory state in EDS[215]. This finding might be a first step in the identification of biomarkers for vEDS. Development or application of improved imaging techniques that can assess arterial wall function would also be welcome.

New therapeutic strategies

Over the past few decades, insights into the mechanisms of other heritable connective tissue disorders helped pave the way towards new therapeutic approaches for EDS. For example, excessive TGFβ signalling has been well documented in Marfan syndrome and other related aortic aneurysm syndromes and is thought to contribute to aortic aneurysm progression and dissection[216]. On the basis of these insights, several clinical trials using traditional pharmacological agents such as the angiotensin II type 1 receptor-blocker losartan (which can attenuate TGFβ signalling) have been performed; however, these studies could not unequivocally confirm the benefit of this treatment in patients with Marfan syndrome despite dramatic effects in mouse models[217]. Research on the pathogenesis of EDS is expected to be faced with the same opportunities and challenges but might open doors towards targeted therapies. For example, preclinical investigation into vEDS has been severely hampered by the lack of animal models that recapitulate the vascular pathology or molecular mechanisms of the condition. However, several vEDS mouse models have now been identified, and studies are beginning to emerge that provide experimental justification for clinical evaluation of targeted therapies. For example, *Col3a1* haploinsufficient mice have increased matrix metallopeptidase 9 (MMP9) levels, and chronic treatment with the MMP inhibitor doxycycline normalized MMP9 activity and aortic collagen content and prevented the development of spontaneous aortic lesions[218,219]. In another vEDS mouse model, harbouring an in-frame *Col3a1* deletion of exons 33–39, both celiprolol and doxycycline, but not losartan, improved the biomechanical integrity of the aortic wall[220]. Two knock-in mouse models, each harbouring a unique glycine substitution in the triple helical domain of type III collagen, have been shown to faithfully mimic human vEDS, with mice dying prematurely from spontaneous aortic rupture[221]. Transcriptome analyses on the descending thoracic aorta revealed abnormalities in the PLC–IP_3–PKC–ERK pathway, and treatment of the mutant mice with inhibitors of ERK1/2 or PKCβ prevented death due to spontaneous aortic rupture[221]. Additional studies will be needed to fully understand the crosstalk between these treatments and the specific underlying defects, and the events by which they influence collagen content in the connective tissues and eventually the biomechanical integrity of the arterial wall.

For dominantly inherited disorders, targeted loss of expression of the deleterious allele in disorders where haploinsufficiency is a much milder condition (for example, vEDS) has some promise. Allele-specific RNA interference targeted to the defective allele in fibroblasts from an individual with vEDS reduced expression of the disease-causing allele and was accompanied by the reduction of the unfolded protein response of the ER, and restoration of collagen fibril formation[222]. This concept could be developed into a promising approach towards personalized treatment strategies. The biggest challenge for genetic approaches, including cell-based replacement, is the need for ubiquitous targeting in an efficient manner that avoids off-target effects. Enzyme replacement for proteins that act in the ECM might be

considered in dEDS, although the cost of development may be high compared with the target number.

Challenges of designing clinical trials

Major hurdles to the development of pharmacological and non-pharmacological treatment strategies, such as orthopaedic and vascular surgery, physiotherapy, pain management and prevention of vascular rupture, are patient recruitment and a clinical trial design that can definitively determine whether a therapy is effective. The EDS types are individually rare, their phenotypes are variable, and the natural history is not well documented, so trials are by definition small and often underpowered. Recruiting homogeneous patient populations is difficult, and robust outcome measures are often lacking. Efforts to improve these clinical trials are being put in place and include creation of patient registries with clinical and molecular data, stimulating international collaboration to recruit larger patient cohorts and improving trial design where possible.

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