The 2017 International Classification of the Ehlers–Danlos Syndromes

FRANSISKA MALFAIT,* CLAIR FRANCOMANO, PETER BYERS, JOHN BELMONT, BRITTA BERGLUND, JAMES BLACK, LARA BLOOM, JESSICA M. BOWEN, ANGELA F. BRADY, NIGEL P. BURROWS, MARCO CASTORI, HELEN COHEN, MARINA COLOMBI, SERWET DEMIRDAS, JULIE DE BACKER, ANNE DE PAEPE, SYLVIE FOURNEL-GIGLEUX, MICHAEL FRANK, NEETI GHALI, CECILIA GIUNTA, RODNEY GRAHAME, ALAN HAKIM, XAVIER JEUNEMAITRE, DIANA JOHNSON, BIRGIT JUUL-KRISTENSEN, INES KAPFERER-SEEBAKER, HANADI KAZKAZ, TOMOKI KOSHO, MARK E. LAVALLEE, HOWARD LEVY, ROBERTO MENDOZA-LONDONO, MELANIE PEPIN, F. MICHAEL POPE, EYAL REINSTEIN, LEEMA ROBERT, MARIANNE ROHRBACH, LYNN SANDERS, GLENDRA J. SOBEY, TIM VAN DAMME, ANTHONY VANDERSTEEN, CAROLINE VAN MOURIK, NICOL VOERMANS, NIGEL WHEELDON, JOHANNES ZSCHOCKE, AND BRAD TINKLE

The Ehlers–Danlos syndromes (EDS) are a clinically and genetically heterogeneous group of heritable connective tissue disorders (HCTDs) characterized by joint hypermobility, skin hyperextensibility, and tissue fragility. Over the past two decades, the Villefranche Nosology, which delineated six subtypes, has been widely used as the standard for clinical diagnosis of EDS. For most of these subtypes, mutations had been identified in collagen-encoding genes, or in genes encoding collagen-modifying enzymes. Since its publication in 1998, a whole spectrum of novel EDS subtypes has been described, and mutations have been identified in an array of novel genes. The International EDS Consortium proposes a revised EDS classification, which recognizes 13 subtypes. For each of the subtypes, we propose a set of clinical criteria that are suggestive for the diagnosis. However, in view of the vast genetic heterogeneity and phenotypic variability of the EDS subtypes, and the clinical overlap between EDS subtypes, but also with other HCTDs, the definite diagnosis of all EDS subtypes, except for the hypermobile type, relies on molecular confirmation with identification of (a) causative genetic variant(s). We also revised the clinical criteria for hypermobile EDS in order to allow for a better distinction from other joint hypermobility disorders. To satisfy research needs, we also propose a pathogenetic scheme, that regroups EDS subtypes for which the causative proteins function within the same pathway. We hope that the revised International EDS Classification will serve as a new standard for the diagnosis of EDS and will provide a framework for future research purposes. © 2017 Wiley Periodicals, Inc.

KEY WORDS: classification; Ehlers–Danlos syndromes; genetic basis; collagen

INTRODUCTION

The Ehlers–Danlos syndromes (EDS) are a heterogeneous group of heritable connective tissue disorders (HCTDs) characterized by joint hypermobility, skin hyperextensibility, and tissue fragility. The clinical and genetic heterogeneity of this condition has long been recognized. The 1988 “Berlin Nosology” recognized 11 subtypes, defined by Roman numerals, based on
clinical findings and mode of inheritance [Beighton et al., 1988]. The subjective interpretation of several semi-quantitative clinical signs, such as joint hypermobility, skin hyperextensibility, tissue fragility and bruising, however, led to clinical uncertainty, diagnostic confusion regarding the type of EDS and the inclusion of phenotypically similar conditions under the broad diagnosis of EDS. With the elucidation of the biochemical and molecular basis of many of these EDS types, a revised classification, the “Villefranche Nosology,” was published in 1998 [Beighton et al., 1998]. This classification delineated six subtypes, for which major and minor clinical criteria were defined, and which included the biochemical and molecular basis, when known. The Roman numerals were substituted by a descriptive name, which captured the characteristic manifestations of each type. One underlying assumption was that most, if not all, of these types of EDS were a consequence of alterations in fibrillar collagen genes or in genes that encoded collagen modifiers.

**With the elucidation of the biochemical and molecular basis of many of these EDS types, a revised classification, the “Villefranche Nosology,” was published in 1998. This classification delineated six subtypes, for which major and minor clinical criteria were defined, and which included the biochemical and molecular basis, when known.**

Over the past two decades the Villefranche Nosology has served its purpose and has been widely used as the standard for the clinical diagnosis of EDS, and for clinical research on various aspects of these conditions. However, since its publication, a whole spectrum of novel EDS subtypes has been described, and with the advent of next-generation sequencing (NGS) facilities, mutations have been identified in an array of new genes, that are not always, at first sight, involved in collagen biosynthesis and/or structure. As such, the Villefranche classification is showing its age. Furthermore, in the persistent lack of a genetic defect, there is a dire need for a better clinical definition of the hypermobile type of EDS and its delineation from other hypermobility disorders. Therefore, we undertook a comprehensive review of the EDS-related literature, and, based on our findings, revised the EDS Classification.

**The 2017 International Classification for the Ehlers–Danlos Syndromes**

The new classification recognizes 13 subtypes, as outlined in Table I. After careful discussions whether to maintain a clinically orientated classification versus a genetic classification, we propose to maintain a clinical classification, in which the previously established descriptive names are maintained, since they are generally accepted and widely used in the medical, scientific and patient community. For newly defined EDS phenotypes, we propose a novel descriptor that captures the characteristic manifestations of the phenotype.

We included all phenotypes that present the basic clinical hallmarks of EDS, that is joint hypermobility, skin hyperextensibility and tissue fragility. In particular, such features should distinguish the redefined hypermobile type (hypermobile EDS, hEDS) from other joint hypermobility disorders (See also “A framework for the classification of Joint Hypermobility and Related Conditions” by Castori et al., this issue). Some of the phenotypes clinically overlap with other HCTDs, such as “myopathic EDS,” which is caused by heterozygous or biallelic mutations in COL12A1 (mEDS) and which clinically overlaps with Bethlem Myopathy, and “spondylo dysplastic EDS” caused by biallelic B3GALT6 mutations (spEDS-B3GALT6), which clinically overlaps with spondylo-epimetaphyseal dysplasia with joint laxity type 1 (SEMD-JL1).

Since several patients with these conditions are clinically suspected to have a form of EDS, we believe that inclusion in the EDS classification is justified. This is also the case for Brittle Cornea Syndrome. We currently did not retain the filaminaA-related periventricular nodular heterotopia (PVNH) with EDS-features within the classification, as the majority of patients primarily present with a neurological phenotype. A minority of patients has varying features of a HCTD, which may include life-threatening aneurysms, however, there is insufficient published data to reliably differentiate and prognosticate PVNH from PVNH-EDS. We recommend that in- or exclusion of these conditions in the EDS classification is reviewed in future years, when more information becomes available.

In line with the 1997 Villefranche Nosology, we propose a set of major and minor clinical criteria for each EDS subtype. A major criterion has high diagnostic specificity because it is present in the vast majority of the affected individuals and/or it is characteristic for the disorder and allows differentiation from other EDS subtypes and/or other HCTDs. A minor criterion is a sign of lesser diagnostic specificity, but its presence supports the diagnosis. For each of the subtypes, we defined minimal major ± minor clinical criteria that are suggestive for the diagnosis of a specific subtype. However, in view of the vast genetic heterogeneity and phenotypic variability of the EDS subtypes, and the clinical overlap between many of these subtypes, but also with other HCTDs, the definite diagnosis relies for all subtypes, except hEDS, on molecular confirmation with identification of (a) causative variant(s) in the respective gene. A molecular diagnosis is extremely important for counseling.
purposes, as it allows confirmation of the precise diagnosis and gives information on inheritance pattern, recurrence risk and prognosis, and it may guide management. Moreover, it allows for the formation of homogeneous cohorts for research purposes, and future therapeutic interventions. Since the genetic basis of hEDS is still unknown, the diagnosis of this subtype rests on clinical findings, as delineated in the revised criteria for hEDS.

In view of the vast genetic heterogeneity and phenotypic variability of the EDS subtypes, and the clinical overlap between many of these subtypes, but also with other HCTDs, the definite diagnosis relies for all subtypes, except hEDS, on molecular confirmation with identification of (a) causative variant(s) in the respective gene.

Molecular diagnostic strategies should rely on NGS technologies, which offer the potential for parallel sequencing of multiple genes. Targeted resequencing of a panel of genes, for example, COL5A1, COL5A2,
COL1A1 and COL1A2, is a time- and cost-effective approach for the molecular diagnosis of the genetically heterogeneous EDS. When no mutation (or in case of an autosomal recessive condition only one mutation) is identified, this approach should be complemented with a copy number variant (CNV) detection strategy to identify large deletions or duplications, for example Multiplex Ligation-dependent Probe Amplification (MLPA), qPCR, or targeted array analysis. Alternatively, or in a second phase, whole exome sequencing (WES) or whole genome sequencing (WGS) and RNA sequencing techniques can be used, with data-analysis initially focusing on the genes of interest for a given EDS subtype. In absence of the identification of a causal mutation, this approach allows to expand the analysis to other genes within the genome. This is particularly interesting in view of the clinical overlap between EDS subtypes and with other HCTDs, and the observation that in an important proportion of EDS-patients, no pathogenic variants are identified in any of the known EDS-associated genes. The interpretation of variants of uncertain significance (VUS), especially missense variants, should include correlation with the complete clinical phenotype. In keeping with the ACMG guidelines, variants that are supported by some evidence of pathogenicity (e.g., high in silico scores, presence in a functionally active domain) can be considered “likely pathogenic.” Familial segregation studies may help to interpret the pathogenicity of the variant, and for some genes, ultrastructural, biochemical and/or functional protein assays are available, as outlined below. Individuals harboring such a “likely pathogenic” variant should be followed clinically. Initial counseling for such patients should point out that the true significance of the variant will not be known until these additional tests are completed. In the longer term, assignment of pathogenicity is likely to be facilitated by data from large-scale genome-sequencing projects in patient and control cohorts [Weerakkody et al., 2016].

For patients who fulfill the set of minimal clinical requirements for a specific EDS subtype, but (i) who have no access to molecular confirmation; (ii) in whom one or more VUS is/are identified in one the EDS subtype-specific genes; or (iii) in whom no causative variants are identified in any of the EDS-subtype-specific genes, a provisional clinical diagnosis” of an EDS subtype can be made, and patients should be followed clinically. However, alternative diagnoses and hence expanded molecular testing should be considered.

PATHOGENETIC MECHANISMS UNDERLYING THE EHLERS–DANLOS SYNDROMES

While the proposed clinically oriented classification aims to be user-friendly for the EDS non-specialist, and offers the affected patients and their family members a “descriptive” diagnosis that he or she can identify with, a genetic classification provides a better framework for research purposes and for the development of future treatment strategies. To satisfy both clinical and research needs, we propose, in addition to the clinical classification, a pathogenetic scheme, that regroups EDS subtypes for which the proteins, coded by the causative genes, function within the same pathway, and which are likely to have shared pathogenic mechanisms, based on current knowledge (Table II). A similar regrouping of osteogenesis imperfecta (OI) subtypes by gene function was proposed and is widely adapted in clinical and research settings.

CLASSIFICATION OF EDS

Classical EDS (cEDS)

- Inheritance
  - Autosomal dominant
  - Major criteria
    1. Skin hyperextensibility
    2. Generalized joint hypermobility
    3. Skin fragility (or traumatic splitting)
    4. Molluscoid pseudotumors
    5. Subcutaneous spheroids
    6. Hernia (or history thereof)
    7. Epicanthal folds
    8. Complications of joint hypermobility (e.g., sprains, luxation/subluxation, pain, flexible flatfoot)
    9. Family history of a first degree relative who meets clinical criteria

Skin extensibility should be measured by pinching and lifting the cutaneous and subcutaneous layers of the skin on the volar surface at the middle of the non-dominant forearm as described in Remvig et al. [2009]. Skin is hyperextensible if it can be stretched over a standardized cut-off in three of the following areas: 1.5 cm for the distal part of the forearms and the dorsum of the hands; 3 cm for neck, elbow, and knees.

1Skin extensibility should be measured by pinching and lifting the cutaneous and subcutaneous layers of the skin on the volar surface at the middle of the non-dominant forearm as described in Remvig et al. [2009]. Skin is hyperextensible if it can be stretched over a standardized cut-off in three of the following areas: 1.5 cm for the distal part of the forearms and the dorsum of the hands; 3 cm for neck, elbow, and knees.

2Abnormal scarring can range in severity. Most patients have extensive atrophic scars at a number of sites (Fig. 1). These can sometimes be haemosiderotic. A minority of patients are more mildly affected.

3GJH is evaluated according to the Beighton score; a Beighton score of ≥5 is considered positive for the presence of GJH (Fig. 2). Since laxity decreases with age, patients with a Beighton score ≤5/9 may be considered positive based on their historical observations (see “five-point questionnaire (5PQ)” (Table III).

4Easy bruising can occur anywhere on the body, including unusual sites. The pretilial area often remains stained with hemosiderin from previous bruises.

5Subjective abnormality of the skin texture is appreciable by touching the skin.

6Molluscoid pseudotumors are fleshy lesions associated with scars, found over pressure points (e.g., elbow, fingers).

7Subcutaneous spheroids (Fig. 1F) are small spherical hard bodies, frequently mobile, and palpable on the forearms and shins. Spheroids may be calcified and detectable radiologically.

8Epicanthal folds are often seen in childhood but may also be seen in adults.
TABLE II. Regrouping of the Ehlers-Danlos Syndromes According to Underlying Genetic and Pathogenetic Mechanisms

<table>
<thead>
<tr>
<th>Group</th>
<th>Former nomenclature and other names</th>
<th>Villefranche nomenclature</th>
<th>New Nomenclature</th>
</tr>
</thead>
<tbody>
<tr>
<td>GROUP A: Disorders of collagen primary structure and collagen processing</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Classical EDS (cEDS)</td>
<td>Gravis/EDS I</td>
<td>Classical EDS (cEDS)</td>
<td>130000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>COL5A1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mitis/EDS II</td>
<td>Type V collagen</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>130010</td>
<td>2q32.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>COL5A2</td>
<td>120190</td>
</tr>
<tr>
<td></td>
<td></td>
<td>17q21.33</td>
<td>COL1A1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>130050</td>
<td>2q32.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>COL3A1</td>
<td>120150</td>
</tr>
<tr>
<td></td>
<td></td>
<td>17q21.33</td>
<td>COL1A1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>130060</td>
<td>17q21.33</td>
</tr>
<tr>
<td></td>
<td></td>
<td>COL1A1</td>
<td>120150</td>
</tr>
<tr>
<td>GROUP B: Disorders of collagen folding and collagen cross-linking</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ocular-Scoliotic EDS</td>
<td>Kyphoscoliosis type (kEDS-PLOD1)</td>
<td>225400</td>
<td>1p36.22</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PLOD1</td>
<td>600985</td>
</tr>
<tr>
<td></td>
<td>Cardiac-valvular EDS</td>
<td>225320</td>
<td>7q21.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>COL1A2</td>
<td>120160</td>
</tr>
<tr>
<td>GROUP C: Disorders of structure and function of myomatrix, the interface between muscle and ECM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Classical-like EDS (clEDS)</td>
<td>606408</td>
<td>6p21.33-p21.32</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>TNXB</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Myopathic EDS (mEDS)</td>
<td>616471</td>
<td>6q13-q14</td>
</tr>
<tr>
<td></td>
<td></td>
<td>COL12A1</td>
<td>120320</td>
</tr>
<tr>
<td>GROUP D: Disorders of glycosaminoglycan biosynthesis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human dermatosparaxis EDS</td>
<td>Dermatosparaxis type (dEDS-ADAMTS2)</td>
<td>225410</td>
<td>5q35.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ADAMTS2</td>
<td>604539</td>
</tr>
<tr>
<td></td>
<td></td>
<td>614505</td>
<td>FKBP2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>FKBP12</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Adducted Thumb Clubfoot syndrome</td>
<td>Musculocontractural EDS (mcEDS-CHST14)</td>
<td>601776</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CHST14</td>
<td>608429</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Gene/Locus</th>
<th>OMIM</th>
<th>OMIM</th>
<th>Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gravis</td>
<td>130000</td>
<td>9q34.3</td>
<td>COL5A1</td>
</tr>
<tr>
<td>Mitis</td>
<td>130010</td>
<td>2q32.2</td>
<td>COL5A2</td>
</tr>
<tr>
<td></td>
<td>120090</td>
<td>Type I collagen (p.(Arg312Cys)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>120130</td>
<td>Type III collagen</td>
<td></td>
</tr>
<tr>
<td></td>
<td>130050</td>
<td>2q32.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>130060</td>
<td>17q21.33</td>
<td></td>
</tr>
<tr>
<td></td>
<td>130090</td>
<td>17q21.33</td>
<td></td>
</tr>
<tr>
<td></td>
<td>130150</td>
<td>Total absence of pro-alpha2(I) collagen chains</td>
<td></td>
</tr>
<tr>
<td></td>
<td>225400</td>
<td>1p36.22</td>
<td></td>
</tr>
<tr>
<td></td>
<td>600985</td>
<td>TenascinXB</td>
<td></td>
</tr>
<tr>
<td></td>
<td>225320</td>
<td>7q21.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>600985</td>
<td>TenascinXB</td>
<td></td>
</tr>
<tr>
<td></td>
<td>601776</td>
<td>15q15.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>608429</td>
<td>Dermatan-4 sulfotransferase-1</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Protein Name</th>
<th>Pathways</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysylhydroxylase 1</td>
<td></td>
</tr>
<tr>
<td>FKBP2</td>
<td></td>
</tr>
<tr>
<td>FKBP12</td>
<td></td>
</tr>
<tr>
<td>Dermatan-4 sulfotransferase-1</td>
<td></td>
</tr>
<tr>
<td>Galactosyltransferase I</td>
<td></td>
</tr>
<tr>
<td>Galactosyltransferase II</td>
<td></td>
</tr>
<tr>
<td>Galactosyltransferase III</td>
<td></td>
</tr>
<tr>
<td>Collagen XII</td>
<td></td>
</tr>
<tr>
<td>Collagen XI</td>
<td></td>
</tr>
<tr>
<td>Tenascin X</td>
<td></td>
</tr>
<tr>
<td>Tenascin</td>
<td></td>
</tr>
</tbody>
</table>
Minimal criteria suggestive for cEDS:

- Major criterion (1): skin hyperextensibility and atrophic scarring

Plus
- Either major criterion (2): GJH
- And/or: at least three minor criteria

Confirmatory molecular testing is obligatory to reach a final diagnosis.

Molecular basis

More than 90% of cEDS patients harbor a heterozygous mutation in one of the genes encoding type V collagen (COL5A1 and COL5A2) [Symoens et al., 2012; Ritelli et al., 2013; Zoppi et al., 2015] (see also “Ehlers–Danlos Syndrome, Classical Type,” by Bowen et al., this issue). Rarely, specific mutations in the genes encoding type I collagen can be associated with a cEDS-phenotype. These include the heterozygous COL1A1 c.934C>T, p.(Arg312Cys) substitution [Malfait et al., 2007a]. Patients harboring this mutation are particularly at risk for vascular rupture, whereas patients harboring other COL1A1 arginine-to-cysteine substitutions are associated with other specific phenotypes (see also “Ehlers–Danlos Syndromes, Rare Types,” by Brady et al., this issue). Sodium Dodecyl Sulfate PolyAcrylamide Gel Electrophoresis (SDS PAGE) demonstrates the migration of an extra band in the cell fraction, and sometimes also in the medium fraction. This band, which disappears after reduction with β-mercaptoethanol, consists of disulfide-bonded α chains [Malfait et al., 2007b]. Furthermore, biallelic COL1A2 mutations that lead to complete absence of the proα2(I) collagen chain may also present with a classical EDS-like phenotype, but these patients are at risk for developing severe cardiac-valvular problems. Moreover, inheritance of this condition is autosomal recessive (see also “Cardiac-valvular EDS,” below, and “Ehlers–Danlos Syndromes, Rare Types,” by Brady et al., this issue). SDS PAGE demonstrates complete absence of (pro-) α2 chains of type I (pro) collagen extracted from dermis [Schwarze et al., 2004; Malfait et al., 2006].

Verification of clinical diagnosis

Molecular screening by means of targeted resequencing of a gene panel

<table>
<thead>
<tr>
<th>TABLE II. (Continued)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>GROUP E: Disorders of complement pathway</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>EDSVIII</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>GROUP F: Disorders of intracellular processes†</td>
</tr>
<tr>
<td>Spondylodysplastic EDS</td>
</tr>
<tr>
<td>Brittle Cornea Syndrome</td>
</tr>
<tr>
<td>Brittle Cornea Syndrome</td>
</tr>
<tr>
<td>Unresolved forms of EDS</td>
</tr>
<tr>
<td>Hypomorphic EHS</td>
</tr>
<tr>
<td>Hypomorphic type</td>
</tr>
<tr>
<td>Hypomorphic, type</td>
</tr>
<tr>
<td>Conditions not included in EDS spectrum anymore</td>
</tr>
</tbody>
</table>

Table continued on next page.

IP, inheritance pattern; AD, autosomal dominant; AR, autosomal recessive; X-L, X-linked recessive.

For EDS subtypes implemented in this category, the underlying pathophysiological mechanism is not really understood, and classification within this subgroup is provisional, until further functional information becomes available.

For EDS subtypes implemented in this category, the underlying pathophysiological mechanism is not really understood, and classification within this subgroup is provisional, until further functional information becomes available.
that includes at least the COL5A1, COL5A2, COL1A1, and COL1A2 genes, or by WES or WGS, is indicated. When no mutation is identified, this approach should be complemented with a CNV detection strategy to identify large deletions or duplications.

In case of unavailability of genetic testing, transmission electron microscopy (TEM) findings of collagen flowers on skin biopsy can support the clinical diagnosis, but cannot confirm it. Absence of these confirmatory findings does not exclude the diagnosis, as specific types of mutations (e.g., deep intronic mutations) may go undetected by standard diagnostic molecular techniques; however, alternative diagnoses should be considered in the absence of (a) COL5A1, COL5A2, COL1A1, or COL1A2 mutation(s).

In the absence of these confirmatory findings, transmission electron microscopy (TEM) findings of collagen flowers on skin biopsy can support the clinical diagnosis, but cannot confirm it. Absence of these confirmatory findings does not exclude the diagnosis, as specific types of mutations (e.g., deep intronic mutations) may go undetected by standard diagnostic molecular techniques; however, alternative diagnoses should be considered in the absence of (a) COL5A1, COL5A2, COL1A1, or COL1A2 mutation(s).
Molecular basis
ciEDS is caused by a complete lack of Tenascin XB (TNX) due to biallelic TNXB mutations, that lead to nonsense-mediated mRNA decay, or biallelic deletion of TNXB. As a result the TNX protein is completely absent. TNXB is the only gene associated with ciEDS.

Verification of diagnosis
Molecular analysis of the TNXB gene should be used as the standard confirmatory test. Difficulties in DNA testing are related to the presence of a pseudogene (TNX-A), which is more than 97% identical to the 3′ end of TNXB (exons 32–44). With the only exception of exon 35, which partially shows a TNXB-specific sequence, exon and intron sequences in this region are identical or almost identical in both the gene and the pseudogene. This has implications both for sequencing and deletion/duplication analysis. For sequence analysis of TNXB, two approaches are recommended.

1. Sanger sequencing of the entire TNXB gene.
2. Next-generation sequencing of TNXB + Sanger sequencing of the pseudogene region.

Both approaches will require sequence analysis of the pseudogene-homolog region in a few large multi-exons amplicons.

If no or only one causative mutation is identified by classic sequencing, additional methods that allow detection of large deletions/duplications should be added. So far no method is able to specifically detect TNXB CNVs in the highly homologous exons 32–34 and 36–44. CNV analysis of exon 35 is currently used to detect deletions in this region, including the 30 kb deletion previously described by Schalkwijk et al. [2001].

TNX, a large 450 kDa extracellular matrix glycoprotein, secreted by skin fibroblasts, can be detected with antibodies directed against its carboxyterminal end. Patients with ciEDS are completely depleted of the TNX protein in serum. We refer to the paper of Schalkwijk et al. [2001] for more detailed information concerning the used method to detect TNX.

Absence of these confirmatory findings does not exclude the diagnosis, as specific types of mutations (e.g., deep intronic mutations) may go undetected by standard diagnostic molecular techniques; however, alternative diagnoses should be considered in the absence of a TNXB mutation.

Cardiac-Valvular EDS (cvEDS)

- Inheritance
  - Autosomal recessive

- Major criteria
  1. Severe progressive cardiac-valvular problems (aortic valve, mitral valve)¹⁰
  2. Skin involvement: skin hyperextensibility, atrophic scars, thin skin, easy bruising
  3. Joint hypermobility (generalized or restricted to small joints)

- Minor criteria
  1. Inguinal hernia
  2. Pectus deformity (especially excavatum)
  3. Joint dislocations
  4. Foot deformities: pes planus, pes planovalgus, hallux valgus

- Minimal criteria suggestive for cvEDS:
  - Major Criterion (1): severe progressive cardiac-valvular problems
  - AND a family history compatible with autosomal recessive inheritance
  - Plus
    - Either: one other major criterion
    - And/or: at least two minor criteria

Confirmation molecular testing is obligatory to reach a final diagnosis.

- Molecular basis
  cvEDS is caused by a complete lack of the proα2-chain of type I collagen due to biallelic COL1A2 mutations, that

¹⁰The cardiac-valvular problems were reported in all affected adult individuals, but were absent in the two reported children (both <10 years of age).
¹¹For definition of skin hyperextensibility, see criteria for “Classical EDS.”
lead to nonsense-mediated mRNA decay. COL1A2 is the only gene associated with cvEDS.

- Verification of diagnosis
  Molecular screening by Sanger sequencing of COL1A2, or targeted resequencing of a gene panel that includes COL1A2 is indicated. When no mutation is identified, this approach should be complemented with a CNV detection strategy to identify large deletions or duplications.

  In case of unavailability of genetic testing, SDS PAGE demonstrates total absence of (pro-) α2(I) collagen chains.

  Whereas absence of these confirmatory biochemical findings allows to exclude the diagnosis of cvEDS, absence of confirmatory genetic findings does not exclude the diagnosis, as specific types of mutations (e.g., deep intronic mutations) may go undetected by standard diagnostic molecular techniques.

Vascular EDS (vEDS)

- Inheritance
  Autosomal dominant

- Major criteria
  1. Family history of vEDS with documented causative variant in COL3A1
  2. Arterial rupture at a young age
  3. Spontaneous sigmoid colon perforation in the absence of known diverticular disease or other bowel pathology
  4. Uterine rupture during the third trimester in the absence of previous C-section and/or severe peripartum perineum tears
  5. Carotid-cavernous sinus fistula (CCSF) formation in the absence of trauma

- Minor criteria
  1. Bruising unrelated to identified trauma and/or in unusual sites such as cheeks and back
  2. Thin, translucent skin with increased venous visibility
  3. Characteristic facial appearance
  4. Spontaneous pneumothorax
  5. Acrogeria
  6. Talipes equinovarus
  7. Congenital hip dislocation
  8. Hypermobility of small joints
  9. Tendon and muscle rupture
  10. Keratoconus
  11. Gingival recession and gingival fragility
  12. Early onset varicose veins (under age 30 and nulliparous if female)

- Minimal criteria suggestive for vEDS:
  A family history of the disorder, arterial rupture or dissection in individuals less than 40 years of age, unexplained sigmoid colon rupture, or spontaneous pneumothorax in the presence of other features consistent with vEDS should all lead to diagnostic studies to determine if the individual has vEDS. Testing for vEDS should also be considered in the presence of a combination of the other “minor” clinical features listed above.

---

A family history of the disorder, arterial rupture, or dissection in individuals less than 40 years of age, unexplained sigmoid colon rupture, or spontaneous pneumothorax in the presence of other features consistent with vEDS should all lead to diagnostic studies to determine if the individual has vEDS.

---

Hypermobile EDS (hEDS)

- Inheritance
  Autosomal dominant

- Molecular basis
  Unknown

- Clinical diagnosis
  The diagnosis of hEDS remains clinical due to the limited availability of reliable or appreciable genetic etiology to test for as there is yet no reliable or appreciable genetic etiology to test for in the vast majority of patients. This, in part, likely reflects genetic heterogeneity. In addition, the syndromic presentation may vary according to age and gender. There is also a clinical spectrum ranging from asymptomatic joint hypermobility, through “non-syndromic” hypermobility with secondary manifestations, to hEDS (see “A Framework for the Classification of Joint Hypermobility and Related Conditions” by Castori et al., this issue). A diagnosis of hEDS should be assigned only in those who meet all of the criteria described below, which should help to reduce heterogeneity and facilitate efforts to discover the
underlying genetic cause(s) of the syndrome which, in turn, may help clinical management. Since there is currently no “gold standard” laboratory test to confirm or refute the diagnosis, we anticipate that future research will lead to further revisions of these clinical criteria necessitating regular review of the relevant medical literature. It is also imperative, as this is a clinical diagnosis, to be relatively confident that the patient’s presentation does not represent one of the many other disorders of connective tissue. Therefore, the clinician should be experienced at the physical examination described herein as well the historical and clinical presentation of other HCTD and their diagnoses.

The clinical diagnosis of hEDS needs the simultaneous presence of criteria 1 AND 2 AND 3. Specific annotations and further explanations (i.e., footnotes [FN]) are reported for select features.

Criterion 1: Generalized Joint Hypermobility (GJH)

To date, the Beighton score (Fig. 2) is the most recognized tool for assessing GJH (see “Measurement Properties of Clinical Assessment Methods for Classifying Generalized Joint Hypermobility—a Systematic Review” by Juul-Kristensen et al., this issue). According to the original definition of the Beighton score and its subsequent incorporation into the Villefranche nosology for the hEDS, the cut-off for the definition of GJH is ≥5 points out of 9. However, joint range of motion decreases with age and there is an inverse relationship between age at ascertainment and the Beighton score, so the cut-off of five may prompt an over-diagnosis in children and an under-diagnosis among adults and elders.

In individuals with acquired joint limitations (past surgery, wheelchair, amputations, etc.) affecting the Beighton score calculation, the assessment of GJH may include historical information using the five-point questionnaire (5PQ) (Table III) [Hakim and Grahame, 2003; Mulvey et al., 2013], although this has not been validated in children (see “Measurement Properties of Clinical Assessment Methods for Classifying Generalized Joint Hypermobility—a Systematic Review” by Juul-Kristensen et al., this issue). If the Beighton score is 1 point below the age- and sex-specific cut-off AND the 5PQ is ‘positive’ (= at least two positive items), then a diagnosis of GJH can be made.

For patients with lower Beighton scores, the assessment of other joints is often considered, including temporomandibular joint, shoulder, hip, foot, wrist, ankle, and other digits. Increased ankle and wrist dorsiflexion, increased internal and external hip rotation, and pes planus have been correlated with Beighton score [Smits-Engelsman et al., 2011] However, similar concerns about age, gender, and environmental influences as well as measurement methodology and reliable cut-off values, limit such analysis as too subjective in the determination of GJH. Therefore, the use of such measurements cannot be factored into a diagnostic algorithm at this time. Obviously, more information regarding the assessment methodology (ies) in the determination of GJH is needed (see “Measurement Properties of Clinical Assessment Methods for Classifying Generalized Joint Hypermobility—a

**TABLE III. The Five-Point Questionnaire. Adapted From [Grahame and Hakim, 2003]**

1. Can you now (or could you ever) place your hands flat on the floor without bending your knees?
2. Can you now (or could you ever) bend your thumb to touch your forearm?
3. As a child, did you amuse your friends by contorting your body into strange shapes or could you do the splits?
4. As a child or teenager, did your shoulder or kneecap dislocate on more than one occasion?
5. Do you consider yourself “double-jointed”?

A “yes” answer to two or more questions suggests joint hypermobility with 80–85% sensitivity and 80–90% specificity.
Lastly, the use of the Beighton scoring system is meant to be a diagnostic screening method. It is understood that gender, age, ethnicity, strength training, stretching exercises, and warming up all affect JH and therefore GJH. However, muscular overcompensation, injury and surgery can cause either joint hypermobility or hypomobility. Muscular overcompensation, such as tight hamstrings, can affect the degree of knee extension and lumbar flexion negatively, while stretching exercises and warming up affects positively. Injury can destabilize a joint or alternatively reduce movement. Surgery can similarly affect a joint. For example, a person with lumbar spine fusion may similarly affect a joint. A and B; A and C; B and C; A and B and C

Feature A: systemic manifestations of a more generalized connective tissue disorder (a total of five must be present)\(^2\)

1. Unusually soft or velvety skin\(^{13}\)
2. Mild skin hypertextensibility\(^{14}\)
3. Unexplained striae such as striae distensae or rubrae at the back, groins, thighs, breasts and/or abdomen in adolescents, men or prepubertal women without a history of significant gain or loss of body fat or weight
4. Bilateral piezogenic papules of the heel\(^{15}\)
5. Recurrent or multiple abdominal hernia(s) (e.g., umbilical, inguinal, crural)

\(^{12}\) If marfanoid features are present, consider other conditions such as: Marfan syndrome, Loeys-Dietz syndrome, congenital contractual arachnodactyly, Shprintzen–Goldberg syndrome, Stickler syndrome, Homocystinuria, multiple endocrine neoplasia type 2B, and the familial thoracic aortic aneurysmal disorders [Pyeritz and Loeys, 2012]. Molecular testing for many of these conditions is clinically available.

\(^{13}\) While skin softness and texture remain subjective, it is often very notable in some individuals and useful when present but not quantifiable; we therefore recommend a high threshold for positivity.

\(^{14}\) Skin extensibility as measured by pinching and lifting the cutaneous and subcutaneous layers of the skin on the volar surface at the middle of the non-dominant forearm as described in Remvig et al. [2009]. Skin extensibility of \(>1.5\) cm is considered the upper end of normal. It is likely that the hyper-extensibility of the skin in hEDS overlaps significantly with that of "normal" skin. Therefore, extensibility of more than \(1.5\) cm is "positive." If extensibility \(>2.0\) cm is present in combination with other cutaneous features, such as papyraceous scars, molluscoid pseudotumors and/or subcutaneous spheroids, consider other EDS types as possible alternative diagnoses (mainly cEDS and classical-like EDS).

\(^{15}\) Piezogenic papules are herniations of subcutaneous fat often demonstrable in the heel upon standing (Fig. 3). It is considered uncommon in children but can be found in adults with history of prolonged standing (occupational), marathon runners, or weightlifters [Poppe and Hamm, 2013] However, in a sex- and age-matched study of 29 Dutch EDS patients, piezogenic papules were found in 34.5% but none in the control group [Kahana et al., 1987].

\(^{16}\) Atrophic scarring is defined as scars from linear traumatic lacerations or single-surgery that are unusually shallow (i.e., thin and sunken) and/or wider than the original wound due to impaired repair and subsequent dermal hypo trophy. Atrophic scars as the result of multiple incisions, wound infections, or inflammatory conditions (such as viral infections, cystic acne, etc.) are not to be considered. Elliptical incisions (e.g., for removal of nevi) may be difficult to assess without knowing the size of the original wound. True skin fragility, such as the propensity to have an open wound due to trivial trauma, is not a typical feature of hEDS. Atrophic scarring in hEDS is mildly to moderately different from that usually considered typical of cEDS (Fig. 1).

\(^{17}\) Includes history of dental crowding or orthodontic intervention(s) to correct such problems. Both conditions must be positive to meet this criterion.

\(^{18}\) Some studies show no increase in the frequency of clinically significant MVP [Dolan et al., 1997; McDonnell et al., 2006; Atzinger et al., 2011] and others show an MVP frequency of 28–67% among hEDS patients [Camerota et al., 2014; Kozanoglu et al., 2016]. This feature is included in the diagnostic criteria based on the formation of truly papyraceous and/or hemosideric scars as seen in classical EDS.

**Criterion 2: Two or More Among the Following Features (A–C) MUST Be Present (for Example: A and B; A and C; B and C; A and B and C)**

Feature A: systemic manifestations of a more generalized connective tissue
independently meeting the current diagnostic criteria for hEDS.

Feature C: musculoskeletal complications (must have at least one):

1. Musculoskeletal pain in two or more limbs, recurring daily for at least 3 months
2. Chronic, widespread pain for ≥3 months
3. Recurrent joint dislocations or frank joint instability, in the absence of trauma (a or b)\(^\text{19}\)
   a. Three or more atraumatic dislocations in the same joint or two or more atraumatic dislocations in two different joints occurring at different times
   b. Medical confirmation of joint instability at two or more sites not related to trauma\(^\text{20}\)

**Criterion 3: All the Following Prerequisites MUST Be Met**

1. Absence of unusual skin fragility, which should prompt consideration of other types of EDS
2. Exclusion of other heritable and acquired connective tissue disorders, including autoimmune rheumatologic conditions. In patients with an acquired connective tissue disorder (e.g., lupus, rheumatoid arthritis, etc.), additional diagnosis of hEDS requires meeting both Features A and B of Criterion 2. Feature C of Criterion 2 (chronic pain and/or instability) cannot be counted towards a diagnosis of hEDS in this situation.
3. Exclusion of alternative diagnoses that may also include joint hypermobility by means of hypotonia and/or connective tissue laxity. Alternative diagnoses and diagnostic categories include, but are not limited to, neuromuscular disorders (e.g., myopathic EDS, Bethlem myopathy), other HCTD (e.g., other types of EDS, Loeys–Dietz syndrome, Marfan syndrome), and skeletal dysplasias (e.g., OI). Exclusion of these considerations may be based upon history, physical examination, and/or molecular genetic testing, as indicated.
   • General comment
   Many other features are described in hEDS but most are not sufficiently specific nor sensitive at the moment to be included in formal diagnostic criteria (see “Hypermobile Ehlers–Danlos Syndrome (a.k.a. Ehlers–Danlos Syndrome Type III and Ehlers–Danlos syndrome hypermobility type): Clinical Description, and Natural History” by Tinkle et al., this issue). These include but are not limited to: sleep disturbance, fatigue, postural orthostatic tachycardia, functional gastrointestinal disorders, dysautonomia, anxiety, and depression. These other systemic manifestations may be more debilitating than the joint symptoms, often impair functionality and quality of life, and should always be determined during clinical encounters. While they are not part of the diagnostic criteria, the presence of such systemic manifestations may prompt consideration of hEDS in the differential diagnosis. Future research will need to focus on such symptoms to validate any association with hEDS, describe sub-groups or sub-phenotypes, and be focused on evidence-based management of the symptoms in the context of hEDS.

**Arthrochalasia EDS (aEDS)**

- Inheritance
  - Autosomal dominant
- Major criteria
  1. Congenital bilateral hip dislocation\(^\text{21}\)
  2. Severe GJH, with multiple dislocations/subluxations\(^\text{22}\)
  3. Skin hyperextensibility\(^\text{22}\)
    • Minor criteria
      1. Muscle hypotonia
      2. Kyphoscoliosis
      3. Radiologically mild osteopenia
      4. Tissue fragility, including atrophic scars
      5. Easy bruisable skin
    • Minimal criteria suggestive for aEDS:
      - Major criterion (1): Congenital bilateral hip dislocation
      - Either major criterion (3): skin hyperextensibility
      - Or major criterion (2): severe GJH with multiple dislocations/subluxations and at least two other minor criteria
    Confirmatory molecular testing is obligatory to reach a final diagnosis.
    • Molecular basis
      aEDS is caused by heterozygous mutations in either COL1A1 or COL1A2, that cause entire or partial loss of exon 6 of the respective gene. No other genes are associated with aEDS.
      • Verification of diagnosis
        Molecular screening by Sanger sequencing of COL1A1 and COL1A2, or targeted resequencing of a gene panel that includes these genes, is indicated. When no mutation is identified, this approach should be complemented with a CNV detection strategy to identify large deletions or duplications.

In case of unavailability of genetic testing, SDS PAGE of the pepsin-digested collagen in the medium or cell layer of cultured dermal fibroblasts demonstrates the presence of a mutant pN\(\alpha1(l)\) or pN\(\alpha2(l)\) chain (precursor procollagen chains in which the carboxy (C)–but not the amino (N)–propetide is cleaved off).

TEM of skin specimens shows loosely and randomly organized collagen fibrils with a smaller and more variable diameter, and an irregular outline. These findings may support the diagnosis, but cannot confirm it.

Absence of a causative mutation in COL1A1 or COL1A2 that leads to

\(^\text{19}\)“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.

\(^\text{20}\)Refers to sites regardless of laterality. For example, right and left patellar instability would count as two. Instability should be evaluated and determined by a qualified practitioner using recommended guidelines.

\(^\text{21}\)All reported aEDS patients had congenital bilateral hip dislocation. One unreported molecularly proven aEDS patient is known to have had congenital unilateral hip dislocation [Byers et al., personal communication].

\(^\text{22}\)For definition of GJH, see criteria for “Classical EDS.”
complete or partial deletion of the exon 6 of either gene excludes the diagnosis of aEDS.

**Dermatosparaxis EDS (dEDS)**

- Inheritance
  - Autosomal recessive
- Major criteria:
  1. Extreme skin fragility with congenital or postnatal skin tears
  2. Characteristic craniofacial features, which are evident at birth or early infancy, or evolve later in childhood
  3. Redundant, almost lax skin, with excessive skin folds at the wrists and ankles
  4. Increased palmar wrinkling
  5. Severe bruising with a risk of subcutaneous hematomas and haemorrhage
  6. Umbilical hernia
  7. Postnatal growth retardation
  8. Short limbs, hands and feet
  9. Perinatal complications due to connective tissue fragility
- Minor criteria
  1. Soft and doughy skin texture
  2. Skin hyperextensibility
  3. Atrophic scars
  4. GJH
  5. Complications of visceral fragility (e.g., bladder rupture, diaphragmatic rupture, rectal prolapse)
  6. Delayed motor development
  7. Osteopenia

**Gene-specific minor criteria**

1. **PLOD1**
   1. Skin fragility (easy bruising, friable skin, poor wound healing, widened atrophic scarring)
   2. Scleral and ocular fragility/rupture
   3. Microcornea
   4. Facial dysmorphism
2. **FKBP14**
   1. Congenital hearing impairment (sensorineural, conductive, or mixed)
   2. Follicular hyperkeratosis
   3. Muscle atrophy
   4. Bladder diverticula

**Molecular basis**

- dEDS is caused by biallelic mutations in ADAMTS2, the gene encoding ADAMTS-2, the main procollagen I N-proteinase. It is the only gene associated with dEDS.

- Verification of diagnosis
  Molecular screening by Sanger sequencing of targeted resequencing of a gene panel that includes ADAMTS2 is indicated. When no, or only one, causative mutation is identified, this approach should be complemented with a CNV detection strategy to identify large deletions or duplications.

In case of unavailability of genetic testing, SDS PAGE demonstrates presence of pNa1(I) and pNa2(I) chains of type I procollagen extracted from dermis in the presence of protease inhibitors or detected in fibroblast cultures.

TEM shows collagen fibrils in affected skin specimens with a hieroglyphic pattern. These ultrastructural findings are usually typical but may be almost indistinguishable from those observed in aEDS. As such, they are not sufficient to confirm the diagnosis.

Absence of these confirmatory findings does not exclude the diagnosis of dEDS, as specific types of mutations (e.g., deep intronic mutations) may go undetected by standard diagnostic molecular techniques; however, alternative diagnoses should be considered in the absence of ADAMTS2 mutations.

**Kyphoscoliotic (kEDS)**

- Inheritance
  - Autosomal recessive
  1. Congenital muscle hypotonia
  2. Congenital or early onset kyphoscoliosis (progressive or non-progressive)
  3. GJH with dislocations/subluxations (shoulders, hips, and knees in particular)

**Molecular basis**

- dEDS is caused by biallelic mutations in ADAMTS2, the gene encoding ADAMTS-2, the main procollagen I N-proteinase. It is the only gene associated with dEDS.

- Verification of diagnosis
  Molecular screening by Sanger sequencing of targeted resequencing of a gene panel that includes ADAMTS2 is indicated. When no, or only one, causative mutation is identified, this approach should be complemented with a CNV detection strategy to identify large deletions or duplications.

In case of unavailability of genetic testing, SDS PAGE demonstrates presence of pNa1(I) and pNa2(I) chains of type I procollagen extracted from dermis in the presence of protease inhibitors or detected in fibroblast cultures.

TEM shows collagen fibrils in affected skin specimens with a hieroglyphic pattern. These ultrastructural findings are usually typical but may be almost indistinguishable from those observed in aEDS. As such, they are not sufficient to confirm the diagnosis.

Absence of these confirmatory findings does not exclude the diagnosis of dEDS, as specific types of mutations (e.g., deep intronic mutations) may go undetected by standard diagnostic molecular techniques; however, alternative diagnoses should be considered in the absence of ADAMTS2 mutations.

**Kyphoscoliotic (kEDS)**

- Inheritance
  - Autosomal recessive
  1. Congenital muscle hypotonia
  2. Congenital or early onset kyphoscoliosis (progressive or non-progressive)
  3. GJH with dislocations/subluxations (shoulders, hips, and knees in particular)

**Molecular basis**

- dEDS is caused by biallelic mutations in ADAMTS2, the gene encoding ADAMTS-2, the main procollagen I N-proteinase. It is the only gene associated with dEDS.

- Verification of diagnosis
  Molecular screening by Sanger sequencing of targeted resequencing of a gene panel that includes ADAMTS2 is indicated. When no, or only one, causative mutation is identified, this approach should be complemented with a CNV detection strategy to identify large deletions or duplications.

In case of unavailability of genetic testing, SDS PAGE demonstrates presence of pNa1(I) and pNa2(I) chains of type I procollagen extracted from dermis in the presence of protease inhibitors or detected in fibroblast cultures.

TEM shows collagen fibrils in affected skin specimens with a hieroglyphic pattern. These ultrastructural findings are usually typical but may be almost indistinguishable from those observed in aEDS. As such, they are not sufficient to confirm the diagnosis.

Absence of these confirmatory findings does not exclude the diagnosis of dEDS, as specific types of mutations (e.g., deep intronic mutations) may go undetected by standard diagnostic molecular techniques; however, alternative diagnoses should be considered in the absence of ADAMTS2 mutations.

**Kyphoscoliotic (kEDS)**

- Inheritance
  - Autosomal recessive
  1. Congenital muscle hypotonia
  2. Congenital or early onset kyphoscoliosis (progressive or non-progressive)
  3. GJH with dislocations/subluxations (shoulders, hips, and knees in particular)

**Molecular basis**

- dEDS is caused by biallelic mutations in ADAMTS2, the gene encoding ADAMTS-2, the main procollagen I N-proteinase. It is the only gene associated with dEDS.

- Verification of diagnosis
  Molecular screening by Sanger sequencing of targeted resequencing of a gene panel that includes ADAMTS2 is indicated. When no, or only one, causative mutation is identified, this approach should be complemented with a CNV detection strategy to identify large deletions or duplications.

In case of unavailability of genetic testing, SDS PAGE demonstrates presence of pNa1(I) and pNa2(I) chains of type I procollagen extracted from dermis in the presence of protease inhibitors or detected in fibroblast cultures.

TEM shows collagen fibrils in affected skin specimens with a hieroglyphic pattern. These ultrastructural findings are usually typical but may be almost indistinguishable from those observed in aEDS. As such, they are not sufficient to confirm the diagnosis.

Absence of these confirmatory findings does not exclude the diagnosis of dEDS, as specific types of mutations (e.g., deep intronic mutations) may go undetected by standard diagnostic molecular techniques; however, alternative diagnoses should be considered in the absence of ADAMTS2 mutations.

**Kyphoscoliotic (kEDS)**

- Inheritance
  - Autosomal recessive
  1. Congenital muscle hypotonia
  2. Congenital or early onset kyphoscoliosis (progressive or non-progressive)
  3. GJH with dislocations/subluxations (shoulders, hips, and knees in particular)

**Molecular basis**

- dEDS is caused by biallelic mutations in ADAMTS2, the gene encoding ADAMTS-2, the main procollagen I N-proteinase. It is the only gene associated with dEDS.

- Verification of diagnosis
  Molecular screening by Sanger sequencing of targeted resequencing of a gene panel that includes ADAMTS2 is indicated. When no, or only one, causative mutation is identified, this approach should be complemented with a CNV detection strategy to identify large deletions or duplications.

In case of unavailability of genetic testing, SDS PAGE demonstrates presence of pNa1(I) and pNa2(I) chains of type I procollagen extracted from dermis in the presence of protease inhibitors or detected in fibroblast cultures.

TEM shows collagen fibrils in affected skin specimens with a hieroglyphic pattern. These ultrastructural findings are usually typical but may be almost indistinguishable from those observed in aEDS. As such, they are not sufficient to confirm the diagnosis.

Absence of these confirmatory findings does not exclude the diagnosis of dEDS, as specific types of mutations (e.g., deep intronic mutations) may go undetected by standard diagnostic molecular techniques; however, alternative diagnoses should be considered in the absence of ADAMTS2 mutations.

**Kyphoscoliotic (kEDS)**

- Inheritance
  - Autosomal recessive
  1. Congenital muscle hypotonia
  2. Congenital or early onset kyphoscoliosis (progressive or non-progressive)
  3. GJH with dislocations/subluxations (shoulders, hips, and knees in particular)

**Molecular basis**

- dEDS is caused by biallelic mutations in ADAMTS2, the gene encoding ADAMTS-2, the main procollagen I N-proteinase. It is the only gene associated with dEDS.

- Verification of diagnosis
  Molecular screening by Sanger sequencing of targeted resequencing of a gene panel that includes ADAMTS2 is indicated. When no, or only one, causative mutation is identified, this approach should be complemented with a CNV detection strategy to identify large deletions or duplications.

In case of unavailability of genetic testing, SDS PAGE demonstrates presence of pNa1(I) and pNa2(I) chains of type I procollagen extracted from dermis in the presence of protease inhibitors or detected in fibroblast cultures.

TEM shows collagen fibrils in affected skin specimens with a hieroglyphic pattern. These ultrastructural findings are usually typical but may be almost indistinguishable from those observed in aEDS. As such, they are not sufficient to confirm the diagnosis.

Absence of these confirmatory findings does not exclude the diagnosis of dEDS, as specific types of mutations (e.g., deep intronic mutations) may go undetected by standard diagnostic molecular techniques; however, alternative diagnoses should be considered in the absence of ADAMTS2 mutations.

**Kyphoscoliotic (kEDS)**

- Inheritance
  - Autosomal recessive
  1. Congenital muscle hypotonia
  2. Congenital or early onset kyphoscoliosis (progressive or non-progressive)
  3. GJH with dislocations/subluxations (shoulders, hips, and knees in particular)
- Minimal criteria suggestive for kEDS:
  - Major criterion (1): congenital muscle hypotonia
  - AND major criterion (2): congenital or early-onset kyphoscoliosis
  - Either major criterion (3): GJH
  - And/or three minor criteria (either general or gene-specific criteria)

Confirmatory molecular testing is obligatory to reach a final diagnosis.

- Molecular basis
  The majority of patients with kEDS harbor biallelic mutations in PLOD1, the gene encoding the collagen-modifying enzyme procollagen-lysin, 2-oxoglutarate 5-dioxygenase 1 (PLOD1 or LH1 [lysylhydroxylase1]).
  - Recently, biallelic mutations have been identified in FKBP14, encoding FKBP22, a member of the F506-binding family of peptidyl-prolyl cis-trans isomerases, in patients displaying a phenotype that clinically overlaps with kEDS-PLOD1 [Baumann et al., 2012].
  - Verification of diagnosis
    - Laboratory confirmation of kEDS should start with the quantification of deoxypyridinoline (Dpyr or LP for lysyl-pyridinoline) and pyridinoline (Pyr or HP for hydroxylysyl-pyridinoline) cross-links in urine quantitated by means of high-performance liquid chromatography (HPLC). An increased Dpyr/Pyr ratio is a highly sensitive and specific test for kEDS caused by biallelic PLOD1 mutations (kEDS-PLOD1), but is normal for biallelic FKBP14 mutations (kEDS-FKBP14).
    - The normal ratio of Dpyr/Pyr cross-links is approximately 0.2, whereas in kEDS-PLOD1 the ratio is significantly increased (approximately 10–40 times increase, range 2–9). This method is fast and cost-effective and it can also be used to determine the pathogenic status of a VUS in PLOD1.
    - SDS–PAGE may detect faster migration of underhydroxylated collagen chains and their derivatives in kEDS-PLOD1 but not in kEDS-FKBP14. However, abnormalities in migration can be subtle.

Molecular analysis for kEDS-PLOD1 may start with MLPA analysis of PLOD1, for the evaluation of the common intragenic duplication in PLOD1 caused by an Alu-Alu recombination between introns 9 and 16 (the most common mutant allele) [Hautala et al., 1993].

Molecular screening by means of targeted resequencing of a gene panel that includes PLOD1 and FKBP14, is indicated when MLPA of PLOD1 fails to identify the common duplication. Such a gene panel may also include other genes associated with phenotypes that clinically overlap with kEDS, such as ZNF469, PRDM5, B4GALT7, B3GALT6, SLC39A13, CHST14, and DSE. Alternatively, WES may be performed. When no, or only one, causative mutation is identified, this approach should be complemented with a CNV detection strategy to identify large deletions or duplications in these genes.

TEM on skin specimens has shown variable diameters and abnormal contours of the collagen fibrils and irregular interfibrillar space, but these abnormalities are not unique to this condition. As such, whereas TEM on a skin biopsy can support diagnosis, it cannot confirm it.

Whereas absence of an abnormal urinary LP/HP ratio excludes the diagnosis of kEDS-PLOD1, absence of the confirmatory genetic findings does not exclude the diagnosis of kEDS, as specific types of mutations (e.g., deep intronic mutations) may go undetected.

Figure 3. Piezogenic papules of the feet which are subcutaneous fat herniations through the fascia. They often appear as blanching white nodules only while bearing weight.
Brittle Cornea Syndrome (BCS)

- Inheritance
  - Autosomal recessive
- Major criteria
  1. Thin cornea, with or without rupture (central corneal thickness often <400 μm)
  2. Early onset progressive keratoconus
  3. Early onset progressive keratoglobus
  4. Blue sclerae
- Minor criteria
  1. Encleuption or corneal scarring as a result of previous rupture
  2. Progressive loss of corneal stromal depth, especially in central cornea
  3. High myopia, with normal or moderately increased axial length
  4. Retinal detachment
  5. Deafness, often with mixed conductive and sensorineural components, progressive, higher frequencies often more severely affected (“slopping” pure tone audiogram).
  6. Hypercompliant tympanic membranes
  7. Developmental dysplasia of the hip
  8. Hypotonia in infancy, usually mild if present
  9. Scoliosis
  10. Arachnodactyly
  11. Hypermobility of distal joints
  12. Pes planus, hallux valgus
  13. Mild contractures of fingers (especially 5th)
  14. Soft, velvety skin, translucent skin
- Minimal criteria suggestive for kEDS:
  - Major criterion (1): thin cornea, with or without rupture (central corneal thickness often <100 micrometer)
- Plus
  - Either: at least one other major criterion
  - And/or three other minor criteria

- Verification of diagnosis

  Molecular screening by means of targeted resequencing of a gene panel that includes ZNF469 and PRDM5 is indicated. Such a gene panel may also include other genes associated with phenotypes that clinically overlap with BCS, such as PLOD1, FKBP14, B4GALT7, B3GALT6, SLC39A13, CHST14 and DSE. Alternatively, WES may be performed. When no, or only one, causative mutation is identified, this approach should be complemented with a CNV detection strategy to identify large deletions or duplications in these genes.

  Absence of these confirmatory findings does not exclude the diagnosis, as specific types of mutations (e.g., deep intronic mutations) may go undetected by standard diagnostic molecular techniques, and other, yet unknown genes, might be associated with BCS.

Spondylohydplastic EDS (spEDS)

- Inheritance
  - Autosomal recessive
- Major criteria
  1. Short stature (progressive in childhood)
  2. Muscle hypotonia (ranging from severe congenital, to mild later-onset)
  3. Bowing of limbs
- Minor criteria
  1. Skin hyperextensibility, soft, doughy skin, thin translucent skin
  2. Pes planus

- Molecular basis

  BCS is caused by biallelic mutations in either ZNF469, encoding ZNF469, a zinc finger protein of unknown function, or PRDM5, encoding a DNA-binding transcription factor of the PR/SET protein family that lacks the intrinsic histon methyltransferase activity. At least one family with a clinical BCS phenotype did not harbor mutations in these genes, suggesting that at least one other gene might be associated with BCS [Rohrbach et al., 2013].

- Gene-specific minor criteria
  - B4GALT7
    - Radioulnar synostosis
    - Bilateral elbow contractures or limited elbow movement
    - GJH
    - Single transverse palmar crease
    - Characteristic craniofacial features
    - Characteristic radiographic findings
    - Severe hypermetropia
    - Clouded cornea
  - B3GALT6
    - Kyphoscoliosis (congenital or early onset, progressive)
    - Joint hypermobility, generalized or restricted to distal joints, with joint dislocations
    - Joint contractures (congenital or progressive) (especially hands)
    - Peculiar fingers (slender, tapered, arachnodactyly, spatulate, with broad distal phalanges)
    - Talipes equinovarus
    - Characteristic craniofacial features
    - Tooth discoloration, dysplastic teeth

33Characteristic craniofacial features associated with biallelic B4GALT7 mutations include: triangular face, wide-spaced eyes, proptosis, narrow mouth, low-set ears, sparse scalp hair, abnormal dentition, flat face, wide forehead, blue sclerae, and cleft palate/bifid uvula.

34Reported radiographic findings associated with biallelic B4GALT7 mutations include: include radioulnar synostosis, metaphyseal flaring, osteopenia, radial head subluxation or dislocation, and short clavicles with broad medial ends.

35Characteristic craniofacial features associated with biallelic B3GALT6 mutations include: midfacial hypoplasia, frontal bossing, proptosis, or prominent eyes, blue sclerae, downslanting palpebral fissures, depressed nasal bridge, long upperlip, low-set ears, micrognathia, abnormal dentition, cleft palate, sparse hair.
– Characteristic radiographic findings
– Osteoporosis with multiple spontaneous fractures
– Ascending aortic aneurysm
– Lung hypoplasia, restrictive lung disease
– SLC39A13:
  – Protuberant eyes with bluish sclerae
  – Hands with finely wrinkled palms
  – Atrophy of the thenar muscles, and tapering fingers
  – Hypermobility of distal joints
  – Characteristic radiologic findings

• Minimal criteria suggestive for spEDS:
  – Major criterion (1): short stature
  – AND major criterion (2): muscle hypertonia
  – Plus
  – Characteristic radiographic abnormalities and at least three other minor criteria (general or type-specific)
  – Confirmatory molecular testing is obligatory to reach a final diagnosis

• Molecular basis
  – Biallelic mutations in B4GALT7, encoding galactosyltransferase I (β1,4-galactosyltransferase 7 or \( \beta4GalT7 \)), which catalyzes the transfer of the first galactose to the xylose residue in tetrasaccharide linker region of glycosaminoglycans (GAGs).
  – Biallelic mutations in B3GALT6, encoding galactosyltransferase II (β1,3-galactosyltransferase 6 or \( \beta3GalT6 \)), which catalyzes the transfer of the second galactose to the first galactose residue in tetrasaccharide linker region of GAGs.

– Biallelic mutations in SLC39A13, encoding the homodimeric transmembrane Zrt/irt-like protein 13 (ZIP13) protein, a member of the SLC39A/ZIP family that regulates the influx of Zn into the cytosol.

• Verification of diagnosis
  – Molecular screening by means of targeted resequencing of a gene panel that includes B4GALTT, B3GALT6, and SLC39A13 is indicated. Such a gene panel may also include other genes associated with phenotypes that clinically overlap with spEDS, such as PLOD1, FKBP14, ZNF469, PRDM5, CHST14, and DSE. Alternatively, WES may be performed. When no, or only one, causative mutation is identified, this approach should be complemented with a CNV detection strategy to identify large deletions or duplications in these genes.

For definitive proof of GAG deficiency (B4GALT7 and B3GALT6 mutations), biochemical methods to assess GAG synthesis in patients’ cultured fibroblasts are currently available in many specialized laboratories [Talhaoui et al., 2010]. The laboratory measurement of urinary pyridinolines, lysyl-pyridinoline (LP) and hydroxylysyl-pyridinoline (HP) quantitated by HPLC allows the detection of an increased ratio LP/HP to approximately 1, (compared to a normal value of approximately 0.2) in patients with mutations in SLC39A13 [Giunta et al., 2008]. This fast and cost-effective method can also be used to determine the pathogenic status of a VUS (see also “verification of diagnosis” in kEDS–PLOD1).

Absence of confirmatory genetic findings does not exclude the diagnosis of spEDS, as specific types of mutations (eg deep intronic mutations) may go undetected by standard diagnostic molecular techniques, and still other, yet to be discovered, genes may be associated with these phenotypes. In case no B4GALT7, B3GALT6, or SLC39A13 mutations are identified, alternative diagnoses should however be considered.

Musculocontractural EDS (mcEDS)

• Inheritance
  – Autosomal recessive

• Major criteria
  1. Congenital multiple contractures, characteristically adduction-flexion contractures and/or talipes equinovarus (clubfoot)
  2. Characteristic craniofacial features, which are evident at birth or in early infancy
  3. Characteristic cutaneous features including skin hyperextensibility, easy bruising, skin fragility with atrophic scars, increased palmar wrinkling

• Minor criteria
  1. Recurrent/chronic dislocations
  2. Pectus deformities (flat, excavated)
  3. Spinal deformities (scoliosis, kyphoscoliosis)
  4. Peculiar fingers (tapering, slender, cylindrical)
  5. Progressive fingers (tapering, slender, cylindrical)
  6. Large subcutaneous hematomas
  7. Chronic constipation
  8. Colonic diverticula
  9. Pneumothorax
  10. Nephrolithiasis/cystolithiasis
  11. Hydronephrosis
  12. Cryptorchidism in males
  13. Strabismus
  14. Refractive errors (myopia, astigmatism)
  15. Glaucma/elevated intraocular pressure

36 Reported radiographic features associated with biallelic B3GALT6 mutations include: platyspondyly, anterior beak of vertebral body, short ilium, prominent lesser trochanter, acetabular dysplasia, metaphyseal flaring, metaphyseal dysplasia of femoral head, elbow malalignment, residual head dislocation, overtubulation, bowing of long bones, generalized osteoporosis, healed fractures. Craniosynostosis and radioulnar dysostosis has been reported in one patient.

37 Reported radiologic findings associated with biallelic SLC39A13 mutations include: mild to moderate platyspondyly, mild to moderate osteopenia of the spine, small ilium, flat proximal femoral epiphyses, short, wide femoral necks.

38 Characteristic craniofacial include: large fontanelle, hypertelorism, short and downsloping palpebral fissures, blue sclerae, short nose with hypoplastic columella, low-set and rotated ears, high palate, long philtrum, thin upper lip vermilion, small mouth, microretrognathia.

39 For definition of skin hyperextensibility, see criteria for “Classical EDS.”

40 The phenotypic features in the three reported patients with EDS caused by DSE deficiency seem to be milder than those in patients with EDS caused by D4ST1-deficiency, but identification of additional patients with DSE-deficiency is needed to confirm this correlation.
Verification of diagnosis

Confirmatory molecular testing is obligatory to reach a final diagnosis.

Molecular basis
mcEDS is caused by biallelic mutations in CHST14, encoding D4ST1, a single-exon gene encoding carbohydrate sulfotransferase 14 or dermanan 4-O-sulfotransferase 1, an enzyme involved in the biosynthesis of the GAG dermanan sulfate. It catalyzes 4-O-sulfation of N-acetylglactosamine (GalNAc) in the sequence “L-iduronic acid (IdoA)-GalNAc,” immediately after epimerization of D-glucuronic acid (GlcA) to IdoA by dermanan sulfate epimerase (DSE).

Myopathic EDS (mEDS)

- Inheritance: Autosomal dominant or autosomal recessive
- Major criteria:
  1. Congenital muscle hypotonia, and/or muscle atrophy, that improves with age
  2. Proximal joint contractures (knee, hip, and elbow)
  3. Hypermobility of distal joints
- Minor criteria:
  1. Soft, doughy skin
  2. Atrophic scarring

Myopathic EDS (mEDS)

- Inheritance: Autosomal dominant or autosomal recessive
- Major criteria:
  1. Congenital muscle hypotonia, and/or muscle atrophy, that improves with age
  2. Proximal joint contractures (knee, hip, and elbow)
  3. Hypermobility of distal joints
- Minor criteria:
  1. Soft, doughy skin
  2. Atrophic scarring

Periodontal EDS (pEDS)

- Inheritance: Autosomal dominant
- Major criteria
  - Severe and intractable periodontitis of early onset (childhood or adolescence)
  - Lack of attached gingiva
  - Pretibial plaques
  - Family history of a first-degree relative who meets clinical criteria
- Minor criteria
  - Easy bruising
  - Joint hypermobility, mostly distal joints
  - Skin hyperextensibility and fragility, abnormal scarring (wide or atrophic)
  - Increased rate of infections
  - Hernias
  - Marfanoid facial features
  - Acrogeria
  - Prominent vasculature
- Minimal criteria suggestive for pEDS:
  - Major criterion (1): severe and intractable periodontitis of early onset (childhood or adolescence)
  - OR major criterion (2): lack of attached gingiva
  - Plus: At least two other major criteria and one minor criterion
- Confirmatory molecular testing is obligatory to reach a final diagnosis.
- Molecular basis
  pEDS is caused by heterozygous gain-of-function mutations in C1R or C1S, encoding subunits C1r and C1s of the first component of the classical complement pathway.
- Verification of diagnosis
  Identification of known or compatible mutations by sequence analysis of C1R and C1S. Large deletions or null mutations that completely remove C1r or C1s protein function do not cause pEDS.

At present it cannot be stated whether absence of a C1R or C1S mutations excludes the diagnosis because the experience with the molecular diagnosis is limited.

CONCLUDING REMARKS

We hope that the revised International EDS criteria will serve as a new, albeit provisional, standard for the diagnosis of EDS. Our proposal has the aim of facilitating accurate and timely diagnosis, and improve the diagnostic uniformity for clinical and research purposes, genetic counseling, management, natural history studies, and identification of potential areas of research. Future revision of this EDS Classification will be planned within the framework of the International EDS Consortium and the Ehlers–Danlos Society.

ACKNOWLEDGMENTS

We are very grateful for the cooperation of many patients and their families all over the world. We thank Professor Stephen Robertson and PVNH support for their help with PVNH-EDS. We also wish to acknowledge the contributions of many professionals and lay persons alike to this endeavour. We would like to thank our generous sponsors including, but not limited to, The Ehlers–Danlos National Foundation, the Ehlers–Danlos Support UK, and the Ehlers–Danlos Society. Frantiscka Malift is Senior Clinical Investigator at the Fund for Scientific Research Flanders.

REFERENCES


cross-sectional study cataloging human variation. Physiotherapy 102:50–56.


