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Looking back and beyond the 2017 diagnostic criteria for hypermobile Ehlers-Danlos syndrome: A retrospective cross-sectional study from an Italian reference center

Marco Ritelli[1] | Nicola Chiarelli[1] | Valeria Cinquina[1] | Marika Vezzoli[2] | Marina Venturini[3] | Marina Colombi[1]

[1]Division of Biology and Genetics, Department of Molecular and Translational Medicine, University of Brescia, Brescia, Italy

[2]Unit of Biostatistics, Department of Molecular and Translational Medicine, University of Brescia, Brescia, Italy

[3]Division of Dermatology, Department of Clinical and Experimental Sciences, Spedali Civili University Hospital, Brescia, Italy

Marina Colombi, Division of Biology and Genetics, Department of Molecular and Translational Medicine, University of Brescia, Viale Europa 11, 25123 Brescia, Italy.
marina.colombi@unibs.it

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Abstract

The most common conditions with symptomatic joint hypermobility are hypermobile Ehlers-Danlos syndrome (hEDS) and hypermobility spectrum disorders (HSD). Diagnosing these overlapping connective tissue disorders remains challenging due to the lack of established causes and reliable diagnostic tests. hEDS is diagnosed applying the 2017 diagnostic criteria, and patients with symptomatic joint hypermobility but not fulfilling these criteria are labeled as HSD, which is not officially recognized by all healthcare systems. The 2017 criteria were introduced to improve diagnostic specificity but have faced criticism for being too stringent and failing to adequately capture the multisystemic involvement of hEDS. Herein, we retrospectively evaluated 327 patients from 213 families with a prior diagnosis of hypermobility type EDS or joint hypermobility syndrome based on Villefranche and Brighton criteria, to assess the effectiveness of the 2017 criteria in distinguishing between hEDS and HSD and document the frequencies of extra-articular manifestations. Based on our findings, we propose that the 2017 criteria should be made less stringent to include a greater number of patients who are currently encompassed within the HSD category. This will lead to improved diagnostic accuracy and enhanced patient care by properly capturing the diverse range of symptoms and manifestations present within the hEDS/HSD spectrum.

KEYWORDS

Beighton score, diagnostic criteria, hypermobile Ehlers-Danlos syndrome, hypermobility spectrum disorders, joint hypermobility, multisystemic manifestations

1 | INTRODUCTION

The Ehlers-Danlos syndromes (EDS) are a group of 14 clinically variable and etiologically heterogeneous heritable connective tissue disorders (HCTDs) primarily characterized by cutaneous manifestations, generalized joint hypermobility (gJHM), and varying degrees of tissue fragility. Most types of EDS are caused by mutations in genes encoding collagens and enzymes involved in their biosynthesis or in the maintenance of extracellular matrix homeostasis (Malfait et al., 2017, 2020). The term hypermobility spectrum disorders (HSD) refers to another group of clinically significant conditions associated with JHM with a wide range in both the type and severity of patients' symptoms,

extending from asymptomatic or paucisymptomatic JHM, or hypermobility affecting only one or few joint(s), to gJHM, dislocations, and recurrent, persistent and/or chronic pain that significantly impacts patients' quality of life (Castori et al., 2017).

Although EDS is currently considered rare with an expected prevalence of 1:5000 (Tinkle et al., 2017), certain forms are far more frequent than others. Of the 14 recognized subtypes, hypermobile EDS (hEDS) is the most common form and is estimated to account for over 90% of EDS diagnoses, with a higher incidence in females (Tinkle et al., 2017). However, recent research from the UK reported that the combined prevalence of hEDS and HSD is closer to 1:500 (Demmler et al., 2019), and estimates from the NIH "All of Us" database suggest 1:300. Unfortunately, unlike the other types of EDS, hEDS and HSD do not have a known molecular basis; hence, given the significant clinical overlap and the lack of any validated diagnostic biomarker, it is extremely difficult to differentiate between the two conditions (Atwell et al., 2021; Gensemer et al., 2021; Scicluna et al., 2021; Tinkle et al., 2009).

The challenging clinical diagnosis of these patients has been widely recognized among experts since the 90s and reflects the intricate history of the different diagnostic sets that have been established over the years. The Villefranche classification in 1998 initially proposed the clinical criteria for hypermobility type EDS (ht-EDS) showing clinical similarity with joint hypermobility syndrome (JHS), a condition associated with JHM and musculoskeletal and systemic symptoms as defined by the Brighton criteria (Table 1) (Grahame et al., 2000; Hakim & Grahame, 2003). Although JHS was initially envisioned as distinct from ht-EDS, clinical practice later suggested that both conditions should be considered a single phenotypic entity (JHS/ht-EDS) (Colombi et al., 2015; Tinkle et al., 2009). The 2017 classification of EDS (Malfait et al., 2017) abolished the dual nature of these overlapping phenotypes and proposed a set of more stringent criteria recognizing a single entity defined as hEDS (Table 1).

As a result, the terms JHS, ht-EDS and JHS/ht-EDS have been withdrawn, and individuals with these previous diagnoses who do not meet the 2017 criteria for hEDS and who do not exhibit signs and symptoms of other JHM-associated conditions are now classified as having HSD (Castori et al., 2017). However, shortly after the updated diagnostic criteria were introduced, several authors raised concerns about their limits, since they neither effectively recognize the more severely affected patients nor account for the numerous extra-musculoskeletal manifestations of hEDS (Aubry-Rozier et al., 2021; Castori, 2021; Copetti et al., 2019; Hakim, 2019; Hakim et al., 2021; Martinez et al., 2021; Morlino et al., 2019; Williams, 2019; Yew et al., 2021). These associated conditions are nowadays recognized, with varying levels of evidence, as JHM-associated comorbidities and include chronic fatigue, pelvic floor problems, bladder dysfunction, various dysautonomic features (e.g., orthostatic decompensation, unstable cardiac rhythms and rates, postural orthostatic tachycardia syndrome [POTS], and gastrointestinal dysfunction), neurological involvement, behavioral disturbances, psychological distress, and immune system alterations such as mast cell disorders (Atwell et al., 2021; Brock et al., 2021; Castori et al., 2017; Celletti et al., 2020; Fernandez et al., 2022; Lam et al., 2021; Malfait et al., 2017; Malfait et al., 2020; Mathias et al., 2021; Pietri-Toro et al., 2023; Rashed et al., 2022; Ruiz Maya et al., 2021; Thwaites et al., 2022; Tinkle et al., 2017; Vermeulen et al., 2022; Wasim et al., 2019). These comorbidities are common not only in hEDS but also in HSD, although their exact incidence has not been extensively documented thus far. Hence, considering the significant clinical overlap, a fervent debate is taking place, with some experts deeming that hEDS and HSD are in essence the same condition along a spectrum (hEDS/HSD), while others believe that they are separate, distinct conditions. Only the identification of the underlying genetic etiology(ies) of hEDS and HSD, which is (are) most likely oligogenic or multifactorial, and/or the development of definitive diagnostic tests will allow the two conditions to be differentiated or merged into a new entity, which may even exist outside the current EDS classification.

Currently, for physicians treating these patients, the principles and types of multidisciplinary management, including the identification of the JHM-associated comorbidities that often are more debilitating than joint symptoms, are essentially the same for hEDS and HSD, as both conditions require awareness, recognition, validation, and care (Anderson & Lane, 2021; Atwell et al., 2021; Bennett et al., 2022; Demes et al., 2020; Estrella & Frazier, 2023; Robbins, 2022; Spanhove et al., 2023; Yew et al., 2021).

Herein, we present a retrospective cross-sectional analysis on a cohort of 327 patients from 213 different families. The main goals of the study were to compare patients diagnosed with hEDS and HSD using the 2017 diagnostic criteria, to evaluate the effectiveness of the included signs and symptoms in distinguishing between the two disorders, and to comprehensively document the frequencies of comorbid conditions to gain a comprehensive understanding of the multisystemic involvement in both hEDS and HSD. These findings may provide valuable insights for revising and improving the current diagnostic criteria for hEDS.

2 | PATIENTS AND METHODS

2.1 | Study design

This study was approved by the local Ethical Committee (ASST degli Spedali Civili di Brescia, protocol number NP4244) and involves a cohort of patients evaluated in an Italian specialized outpatient clinic for the diagnosis and management of HCTDs at the Spedali Civili University Hospital of Brescia. All patients included in this study were diagnosed before March 2017 as ht-EDS or JHS respectively according to the Villefranche nosology (Beighton et al., 1998) and the Brighton criteria (Grahame et al., 2000) and their subsequent modifications (Ross & Grahame, 2011). After the publication of the revised classification of EDS (Malfait et al., 2017), we reviewed the clinical records and reclassified the patients as either hEDS or HSD based on whether they met the 2017 hEDS criteria. It should be noted that we did not remove the previous diagnoses in all cases, but rather applied the 2017 criteria and noted which, if any, of the criteria they met. For

TABLE 1 Summary of the diagnostic criteria for assessing ht-EDS, JHS, and hEDS.

VILLEFRANCHE CRITERIA for ht-EDS (Beighton et al., 1998)	2017 CRITERIA for hEDS (Malfait et al., 2017)
Major criteria • *Generalized joint hypermobility (BS ≥ 5)* • *Skin involvement (hyperextensibility and/or smooth, velvety skin)* **Minor criteria** • *Recurring joint dislocations* • *Chronic joint/limb pain* • *Positive family history* ***Agreement***: *Both major criteria (irrespectively of the presence/absence of minor criteria which are considered supportive)* **BRIGHTON CRITERIA for JHS** (Grahame et al., 2000) **Major criteria** • *A Beighton score ≥4/9* • *Arthralgia for longer than 3 months in 4 or more joints* **Minor criteria** • *A Beighton score of 1–3, (0, 1, 2, or 3 if aged >50 years)* • *Arthralgia (>3 months) in 1 to 3 joints or back pain (>3 months), spondylosis, spondylolysis/spondylolisthesis* • *Dislocation/subluxation in more than one joint, or in one joint on more than one occasion* • *Soft tissue rheumatism >3 lesions* (e.g., *epicondylitis, tenosynovitis, bursitis*) • *Marfanoid habitus (tall, slim, span/height ratio >1.03, upper: lower segment ratio less than 0.89, arachnodactyly [positive Steinberg/wrist signs])* • *Abnormal skin: striae, hyperextensibility, thin skin, papyraceous scarring* • *Eye signs: drooping eyelids, myopia, or antimongoloid slant* • *Varicose veins, hernia, or uterine or rectal prolapse* ***Agreement***: Both major, or 1 major and 2 minor, or 4 minor criteria, or 2 minor criteria with an affected first degree relative (Ross & Grahame, 2011).	**CRITERION 1** *Presence of generalized joint hypermobility (gJHM)* **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* In individuals with acquired joint limitations (past surgery, wheelchair, amputations, etc.) affecting the Beighton score calculation, the assessment of gJHM may include historical information using the 5-point questionnaire (5PQ) (Hakim & Grahame, 2003). If the Beighton score is 1 point below the age-specific cut-off AND the 5PQ is "positive" (at least two positive items), then a diagnosis of gJHM 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'?* **CRITERION 2** *Two or more among features (A–C) MUST be present (A and B; A and C; B and C; A and B and C)* • **Feature A (*must have at least 5*)** • *Unusually soft or velvety skin* • *Mild skin hyperextensibility (>1.5 cm)* • *Striae distensae/rubrae*: striae distensae or 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 heels* • *Recurrent or multiple abdominal hernia(s)* (e.g., umbilical, inguinal, crural) • *Atrophic scarring involving at least two sites* • *Pelvic floor, rectal, and/or uterine prolapse* 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: Positive wrist sign (Steinberg sign) on both sides Positive thumb sign (Walker sign) on both sides • *Arm span-to-height ≥1.05* • *Mitral valve prolapse (MVP)* • *Aortic root dilatation with Z-score > +2* • **Feature B:** *Positive family history of hEDS with at least one first-degree relative independently meeting hEDS criteria* • **Feature C (*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 or frank joint instability in the absence of trauma* **CRITERION 3** Exclusion of other conditions • Other EDS types • Other heritable/acquired connective tissue disorders • Alternative diagnoses ***Agreement***: *Simultaneous presence of all criteria*

adults aged 18 years or older with a BS <5, we assessed the presence of historical JHM using the 5PQ (Hakim & Grahame, 2003). In case of a suspected overlap with other acquired or hereditary CTDs, we extended the differential diagnosis and evaluation to include autoimmune rheumatologic screening, skeletal X-ray, standard bone densitometry, ophthalmological examination, magnetic resonance imaging

and/or heart ultrasound, and other selected supplementary evaluations (e.g., audiometry, baseline bone metabolism serum, and urine analyses). When necessary, we applied other sets of criteria, such as the Ghent criteria for Marfan syndrome (Loeys et al., 2010) along with appropriate molecular studies. Genetic testing included *COL5A1/A2* or *TNXB* analyses to exclude classical and classical-like EDS in patients with a borderline cutaneous phenotype or a custom-made NGS panel comprising most EDS genes and those of some related disorders for more complex phenotypes (Ritelli, Venturini, et al., 2020; Rymen et al., 2019).

To study the multisystemic nature of hEDS and HSD, we also documented, for each proband, a set of signs and symptoms not included in the 2017 nosology through direct clinical evaluation or review of patient-provided medical reports. In total, we recorded 95 distinct issues, comprising 12 mucocutaneous, 10 osteoarticular, 18 orthopedic, 4 muscular, 11 gastrointestinal, 7 cardiovascular, 15 neuropsychiatric, 6 uro-gynecological, 7 immunological/atopic, and 5 ocular dysmorphic features (Additional Table 1).

In addition to the nosological criteria outlined in Table 1, the recorded mucocutaneous features included keratosis pilaris, light blue sclerae, easy bruising, gingival inflammation/recessions, resistance to local anesthetic drugs, and uvula abnormalities (elongated, hypoplastic). Osteoarticular features comprised congenital hip dysplasia, temporomandibular joint dysfunction, early osteoarthritis, walking difficulties, and limited walking autonomy. Orthopedic issues comprised clubfeet, pes planus/cavus, scoliosis, spine curvature anomalies (cervical, dorsal, lumbar hyper[hypo]kyphosis/lordosis), disc hernias/protrusions, lombosciatalgy, spinal surgery, arthrodesis, cubita/genua valga, halluces valgi, minor asymmetry at lower limbs (anatomical or functional anisomelia) or at other body areas, osteopenia (non-postmenopausal or early in men), snapping hip, pectus excavatum/carinatum. Muscular features were mild muscle hypotonia, recurrent myalgias and cramps, fibromyalgia, and involuntary muscle contractions. Gastrointestinal features covered dysphagia, gastroesophageal reflux, hiatal hernia, defecatory dysfunction, food intolerances, dolichocolon, delayed gastric/bowel/colonic transit, irritable bowel disease, celiac disease, abdominal pain, and visceroptosis. Cardiovascular signs were valvular regurgitation with mild hemodynamic involvement, low progressive aortic root dilatation, varicose veins, capillary fragility, recurrent epistaxis or gingival bleeding, and Raynaud's phenomenon/acrocyanosis/livedo reticularis. Neuropsychiatric features comprised chronic fatigue, clumsiness, delayed motor development, impaired memory and concentration, neuropathic pain, paresthesia, allodynia, headache/migraine, somatosensory/central sensitization or amplification, anxiety/panic/fears, sleep disturbances, depression, and obsessive-compulsive trait. A subset of patients underwent clinically indicated Tilt-table testing for symptoms suggestive of autonomic dysfunction. Investigated uro-gynecological features included meno/metrorrhagia, disabling dysmenorrhea, postpartum hemorrhage, urinary stress incontinence, and neurological bladder. Immunological features included asthma, atopic dermatitis, anaphylaxis, rhinitis/rhinoconjunctivitis, angioedema, allergy/atopy, and pruritus. Ocular signs included myopia, palpebral ptosis, strabismus, xerophthalmia, and diplopia.

2.2 | Statistical analyses

Assessment between the presence/absence of investigated features in hEDS and HSD was performed with the chi-square test with Yates's correction or Fisher's exact test whenever the count was insufficient. Analysis was carried out with the GraphPad Software and considering significant *p*-values to be less than 0.05.

To investigate whether the 2017 nosological criteria alone and/or the entire set of multisystemic clinical manifestation can effectively differentiate patients with hEDS and HSD, we performed cluster analyses. Given the diverse nature of the variables used (quantitative and qualitative), we employed a machine learning approach (Azzolina et al., 2019) to evaluate the distances between our observations (patients). In detail, we ran an unsupervised random forest (RF), which is a peculiar clustering procedure able to deal with mixed type data since the process of growing and splitting a tree naturally accommodates both continuous and categorical data (Breiman, 2001; Garrafa et al., 2021). Unsupervised machine learning algorithms generate homogenous clusters of observations by translating the analysis into a supervised classification problem. The basic approach involves creating an artificial dataset and combining it with the original data. An RF classification model is then trained on the combined original and artificial data. The RF model produces a proximity matrix, which contains measures of similarity between observations in the dataset. Standard clustering techniques, such as partitioning around medoids (PAM), can then be applied to the proximity matrix to identify clusters within the data (Reynolds et al., 2006; Salvi et al., 2019). To determine the optimal number (k) of clusters in the data, we used the silhouette method, which allows the interpretation and validation of consistency within clusters of data. This method computes silhouette coefficients (which range from -1 to $+1$) measuring how similar an observation is to its own cluster (cohesion) compared to other clusters (separation). Coefficients values close to 1 indicate that the observation is well matched to its own cluster and poorly matched to neighboring clusters. The procedure is repeated for each observation in the sample and the silhouette score (SS) is the mean of these coefficient values. For identifying the optimal k, we have picked a range of candidate values (from a minimum of 2 till the number of observations in the dataset minus 1), then applying the PAM algorithm for each of these values. The optimal number of clusters corresponds to the highest SS.

We first performed this procedure both on the entire cohort and probands with the following nosological criteria and scores: BS (from 0 to 9), features A items (from 0 to 12), presence or lack of positive family history (scored as 1 or 0), and features C items (from 0 to 3, with chronic generalized pain scoring 2 points and recurrent musculoskeletal pain scoring 1 point). Second, cluster analysis with the full set of probands' clinical manifestations included the BS (from 0 to 9) as well as the counts of features in these categories: mucocutaneous (from 0 to 12), osteoarticular (from 0 to 9, gJHM according to the 2017 nosology was excluded as we considered the BS), orthopedic (from 0 to 18, with scoliosis >40° scoring 2 points and mild scoliosis scoring 1 point), muscular (from 0 to 4), gastrointestinal (from 0 to 10), cardiovascular (from 0 to 7), neuropsychiatric (from 0 to 15), uro-gynecological (from 0 to 6 for females and 0 to 3 for males), atopic

(from 0 to 7), and ocular (from 0 to 5) as listed in Additional Table 1. The results of the cluster analysis were visualized using a multidimensional scaling (MDS) plot. MDS is a common approach for graphically representing relationships and similarities between observations. It is based on the proximity matrix extracted from the unsupervised RF and the observations (represented by colored points) are plotted in two dimensions, aiming to approximate their multivariate dissimilarity as closely as possible.

After obtaining the clusters, we used them for stratifying the data by computing the descriptive statistics for the variables that generated them. For quantitative variables, we computed the mean and standard deviation (*SD*), median, first quartile (Q1), third quartile (Q3), and range (minimum–maximum). For categorical variables, frequencies (absolute and percentage values) were computed. To determine any statistically significant differences (p-value <0.05) between the clusters, we applied the Wilcoxon Rank Sum test for quantitative variables and the Fisher's exact test for qualitative variables. All analyses related to the machine learning methods and related descriptive statistics were performed with R, version 4.2.0 (R Foundation).

3 | RESULTS

3.1 | General findings, initial clinical diagnosis, and reclassification according to the 2017 EDS nosology

Comprehensive demographic data of the patients' cohort are shown in Additional Table 2. Complete clinical features by single patient are reported in Additional Table 1 and frequencies of selected features and cluster analyses are reported in Figures 1–5, Additional Tables 1–16, and Additional Figures 1–5. Data are presented for the entire population, for probands and by clinical diagnosis, sex, and age.

Among the 327 individuals described here, 147 were sporadic patients, while 66 probands had at least another affected family member, making a total of 213 index-cases (65.1%) and 114 relatives (34.9%). Most patients were female, with 276 females (84.4%) and 51 males (15.6%) (sex ratio: 5.41). When considering only index cases, the sex ratio was 8.68 (191 females and 22 males). The age range of all patients at their last examination was 2–71 years, with a mean of 33.04 years (*SD* 14.89). Out of all patients, 142 (43.43%) were younger than 30 years, while 185 (56.57%) were aged ≥30 years. For probands, the age at examination ranged from 7 to 70 years, with a mean of 32.91 years (*SD* 12.28). The mean age for female and male probands were 33.24 (*SD* 12.54) and 30.05 (*SD* 9.46) years, respectively. Out of all probands, 89 (41.78%) were younger than 30 years, while 124 (58.22%) were aged ≥30 years.

All patients included in the study fulfilled the criteria for a diagnosis of either ht-EDS or JHS. Specifically, 125 (38.23%) patients of the entire population met the Villefranche nosology and 326 (99.69%) fulfilled the Brighton criteria. Similar frequencies were observed for probands (44.6% for ht-EDS and 100% for JHS). After recategorizing patients according to the 2017 nosology, only 113 patients of the entire cohort met the new criteria for hEDS (34.6%, 102 females and 11 males, sex ratio: 9.27), whereas 214 did not and were therefore classified as having HSD (65.4%, 174 females and 40 males, sex ratio: 4.35). By considering index-cases only, the percentage of individuals meeting the 2017 criteria increased to 40.85% (87/213).

Despite the limited number of males in our cohort, they had a significantly higher overall frequency in HSD compared to hEDS (18.68% vs. 9.73%). In hEDS, 87 individuals were probands (∼77%) and 26 were relatives (23%), while in HSD 126 were probands (∼59%) and 88 were relatives (41.1%). Among hEDS probands, 81 were females (93.10%) and 6 were males (6.90%), resulting in a sex ratio of 13.5. In HSD probands, 110 were females (87.3%) and 16 were males (12.70%), with a sex ratio of 6.88. However, the percentages of male probands in hEDS versus HSD (6.90% vs. 12.70%) did not differ statistically, likely due to the smaller sample size compared to the entire cohort.

In the group of patients who met the Villefranche nosology, about 80% also respected the 2017 hEDS criteria, both in the entire cohort and in probands. On the other hand, only 34.66% of patients and 40.85% of probands previously diagnosed with JHS fulfilled the 2017 hEDS criteria. Additional Tables 3 and 4 present the significant differences in major and minor criteria of the previous diagnostic sets between hEDS and HSD patients and probands, respectively.

3.2 | Diagnostic criteria according to the 2017 EDS nosology

Figure 1 and Additional Figure 1 summarize the occurrences of the three mandatory diagnostic criteria for an hEDS diagnosis observed in the entire cohort and in probands, respectively. Additional Tables 5 and 6 report the overall frequencies and statistically significant differences between hEDS and HSD. The analyses by sex and age (<30 vs. ≥30 years) for both the entire cohort and probands are presented in Additional Tables 7–10.

Concerning criterion 1, only 43.12% of all patients (141/327) satisfied the age-adjusted BS of the revised nosology. This frequency increased slightly to 50.23% (107/213) by considering index-cases only. No statistically significant differences were observed by sex and age both in the entire cohort and in probands. Among individuals who did not meet the 2017 hEDS criteria, 28/214 (13.08%) of all patients and 20/126 (15.87%) of probands nevertheless met criterion 1 and were hence classified as generalized HSD (gHSD). Of the remaining 186 patients not fulfilling criterion 1, 84 (45.16%) met both criteria 2 and 3. Restricting consideration to only the probands, 41/106 (38.67%) met criteria 2 and 3. It is worth noting that 18 of these 84 patients (9 probands) were adults with a BS that was just one point below the age-specific cut-off. Specifically, 8 patients (2 probands) above the age of 50 had a BS of 3, and 10 patients (7 probands) between the ages of 18 and 50 had a BS of 4 (Additional Table 1).

Additional Figure 2 displays the BS distribution in hEDS and HSD, both in the entire cohort and in probands. In hEDS patients, BS of 5 (∼50%) and 6 (∼24%) were the most frequent, whereas a BS of 9 was present in only a small percentage of patients (∼7%). In HSD

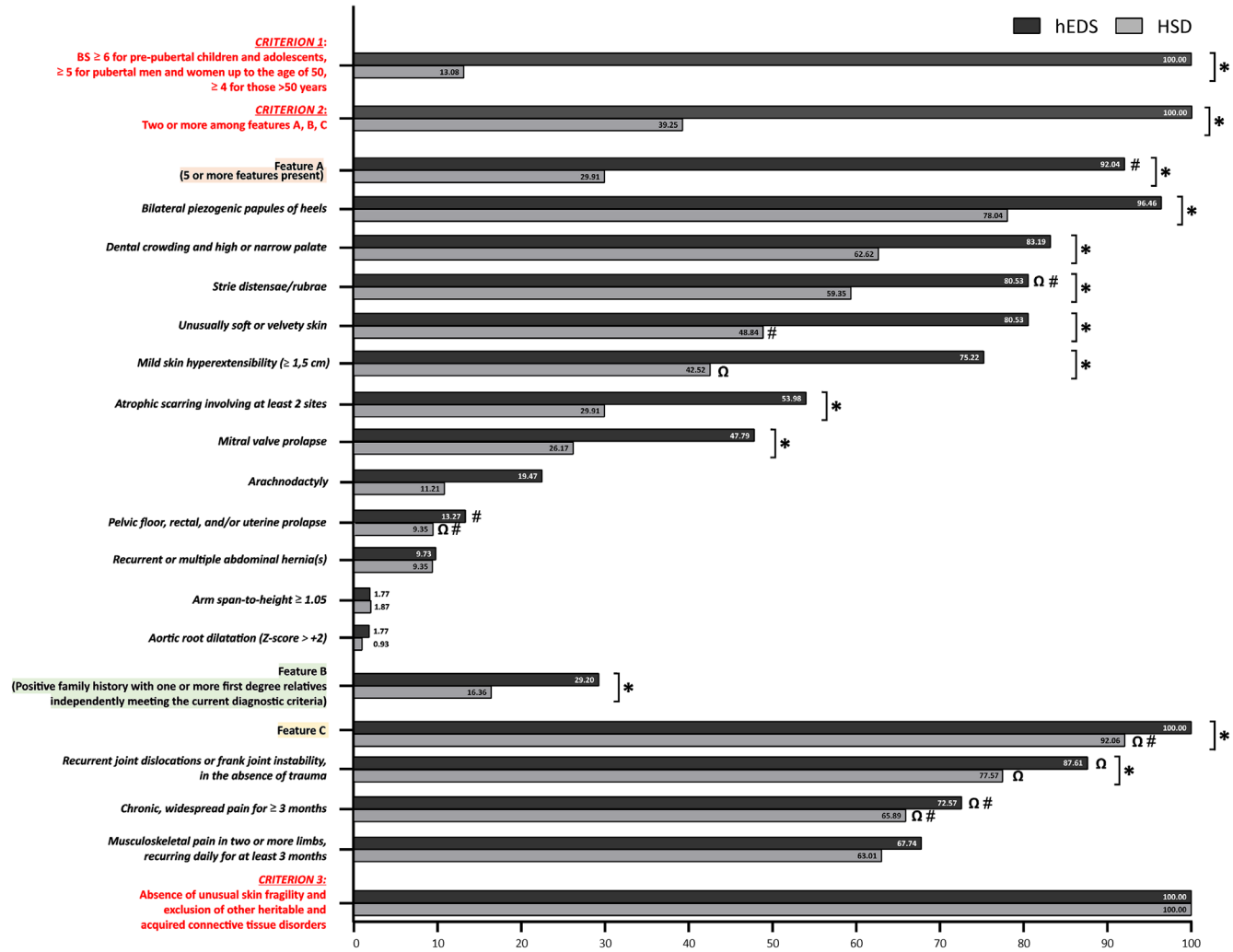

FIGURE 1 Distribution of three mandatory diagnostic criteria for an hypermobile Ehlers-Danlos syndrome (hEDS) diagnosis according to the 2017 Ehlers-Danlos syndrome (EDS) classification in the entire cohort of 327 patients, including 113 hEDS and 214 hypermobility spectrum disorders (HSD) individuals. *Presence of statistically significant differences between hEDS and HSD; Ω presence of statistically significant differences by age (<30 vs. ≥30 years); # presence of statistically significant differences by sex (for frequencies and *p*-values, see Additional Tables 5, 7, and 9).

patients, BS of 3 and 2 accounted for more than 50% of cases. Comparable frequencies were observed in probands for both hEDS and HSD. The introduction of an age-corrected BS in the updated nosology, which considers the notion that JHM decreases with age, is supported by the frequencies of the different BS observed in the two age groups (<30 vs. ≥30 years) both in hEDS and HSD. Indeed, as shown in Additional Figure 3, the percentage of hEDS patients with a BS equal to or above 7 was significantly lower in individuals aged ≥30 years. This inverse correlation was even more pronounced in HSD, as a significant reduction of the BS in older individuals was already evident starting from BS = 5.

Regarding criterion 2, approximately 60% of all patients (197/327) and probands (128/213) met two or more among feature A (multisystemic involvement), B (positive family history), and C (musculoskeletal complaints), without any sex and age bias. In comparison to all hEDS patients who necessarily must meet criterion 2, a significantly lower percentage of HSD patients (39.25%, 84/214) and probands (32.54%, 41/126) fulfilled this criterion.

About feature A, we found that 51.38% (168/327) of patients and 58.69% (125/213) of probands showed at least 5 out of the 12 signs of systemic manifestation. In hEDS, 92.04% (104/113) of patients and 98.85% (86/87) of probands fulfilled feature A, while only about 30% of HSD patients (64/214) and probands (39/126) met this feature. In general, positivity for feature A was more prevalent among individuals aged ≥30 years, although there was a significant *p*-value only in hEDS. Additional Figure 4 shows the distribution of the numbers of positive items of feature A in hEDS and HSD. Both in the entire cohort and in probands, the majority of hEDS individuals (~40%) had the minimum number required, followed by ~34% of people with 6 items; only a few individuals (~1%) showed 9 features, and none had all the 12 signs. In HSD, over 25% of individuals had a number of items that were only one-point below the required

threshold, and a similar percentage showed three items; only 1% did not show any of these items.

In the entire cohort, among the items with frequencies >50%, bilateral piezogenic papules (96.46% vs. 78.04%), dental crowding/high or narrow palate (83.19% vs. 62.62%), striae distensae/rubrae (80.53% vs. 59.35%), unusually soft or velvety skin (80.53% vs. 48.84%), and mild skin hyperextensibility (75.22% vs. 42.52%) were all more prevalent in hEDS compared to HSD. Striae distensae were more common in hEDS females and in individuals aged ≥30 years. Patients aged ≥30 years also exhibited a higher rate of unusually soft or velvety skin, although a significant *p*-value was observed only in HSD. Similarly, mild skin hyperextensibility was more prevalent in males, with a statistical significance only in HSD. Among features with rates <50%, atrophic scarring (53.98% vs. 29.91%) and mitral valve prolapse (MVP) (47.79% vs. 26.17%) were significantly more frequent in hEDS, whereas the difference in arachnodactyly (19.47% vs. 11.21%) was not statistically significant. Additionally, the rarely observed pelvic floor/rectal/uterine prolapse (13.27% vs. 9.35%), abdominal hernias (9.73% vs. 9.35%), arm span-to-height ≥1.05 (1.77% vs. 1.87%), and aortic root dilatation (1.77% vs. 0.93%) did not show any significant differences. Pelvic floor/rectal/uterine prolapse occurred exclusively in females and was more frequent in individuals aged ≥30 years. When considering index-cases, similar rates and differences were observed between hEDS and HSD, as well as in terms of sex and age, with the exceptions that the sex- and age-related differences in striae distensae detected in hEDS were lost.

Concerning feature B, only 20.80% (68/327) of patients and 7.04% (15/213) of probands had one or more first-degree relatives independently meeting the hEDS diagnostic criteria. In the entire cohort, 29.20% (33/113) of hEDS and 16.36% (35/214) of HSD patients respected feature B. When looking at index cases, the difference between hEDS and HSD patients meeting feature B was more pronounced (14.94% vs. 1.59%). In hEDS, 5 of the 13 feature B-positive probands (all females) had only one hEDS relative, 3 had two hEDS parents (on three generations in Family 56), and 5 had in addition to one (or more) hEDS relatives also one or more affected parents who did not fulfill the hEDS criteria (Families 31, 38, 46, 111, and 204 in Additional Table 1). In HSD, the two feature B-positive probands did not meet feature A but fulfilled criterion 2 for the combination B plus C, and both had an hEDS sister. Of the 74 feature B-negative hEDS probands, 17 had one or more parents classified as HSD, 4 had in addition to one hEDS relative also one or more HSD parents (Families 41, 73, 74, and 96 in Additional Table 1), and 53 were sporadic patients. Of the 124 feature B-negative HSD probands, 29 had one or more additional HSD relatives (there were a total of 14 HSD patients over three generations in Family 50), the proband of Family 124 also had two second degree hEDS parents in addition to numerous HSD relatives, and 94 were sporadic patients. Overall, 32 of 66 families with more than one affected member (48.4%) showed co-segregation of either hEDS or HSD.

Regarding feature C, at least one musculoskeletal manifestation was present in 94.80% (310/327) of patients and 98.12% (209/213) of probands. In particular, feature C was met by all hEDS patients and probands, but it was also quite common in HSD. Notably, the difference between hEDS and HSD probands was smaller and not statistically significant (100% vs. 96.83%) when compared to the entire cohort (100% vs. 92.06%), suggesting that probands had a more severe clinical presentation than HSD relatives. In the entire HSD cohort, feature C was more prevalent in females and in individuals aged ≥30 years, but not in probands.

Among the three items of feature C, recurrent joint dislocations/instability were more common in hEDS versus HSD only in the entire cohort (87.61% vs. 77.57%) but not in probands (90.80% vs. 86.51%). This reinforces the idea of a more severe phenotype of HSD probands compared to relatives. Chronic, widespread pain had similar high incidences in hEDS and HSD both in the entire cohort (72.57% vs. 65.89%) and in probands (77.01% vs. 74.60%). Likewise, recurrent musculoskeletal pain, which was considered mutually exclusive with chronic pain, was also prevalent in both hEDS and HSD in the entire cohort (67.76% vs. 63.01%) and in probands (75% vs. 81.25%). Patients without chronic or recurrent pain accounted for only 11.31% (37/327) of the entire cohort, and there was no difference between hEDS and HSD. Notably, when considering index-cases, the overall percentage of probands without pain decreased to about 5%. Concerning sex-related differences, chronic pain and joint dislocations/instability were more common in females in the entire cohort. When considering index-cases, a higher prevalence of females resulted in statistical significance only for chronic pain and only in HSD. Regarding age-related differences, chronic pain was more common in patients aged ≥30 years in both the entire cohort and in probands.

The most prevalent combination that resulted in criteria 2 positivity was A + C, which was present in 83.76% of patients and 97.66% of probands. In the entire cohort, this combination was more common in hEDS (92.04% vs. 72.62%), in which it was also more frequent in individuals aged ≥30 years, while no significant difference was observed in probands (98.85% vs. 95.12%), consistent with the more severe musculoskeletal phenotype of HSD index-cases described above. The combination B + C was met respectively by 32.99% of patients and 11.72% of probands, with no differences among hEDS and HSD and in terms of sex and age. The combination A + B (entire cohort: 17.77%; probands: 9.38%) as well as the simultaneous presence of all features of criterion 2 (entire cohort: 17.26%; probands 9.38%) were both more recurrent in hEDS, although a significant *p*-value was observed only in probands. Indeed, 13.79% of hEDS index-cases and none of HSD fulfilled either A + B or A + B + C, whereas in the entire cohort the rates of these combinations in hEDS and HSD were respectively 21.24% versus 13.10% and 21.24% versus 11.90%. Although these latter 11.90% of HSD patients (10/84, from different families) all had an hEDS first-degree relative, satisfied the multisystemic involvement of feature A, and suffered from musculoskeletal complaints, they were formally classified as HSD only for the absence of a proper BS. Of note, one of these patients (patient 57, family 41 in Additional Table 1), a 47-year-old female having two hEDS daughters and one HSD sister, presented a BS one-point below the age-specific cut-off.

3.3 | Cluster analysis based on nosological criteria

To evaluate the effectiveness of the nosological criteria in differentiating patients who met the 2017 nosology from those who did not, we performed cluster analysis both on the entire cohort and probands. In the entire cohort (Figure 2a, Additional Table 11), consisting of 327 patients (113 with hEDS and 214 with HSD), we identified $k = 4$ optimal clusters by means of the silhouette method, obtaining an SS approximately equal to 1, indicating that observations are, on average, well matched to their own cluster. After stratifying the nosological variables with respect to the clusters obtained, the analysis revealed that there is a significant overlap among the two diagnostic categories, as a considerable number of hEDS and HSD patients clustered together. Specifically, in cluster 1 (shown in red in Figure 2a), which comprised the majority of patients (219/327, 66.96%), we observed nearly the same percentage of hEDS and HSD patients compared to the overall cohorts, with 75 out of 113 (66.37%) being hEDS and 144 out of 214 (67.28%) being HSD. Likewise, in cluster 2 (in green), hEDS and HSD patients grouped together with a slightly higher percentage for HSD (8/113, 7.08% of hEDS, and 44/214, 20.56% of HSD). In contrast, clusters 3 and 4 (in light blue and purple) exclusively included HSD and hEDS patients, respectively, but represented a smaller proportion of the total cohorts (26/214, 12.15% of HSD, and 30/113, 26.4% of hEDS). Looking more closely at the different clusters, we observed that in cluster 4, which comprised 30 hEDS patients who were all negative for feature B, the average BS was 5.13 (range 5–6), the mean number of feature A items was 5.70 (range 5–7), and all patients scored three points for feature C. Contrarywise, in cluster 3, which included 26 HSD patients with no positive family history of hEDS, the average BS was 3.00 (range 2–4), as well as the mean number of feature A items (range 2–4), and all patients scored three points for feature C. In the mixed hEDS/HSD cluster 2 (44 HSD and 8 hEDS), the average BS was 3.37 (range 2–6), the mean number of feature A items was 4.44 (range 3–6), and all patients scored three points for feature C and did not have a first-degree hEDS relative. Finally, in the most enriched hEDS/HSD cluster 1 (144 HSD and 75 hEDS), the mean BS was 4.15 (range 0–9), the average number of feature A items was 4.32 (range 0–9), and the mean number of feature C items was 2.07 (range 0–3). It is noteworthy that 31.05% of the patients (68/219) in this cluster fulfilled feature B and were either hEDS (33/75) or HSD (35/144).

With a few exceptions, cluster analysis using only probands (213 individuals, 87 hEDS, and 126 HSD) generated similar results (Figure 2b, Additional Table 12). Specifically, we found $k = 4$ optimal clusters (with an SS approximately equal to 1), the most enriched of which (cluster 1 in red) encompassed the majority of both hEDS

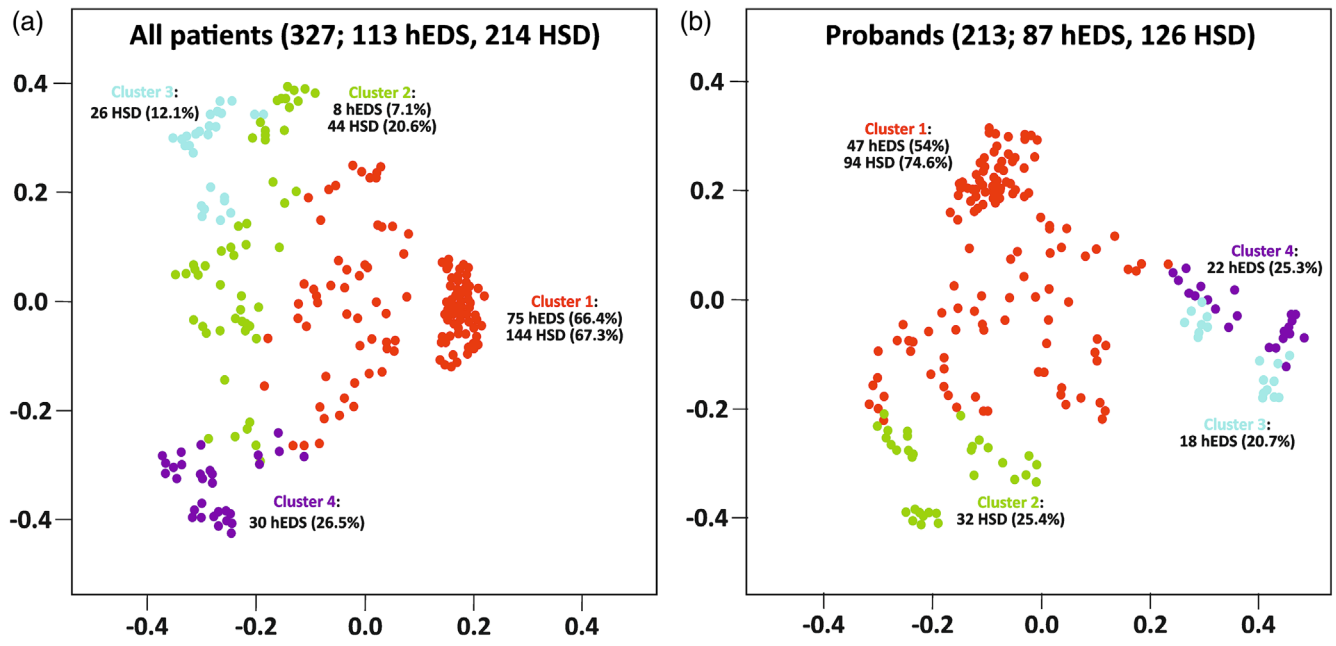

FIGURE 2 Multidimensional scaling plot of the clustered data providing a visual representation of the pattern of proximities among data, where each point (colored respective to the cluster to which it belongs) corresponds to an observation (patient). The two-dimensional plot is based on the proximity matrix extracted from the unsupervised random forest, and the points are visualized such that the distances between them approximate their multivariate dissimilarity as closely as possible. Cluster analysis was performed on the 2017 diagnostic criteria in the entire cohort of 327 patients (a), including 113 hypermobile Ehlers-Danlos syndrome (hEDS) and 214 hypermobility spectrum disorders (HSD) individuals, and on the 213 probands (b), including 87 hEDS and 126 HSD individuals. The optimal number (k) of clusters was identified using the silhouette method, based on the following variables and scores: BS (from 0 to 9), features A items (from 0 to 12), presence or lack of positive family history (scored as 1 or 0), and features C items (from 0 to 3). The percentages of hEDS and HSD patients falling into the different clusters were calculated with respect to the total cohorts (for descriptive statistics related to the variables that generated the clusters, see Additional Tables 11 and 12).

(47/87, 54.02%) and HSD (94/126, 74.60%) probands, and 15 patients (13 hEDS and 2 HSD) fulfilled feature B. The average BS within this cluster was 4.18 (range 1–9), the mean number of feature A items was 4.56 (range 0–9), and the mean number of feature C items was 2.39 (range 0–3). Cluster 2 (in green) comprised the remaining percentage of HSD patients (32/126, 25.39%), exhibiting an average BS of 3.03 (range 2–4), a mean number of feature A items of 4.03 (range 3–5), and all scored three points for feature C. The remaining 40 hEDS probands (40/87, 45.98%) were evenly distributed across cluster 3 (in light blue) and cluster 4 (in purple). These clusters showed similar means in terms of BS (5.39 for cluster 3 and 5.18 for cluster 4), and all patients scored three points for feature C. The differentiating factor between these clusters was the average number of items met for feature A, with patients in cluster 3 having a mean of 5.00 items and those in cluster 4 a mean of 6.41 (range 6–8).

Taken together, these analyses indicate that the boundaries between these diagnostic categories are not well-defined by the current diagnostic criteria, emphasizing the need for a more comprehensive classification framework that can better reflect the complexity and heterogeneity of patients' phenotypes. With this perspective in mind, we documented a set of signs and symptoms not included in the 2017 nosology to gain a more thorough understanding of the multisystemic nature of hEDS and HSD, which may be helpful in developing a more representative picture of the full spectrum of clinical features associated with these disorders.

3.4 | Multisystemic manifestations

In order to investigate the multisystemic nature of hEDS and HSD, we recorded, for each proband, the presence of a set of additional signs and symptoms related to almost all organ systems, including several comorbidities frequently reported in EDS (Tinkle et al., 2017). Figures 3 and 4 and Additional Figure 5 summarize the rates of all investigated

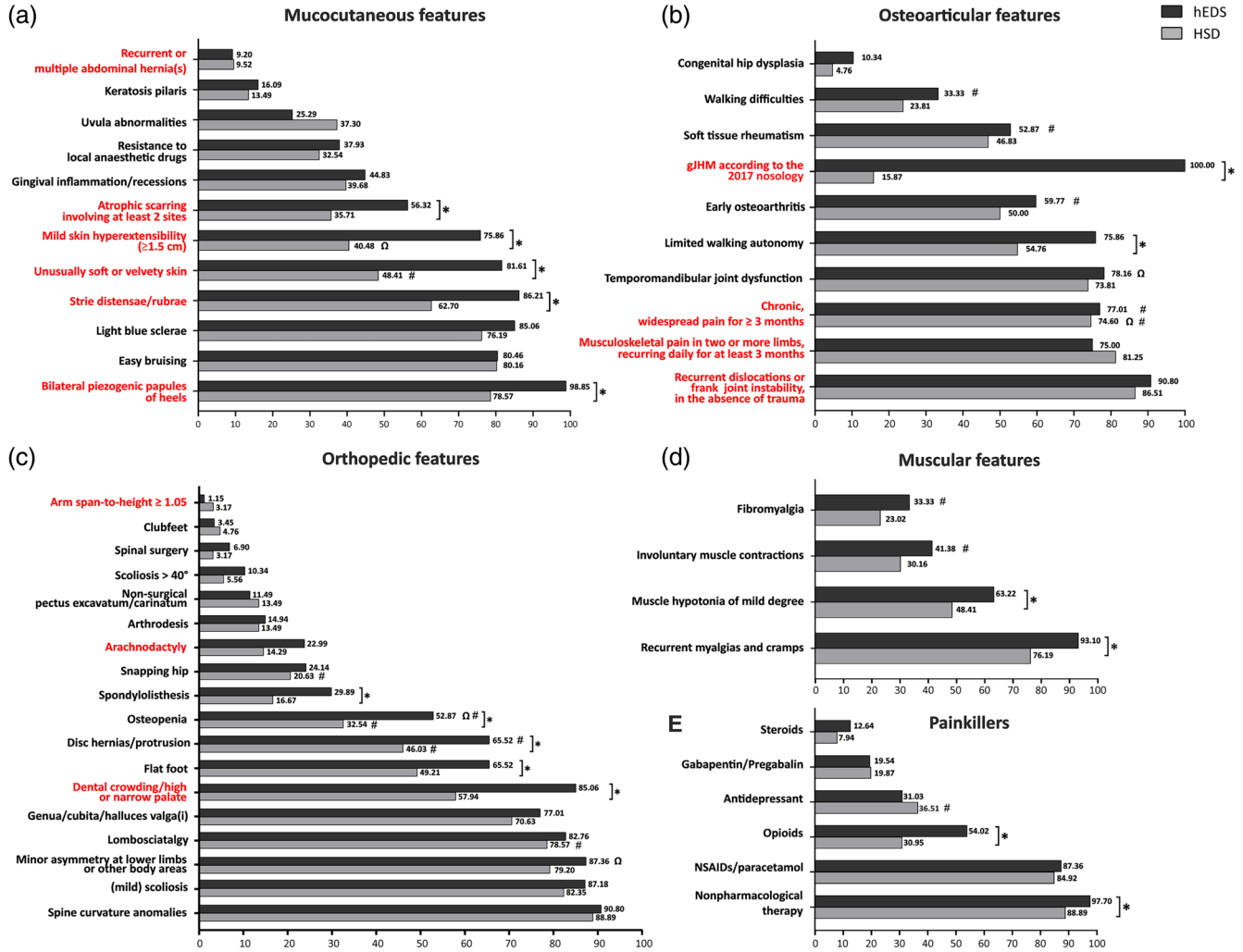

FIGURE 3 Frequencies of mucocutaneous (a), osteoarticular (b), orthopedic (c), and muscular (d) features and of painkiller (e) use in the 213 probands, including 87 hypermobile Ehlers-Danlos syndrome (hEDS) and 126 hypermobility spectrum disorders (HSD) individuals. *Presence of statistically significant differences between hEDS and HSD; Ω presence of statistically significant differences by age (<30 vs. ≥30 years); # presence of statistically significant differences by sex (for frequencies and *p*-values, see Additional Tables 13–15).

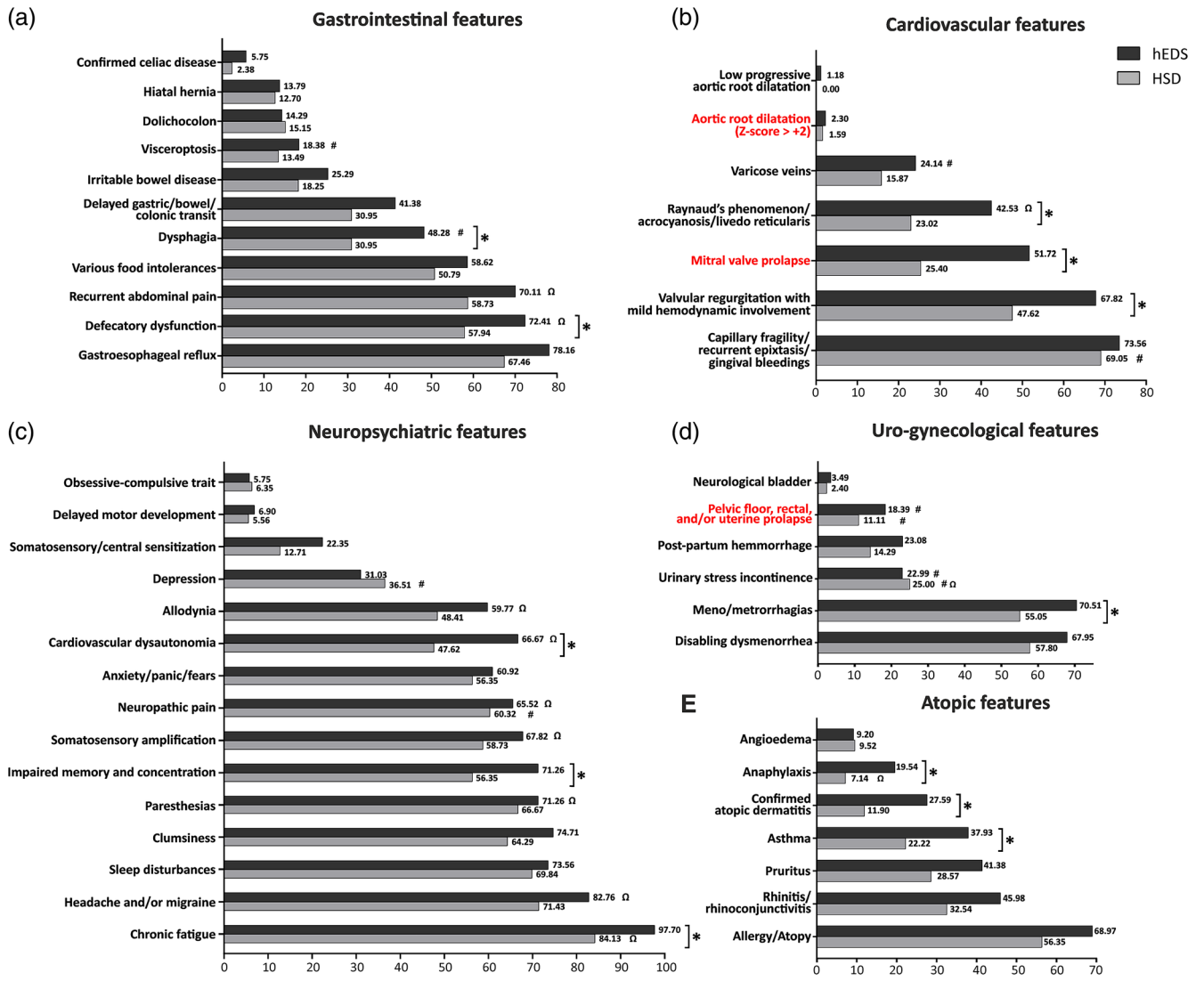

FIGURE 4 Frequencies of gastrointestinal (a), cardiovascular (b), neuropsychiatric (c), uro-gynecological (d), and atopic (e) features in the 213 probands, including 87 hypermobile Ehlers-Danlos syndrome (hEDS) and 126 hypermobility spectrum disorders (HSD) individuals. *Presence of statistically significant differences between hEDS and HSD; Ω presence of statistically significant differences by age (<30 vs. ≥30 years); # presence of statistically significant differences by sex (for frequencies and *p*-values, see Additional Tables 13–15).

mucocutaneous, osteoarticular, orthopedic, muscular, gastrointestinal, cardiovascular, neuropsychiatric, uro-gynecological, immunological/atopic, and ocular features in hEDS and HSD probands, which were analyzed by sex and age (<30 vs. ≥30 years). Additional Tables 13–15 show the corresponding frequencies and statistical significances.

3.4.1 | Mucocutaneous features

Besides the six diagnostic criteria included in feature A of criterion 2, that is, bilateral piezogenic papules (86.85%), striae distensae/rubrae (72.30%), unusually soft or velvety skin (61.97%), mild skin hyperextensibility (54.93%), atrophic scarring involving at least two sites (44.13%), and multiple abdominal hernias (9.39%), we assessed six additional mucocutaneous signs (Figure 3a). Among these, there was a high incidence of easy bruising (80.28%), light blue sclerae (79.81%), and gingival inflammation/recession (41.78%). Local anesthetic drug resistance (34.74%), uvula abnormalities (32.39%), and keratosis pilaris (14.55%) were less frequent. None of these items showed significant differences among hEDS and HSD, as well as nor sex or age biases.

3.4.2 | Osteoarticular features

Apart from criterion 1 (50.23%) and the three items of feature C, that is, musculoskeletal pain in two or more limbs, recurring daily for at least 3 months (78.85%); chronic/widespread pain for more ≥3 months (75.59%); recurrent joint dislocations/frank joint instability in the absence of trauma (88.26%), we documented six additional osteoarticular features (Figure 3b). Among these, temporomandibular joint dysfunction (75.59%), limited walking autonomy (63.30%), early

osteoarthritis (53.99%), and soft tissue rheumatism (49.30%), including bursitis, tendinitis, myofascial pain, epicondylitis, tenosynovitis, and plantar fasciitis, were common in all probands. Walking difficulties (27.70%) and congenital hip dysplasia (7.04%) were less frequent. With the exception of limited walking autonomy, which was more common in hEDS (75.86% vs. 54.76%), there were no significant differences among hEDS and HSD. In hEDS, temporomandibular joint dysfunction affected females more often (82.72% vs. 16.67%), whereas in HSD this issue was more frequent in males (87.50% vs. 71.82%), although not reaching a significant *p*-value. Concerning age-related differences, early osteoarthritis was more frequent in individuals aged ≥30 years both in hEDS (75.00% vs. 41.03%) and HSD (72.37% vs. 16.00%). In addition, older persons also suffered more commonly from soft tissue rheumatisms (hEDS: 64.58% vs. 38.46%; HSD: 53.95% vs. 36.00%) and had more walking difficulties (hEDS: 45.83% vs. 17.95%; HSD: 28.95% vs. 16.00%), though a statistical significance was observed only in hEDS.

3.4.3 | Orthopedic features

Apart from dental crowding and high or narrow palate (69.01%), arachnodactyly (17.84%), and marfanoid habitus (2.35%) included in feature A, we systematically assessed 15 additional orthopedic features (Figure 3c). The most common were spine curvature anomalies (cervical, dorsal, lumbar hyper[hypo]kyphosis/lordosis) (89.67%), mild scoliosis (84.26%), minor asymmetry at lower limbs/other body areas (82.55%), lombosciatalgy (80.28%), genua/cubita/halluces valga(i) (73.24%), pes planus (55.87%), disc hernias/protrusions (53.99%), and osteopenia (non-postmenopausal or early in men) (40.85%). Spondylolisthesis (22.07%), snapping hip (22.07%), arthrodesis (14.08%), nonsurgical pectus excavatum/carinatum (12.68%), scoliosis >40° (7.51%), spinal surgery (4.69%), and clubfeet (4.23%) were less frequent. Pes planus (65.52% vs. 49.21%), spondylolisthesis (29.89% vs. 16.67%), disc hernias/protrusions (65.52% vs. 46.03%), and osteopenia (52.87% vs. 32.54%) were more frequent in hEDS compared to HSD. Minor asymmetry at lower limbs/other body areas (90.12% vs. 50.00%) and osteopenia (56.79% vs. 0%) were more frequent in hEDS females. In both groups, disc hernias/protrusions (hEDS: 81.25% vs. 46.15%; HSD: 67.11% vs. 14.00%) and osteopenia (hEDS: 70.83% vs. 30.77%; HSD: 48.68% vs. 8.00%) were more prevalent in patients aged ≥30 years. Lombosciatalgy (88.16% vs. 64.00%) and snapping hip (27.63% vs. 10.00%) were also more common in older individuals, although with a significant *p*-value only in HSD. Other occasional findings comprised pes cavus, Morton's neuroma, sandal gap, chondromalacia patellae, patellofemoral pain syndrome, Tarlov cysts at the sacral level of the spine, winged scapulae, Madelung deformity of the distal radial physis, and atlanto-axial and cervical instability.

3.4.4 | Muscular features

Among the four investigated muscular signs (Figure 3d), recurrent myalgia and cramps (83.1%) and mild muscle hypotonia (54.66%) were the most common, followed by involuntary muscle contraction (34.74%) and fibromyalgia (27.2%). Recurrent myalgia and cramps (93.10% vs. 76.19%) and mild muscle hypotonia (63.22% vs. 48.41%) were both more frequent in hEDS. hEDS probands aged ≥30 years suffered more frequently from involuntary muscle contraction (56.25% vs. 23.08%) and fibromyalgia (47.92% vs. 15.38%). Sporadic issues were severe, progressive hyposthenia and muscle ruptures.

3.4.5 | Painkillers

The majority of probands, especially those with hEDS (97.70% vs. 88.89%), used physiotherapy to treat musculoskeletal and/or chronic pain (Figure 3e). Both in hEDS and HSD, on-demand use of NSAIDs and/or paracetamol (85.92%) was widespread and often daily. Instead, without any sex-and age-bias, opioid therapy was more common among hEDS probands (54.02% vs. 30.95%). Antidepressants like benzodiazepines were taken by 34.20% of probands, especially at an older age, not only for treating pain but also for anxiety/panic, sleep disturbances, and muscle relaxation. Anti-epileptic/anticonvulsant drugs (gabapentin and pregabalin) and steroids were used respectively by 19.72 and 9.86% of probands. Unfortunately, physiotherapy and painkillers were ineffective in most patients. A small subset of patients reported some therapeutic benefit from cannabis use.

3.4.6 | Gastrointestinal features

We evaluated 11 gastrointestinal features in all probands (Figure 4a). The most common issues were gastroesophageal reflux (71.83%), defecatory dysfunctions (63.85%), recurrent abdominal pain (63.38%), and various food intolerances (53.99%). Less common conditions included dysphagia (38.03%), delayed gastric/bowel/colonic transit (35.21%), irritable bowel disease (21.13%), visceroptosis (15.49%), dolichocolon (14.81%), and hiatal hernia (13.15%). Patients with confirmed celiac disease were very rare (3.76%). Dysphagia (48.28% vs. 30.95%) and defecatory dysfunctions (72.41% vs. 57.94%) were more frequent in hEDS compared to HSD. Defecatory dysfunctions (75.31% vs. 33.33%) and abdominal pain (74.07% vs. 16.67%) were more frequently referred by hEDS females. Visceroptosis was more common in patients aged ≥30 years, although a significant *p*-value was observed only in hEDS (27.08% vs. 7.69%). In the latter, dysphagia was also more frequent in older individuals (62.50% vs. 30.77%). Additional sporadic findings comprised Crohn's disease, ulcerative colitis, recurrent hemorrhoids, and gastroparesis.

3.4.7 | Cardiovascular features

We assessed five additional cardiovascular issues (Figure 4b) in addition to MVP (36.15%) and aortic root dilatation with Z-score >+2 (1.88%) included in feature A. Capillary fragility/recurrent epistaxis and/or gingival bleeding (70.89%) and valvular regurgitation with mild hemodynamic involvement (55.87%) were the most prevalent

problems, whereas Raynaud's phenomenon/acrocyanosis/livedo reticularis (30.99%), and varicose veins (19.25%) were less common. Low progressive aortic root dilatation was a sporadic observation. Valvular regurgitation (67.82% vs. 47.62%) and Raynaud's phenomenon/acrocyanosis/livedo reticularis (42.53% vs. 23.02%) were more frequent in hEDS than in HSD, with the latter also more common in females (45.68% vs. 0%). Varicose veins were seen more frequently in patients aged ≥30 years, with a significant *p*-value only in hEDS (37.50% vs. 7.69%). Capillary fragility was more frequent in older HSD probands (78.95% vs. 54.00%). Bicuspid aortic valve, patent foramen ovale, atrial septal aneurysm, aortic valve calcification, pulmonary artery dilatation, deep venous insufficiency, long QT syndrome, and Wolff-Parkinson-White syndrome were among sporadic findings. One hEDS female suffered from a hemorrhagic stroke, which left her severely disabled.

3.4.8 | Neuropsychiatric features

In both hEDS and HSD, neurological, psychological, and emotional dysfunctions were quite prevalent. The most frequent of the 16 documented issues (Figure 4c) was chronic fatigue (89.67%), which affected hEDS patients more significantly, although the majority of HSD probands (97.70% vs. 84.13%) also complained of it, especially females. Headache/migraine (76.06%), sleep disturbances (71.36%), paresthesia (68.54%), particularly tingling, pricking, and chilling, clumsiness (68.54%), somatosensory amplification (62.44%), impaired memory and concentration (62.44%), neuropathic pain (62.44%), anxiety/panic/fears (58.22%), and allodynia (53.08%) were additional frequent complaints. Except for impaired memory/concentration, which was more prevalent in hEDS (71.26% vs. 56.35%), all these features did not show any difference between hEDS and HSD. Most of these issues were more common in hEDS females, while neuropathic pain showed higher incidence in HSD patients aged ≥30 years. Cardiovascular dysautonomia was verified in 55.40% of probands with hEDS having a greater overall frequency (66.67% vs. 47.62%), especially in females. Nevertheless, among the numerous dysautonomia-related neurological, psychological, and gastrointestinal symptoms, orthostatic intolerance and POTS were found in both groups at comparable rates. Indeed, about 82% of either hEDS or HSD patients who underwent tilt-table testing resulted positive. Chronic depression affected around 34% of hEDS and HSD probands, especially those aged ≥30 years, although with a significant *p*-value only in HSD. Finally, somatosensory/central sensitization (16.75%), delayed motor development (7.51%), and obsessive-compulsive trait (6.10%) were less common. Attention-deficit/hyperactivity disorder, epileptic seizures, and Arnold Chiari malformation were sporadic observations.

3.4.9 | Uro-gynecological features

In addition to pelvic floor/rectal/uterine prolapse (14.08%) included in sign A, we systematically recorded 5 additional uro-gynecological issues (Figure 4d). Disabling dysmenorrhea (62.03%) and meno/metrorrhagia (61.05%) were highly prevalent traits among females of reproductive age, with the latter being more frequent in hEDS (70.51% vs. 55.05%). Then, 81 out of 191 women had at least one pregnancy and postpartum hemorrhage occurred in 15 of them (18.52%), with no difference between hEDS and HSD. Only two individuals had premature rupture of membranes with preterm birth, and there were no instances of uterine rupture. Urinary stress incontinence (24.17%) was more common in females and patients aged ≥30 years, with no difference among hEDS and HSD. Neurological bladder (2.84%), nocturnal polyuria, urinary retention, polycystic ovary syndrome, endometriosis, endometrial cysts, uterine fibromas, and pelvic or vulva varicose veins were uncommon and sporadic findings.

3.4.10 | Atopic features

Immunological concerns were quite common in both hEDS and HSD (Figure 4e). Indeed, 61.5% of probands showing one or more signs and symptoms of allergic/atopic disorders with an overall greater incidence in hEDS (68.97% vs. 56.35%), although this difference was not statistically significant. Recurrent rhinitis/rhinoconjunctivitis (38.03%) and pruritus (33.8%) were the most prevalent issues, followed by asthma (28.64%), confirmed atopic dermatitis (18.31%), and anaphylaxis (12.21%). These 3 latter features were all more prevalent in hEDS in a statistically significant manner (asthma: 37.93% vs. 22.22%; dermatitis: 27.59% vs. 11.90%; anaphylaxis: 19.54% vs. 7.14%), whereas the differences observed for rhinitis/rhinoconjunctivitis (45.98% vs. 32.54%) and pruritus (41.38% vs. 28.57%) did not reach a substantial *p*-value. Anaphylaxis was more common in HSD males (25% vs. 4.55%). Various allergens such as foods, pollen, animal dander, dust mites, insect venoms, metals (above all nickel), and medicines such as NSAID and antibiotics were identified as common triggers. One hEDS proband had multiple chemical sensitivity syndrome. Additional autoimmune/inflammatory disorders that have been previously diagnosed or clinically suspected included Hashimoto thyroiditis (7 hEDS, 11 HSD), Basedow's disease (1 hEDS), Sjögren syndrome (1 hEDS, 4 HSD), Behcet's disease, (1 HSD), Kawasaki disease (1 HSD), and antiphospholipid syndrome (2 hEDS). Besides, 9 patients reported an earlier diagnosis of seronegative spondyloarthritis, comprising ankylosing spondylitis (4 HSD, 2 hEDS), psoriatic arthritis (1 HSD), reactive arthritis (1 HSD), and enteropathic arthritis associated with inflammatory bowel disease (1 HSD). The most common referred features were asymmetrical oligoarthritis, generally in the lower limbs, varying degrees of inflammatory back pain, and enthesitis; an association with the major histocompatibility complex class 1 antigen HLA-B27 was recognized in 6 patients (4 HSD, 2 hEDS). Two probands (1 hEDS, 1 HSD) had previously been diagnosed with rheumatoid arthritis; however, no joint swelling or deformities were observed at examination, and they tested negative for both rheumatoid factor and anticyclic citrullinated peptide antibodies. Finally, antinuclear antibodies were sporadically found in 5 hEDS and 9 HSD probands, not confirmed at further analysis.

3.4.11 | Ocular features

Ocular features were rather uncommon in the entire probands' cohort (Additional Figure 5). Xerophthalmia (38.03%) and myopia >3 (31.46%) were the most common issues, followed by diplopia (15.96%), palpebral ptosis (10.80%), and strabismus (9.39%). These features did not show any difference among hEDS and HSD nor sex or age biases, except for diplopia that was more common in HSD patients aged <30 years.

3.4.12 | Cluster analysis based on all signs and symptoms

To determine whether probands fulfilling the 2017 hEDS nosology ($n = 87$) could be more clearly separated from those not fulfilling the criteria ($n = 126$), we conducted cluster analysis considering all signs and symptoms, including comorbidities, for each individual (Additional Table 1), rather than just the nosological criteria (Figure 2b). Specifically, we aimed to verify if including the entire set of clinical manifestations would result in more distinct clustering of the patients, or if there would still be a significant degree of overlap between hEDS and HSD.

Using silhouette analysis, we determined that $k = 7$ (SS = 0.96) represented the optimal number of clusters (Figure 5). Notably, none of these seven clusters exclusively contained patients from a single diagnostic category (Additional Table 16), highlighting once again the significant overlap in phenotype between hEDS and HSD. Among the clusters, cluster 2 (in purple) and cluster 7 (in green) were the most distant, potentially representing the most extreme variation in phenotype. Cluster 2, which comprised 16 out of the 87 hEDS probands (18.39%) and only 1 out of the 126 HSD individuals, emerged as the most severe end of the spectrum. Indeed, this cluster was characterized by the highest BS average (5.71, range 3–9) and the highest number of signs and symptoms across the organ systems, with mean values of 7.76 (range 6–10) for mucocutaneous, 7.24 (range 5–8) for osteoarticular, 10.29 (range 7–13) for orthopedic, 3.41 (range 2–4) for muscular, 6.94 (range 5–9) for gastrointestinal, 3.88 (range 2–5) for cardiovascular, 11.46 (range 8–15) for neuropsychiatric, 3.35 (range 2–5) for uro-gynecological, 3.59 (range 1–7) for atopic, and 2.0 (range 0–4) for ocular features, respectively. In contrast, cluster 7, with an average BS of 4.38 (range 1–9), exhibited the lowest means of clinical manifestations across all organ systems, placing it at the milder end of the phenotypic spectrum. Indeed, the mean numbers of mucocutaneous, osteoarticular, orthopedic, muscular, gastrointestinal, cardiovascular, neuropsychiatric, uro-gynecological, atopic, and ocular features were 5 (range 3–8), 2.44 (range 0–6), 4.16 (range 0–8), 0.97 (range 0–4), 1.25 (range 0–4), 1.56 (range 0–3), 2.81 (range 0–7), 0.62 (range 0–2), 1 (range 0–5), and 0.44 (range 0–2), respectively. Notably, cluster 7 included 23 out the 126 HSD patients (18.25%) but also 9 out of the 87 hEDS probands (10.34%), thus demonstrating that patients may exhibit a phenotype with a relatively low multisystemic involvement regardless of whether or not they fulfill the 2017 hEDS diagnostic criteria.

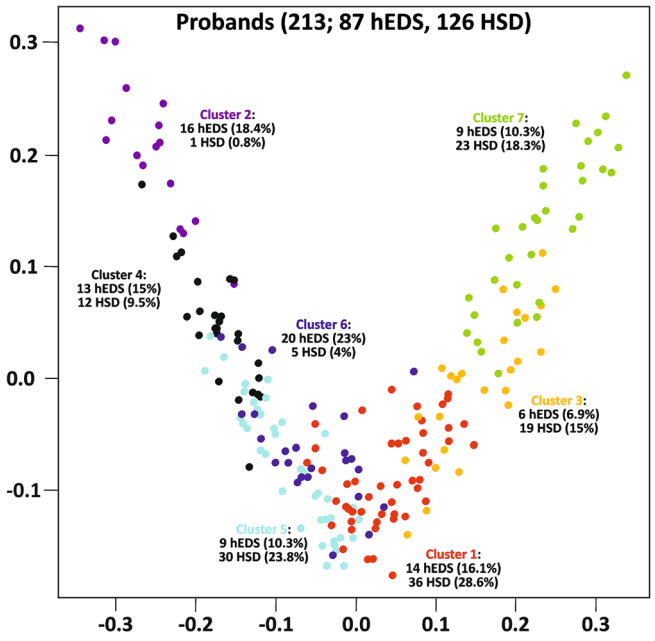

FIGURE 5 Multidimensional scaling plot of the clustered data providing a visual representation of the pattern of proximities among data, where each point (colored respective to the cluster to which it belongs) corresponds to an observation (patient). The two-dimensional plot is based on the proximity matrix extracted from the unsupervised random forest, and the points are visualized such that the distances between them approximate their multivariate dissimilarity as closely as possible. Cluster analysis was performed on all signs and symptoms of the 213 probands, including 87 hypermobile Ehlers-Danlos syndrome (hEDS) and 126 hypermobility spectrum disorders (HSD) individuals. The optimal number (*k*) of clusters was identified using the silhouette method, based on the following variables and scores: BS (from 0 to 9) and the counts of mucocutaneous (from 0 to 12), osteoarticular (from 0 to 9), orthopedic (from 0 to 18), muscular (from 0 to 4), gastrointestinal (from 0 to 10), cardiovascular (from 0 to 7), neuropsychiatric (from 0 to 15), uro-gynecological, atopic (from 0 to 7), and ocular features (from 0 to 5). The percentages of hEDS and HSD patients falling into the different clusters were calculated with respect to the total cohorts (for descriptive statistics related to the variables that generated the clusters, see Additional Table 16).

Adjacent to these two clusters that delineate the outer edges of the phenotypic spectrum in our cohort, we identified cluster 4 (in black) that was positioned near cluster 2, and cluster 3 (in orange) located proximate to cluster 7. Cluster 4 was characterized by a mean BS of 4.28 (range 2–8) and encompassed 15% of the entire hEDS cohort (13/87) and about 10% of those with HSD (13/126). When comparing the mean numbers of signs and symptoms across organ systems, we found that they were similar to those observed in cluster 2, although slightly lower (see Additional Table 16 for details). In contrast, cluster 3, which included 15.7% of the entire HSD cohort (19/126) and 6.89% (6/87) of the hEDS patients was characterized by an average BS of 3.32 (range 1–9) and by mean number of clinical manifestations similar to those observed in cluster 7, although slightly higher (see Additional Table 16 for details).

More than 50% of the entire probands (114/213) fell into the last three clusters, that is, clusters 1, 5, and 6, which partly overlapped not only with each other but also with clusters 3 and 4. In particular, cluster 1 (in red) and 5 (in light blue) were the most enriched, with cluster 1 including 28.57% of all HSD patients (36/126) and 16.09% (14/87) of those with hEDS, and cluster 5 comprising 23.81% of all HSD patients (30/126) and 10.34% of those with hEDS (9/87). Cluster 1 was distinguished by an average BS of 3.80 (range 1–7) and a relatively high number of signs and symptoms, which was slightly lower compared to clusters 2 and 4 but higher than clusters 3 and 7. Indeed, the mean numbers of mucocutaneous, osteoarticular, orthopedic, muscular, gastrointestinal, cardiovascular, neuropsychiatric, urogynecological, atopic, and ocular features in cluster 1 were 5.82 (range 2–9), 5.24 (range 1–8), 7.64 (range 4–11), 1.8 (range 0–4), 2.24 (range 0–6), 1.6 (range 0–5), 7.6 (range 1–12), 1.48 (range 0–4), 1.56 (range 0–5), and 0.78 (range 0–4), respectively. Patients in cluster 5 showed a similar mean of BS (3.59, range 1–7) and overall multisystemic involvement as those in cluster 1, but with higher mean numbers of gastrointestinal (5.62, range 2–8), neuropsychiatric (10.79, range 7–13), and atopic (mean 2.21, range 0–5) features. Finally, cluster 6 (in blue) encompassed the highest percentage of hEDS probands (20/87, 22.98%), as well as a small percentage of those with HSD (5/126, 3.97%). The overall characteristics of this cluster were similar to cluster 2, with patients exhibiting considerable multisystemic involvement across all organ systems (see Additional Table 16 for details) but showing a higher mean of BS (5.64, range 1–9).

In summary, cluster analysis confirmed the substantial overlap in the clinical manifestations of hEDS and HSD patients, highlighting the complex and heterogeneous nature of these two diagnostic categories. While the question of whether hEDS and HSD should be viewed as distinct clinical entities or as part of a broader spectrum remains open, our findings support the need to look beyond current classifications systems. A more unifying framework is required to advance both scientific understanding and clinical practice in this field.

4 | DISCUSSION

In this study, we conducted a retrospective evaluation of 327 Italian patients from 213 families with a prior diagnosis of ht-EDS or JHS based, respectively, on the Villefranche (Beighton et al., 1998) and Brighton criteria (Grahame et al., 2000). Our results provide additional evidence about the limited reliability of the 2017 criteria in distinguishing the severity of symptoms between patients with a diagnosis of hEDS and HSD, as well as the prevalence of extra-articular manifestations. Overall, our results offer an exhaustive clinical context to the ongoing dispute over whether hEDS and HSD should be categorized as distinct disorders or as variants of the same condition. Based on the clinical experience presented here, our previously published findings, showing a common myofibroblast-like cellular phenotype and dysregulated transcriptional profile in hEDS and HSD patients' dermal fibroblasts (Ritelli et al., 2022; Zoppi et al., 2018), and ongoing in vivo studies, we firmly believe that the majority of individuals classified as hEDS and HSD should be placed along a continuous phenotypic spectrum. Considering the clinical and molecular evidence that we have gathered, we advocate for a revision of the 2017 diagnostic criteria. It is our belief that the criteria should be made less stringent to include a greater number of patients who are currently encompassed within the blanket HSD category. By broadening criteria, it will be possible to more accurately capture the diverse range of symptoms and manifestations present within the hEDS and HSD spectrum, ultimately leading to improved diagnostic accuracy and enhanced patient care.

The fact that the current diagnostic criteria are too stringent to capture all patients with hEDS is corroborated by the observation that only ∼35% of our patients of the entire cohort and ∼41% of the probands previously diagnosed as JHS/ht-EDS met the new criteria for hEDS. The Villefranche nosology and 2017 hEDS criteria showed a higher consistency, with ∼80% of patients meeting both, compared to the Brighton criteria, with only 34–41% of JHS patients/probands fulfilling the new hEDS criteria. These differences in mapping across criteria highlight the lack of clarity and uniformity in how these hypermobility conditions were and are identified and classified either in the past or currently (Castori, 2021; Malfait et al., 2020; Tinkle et al., 2009, 2017). In fact, the new 2017 diagnostic criteria aimed to capture a group of homogenous patients with a similar phenotypic presentation to facilitate genomic discoveries but in a syndrome that has widely been recognized to have a vast inter—and intrafamilial variability (Castori, 2021; Hakim et al., 2021; Tinkle et al., 2017), as demonstrated also in the present work.

Of the three mandatory criteria of the 2017 nosology, the main differentiating factor between an hEDS versus HSD diagnosis in our cohort was the BS cut-off for gJHM, with, respectively, ∼57% of patients and ∼50% of probands not meeting criterion 1 and with only ∼13–16% of patients/probands classified as gHSD. Furthermore, among individuals with HSD who did not fulfill criterion 1, ∼45% of patients and ∼39% of probands met both criteria 2 and 3. Remarkably, ∼21% of both these patients/probands had a BS that was just one-point below the age-specific cut-off. As all these HSD patients tested positive for the 5PQ, they could potentially have been diagnosed as hEDS according to recent papers reporting that a diagnosis of gJHM can be made in such cases (Malfait et al., 2020; McGillis et al., 2020; Yew et al., 2021). In this study, we did not adopt this reframing of the 2017 nosology, which, according to our understanding, stated that the 5PQ should only be used in assessing gJHM in those with acquired joint restrictions (Malfait et al., 2017). Despite some conflicting evidence regarding the reliability of the 5PQ and the fact that it has been only validated for adults (Juul-Kristensen et al., 2017), we support its use but strongly recommend that the wider inclusion of patients scoring one-point below the BS cut-off should not be left to personal interpretation but should be explicitly stated. Even with such an inclusion of the 5PQ, criterion 1 remains too stringent and limited to identify patients, often highly symptomatic, who do not meet the BS, especially those with hypermobility in joints not assessed. We believe that these patients do not have a condition other than hEDS and that further research on alternative or highly standardized revised

methods, questionnaires, and criteria to diagnose gJHM is necessary to address issues of lack of validity, subjectivity, inconsistency, and limited assessment scope. It should be noted that the BS, which is currently the accepted method to assess for JHM in the diagnosis of all EDS forms (Juul-Kristensen et al., 2017), was first designed for epidemiological screening purposes in children, not for comprehensive clinical assessment. An advantage of the BS is that it is quick to perform and ideally requires a goniometer only when joint range is ambiguous. However, despite previous research showing good correlation of gJHM with joints tested on the BS system (Smits-Engelsman et al., 2011), it has several substantial limitations including subjective interpretation by practitioners and intrinsic technical inaccuracies that vary depending on the specific physician's experience with the BS. The lack of agreement among practitioners on the appropriate approach for assessing JHM in the BS, such as measuring thumb/wrist mobility with elbow bent versus straight, further restricts accuracy and validity (Remvig et al., 2014). Additionally, the BS is strongly upper limb biased, comprises a restricted number of joints, and evaluates motion in just the sagittal plane of movement. The most major shortcoming of the BS indeed is that it excludes the joints most usually described as unstable by patients, such as shoulder, foot/ankle, and patellofemoral joints (Nicholson et al., 2022). The recently proposed lower limb assessment score (LLAS) and upper limb hypermobility assessment tool (ULHAT), which are both 12-item tests covering the major joints of the upper and lower limbs in multiple planes of movement (Meyer et al., 2017; Nicholson & Chan, 2018), might be effective alternatives, even if these multidimensional examinations need standard operating methods, expert management, and further psychometric testing for validation. Finally, because of developmental changes and evolving phenotype, assessing joint range of motion of infants and children is significantly more complex, and no assessment tools for gJHM have been validated for those under 5 years old (Nicholson et al., 2022).

In our cohort, about 40% of all patients and probands did not fulfill criterion 2, indicating that the specific design of this criterion, which requires a combination among multisystemic involvement (feature A), positive family history with a first-degree relative who independently meets the hEDS criteria (feature B), and musculoskeletal complaints (feature C), is also excessively stringent, resulting in an excess of exclusions of highly symptomatic patients. Given that in our cohort feature A + C was the most common combination resulting in criterion 2 positivity and that no significant differences in musculoskeletal manifestations were observed between patients fulfilling or not the 2017 criteria for hEDS, the main reason for criterion 2 negativity is the lack of the five requested items of feature A. Moreover, based on our observation that about 50% of all families with more than one affected member showed co-segregation of both hEDS and HSD, we strongly believe that feature B should be given far more weight than it is currently, especially for symptomatic patients who do not fulfill criterion 1. In our cohort, for example, 10 patients from different families showed the combination of all three features of criterion 2 but would currently be classified as HSD only due to the lack of a proper BS. In our experience, similar circumstances occurred regularly since the introduction of 2017 diagnostic criteria, and patients find this extremely difficult to understand and accept. To reduce future significant psychological and social consequences for patients who do not meet the new diagnostic criteria but have a first degree-relative who does, we suggest that a diagnosis of hEDS could be made based on a positive 5PQ, regardless of their BS, as long as they have at least one musculoskeletal manifestation (feature C), with or without fulfilling feature A and/or showing other extra-articular manifestation or comorbidities. The same approach of using the 5PQ could also be taken for patients, overall rare and typically males, with a positive family history and insufficient BS who do not show musculoskeletal complications but exhibit multisystemic involvement. Finally, we advise that individuals who have an hEDS relative and show reliable and objective multisystemic involvement, as well as musculoskeletal complaints, should be diagnosed as hEDS, even in the absence of a proper BS. The proposal to assign more importance to positive family history is consistent with the most plausible genetic explanation for both hEDS and HSD. While it is still possible that hEDS (as well as HSD) may be associated with variation in a single gene with many alleles and small effects of multiple modifier genes, it is much more likely that hEDS/HSD represents a set of oligogenic conditions resulting from the simultaneous presence of multiple low-penetrant alleles of a few or several genes. In this view, the tendency toward reduced BSs and numbers of multisystemic signs and symptoms in secondary family members provides strong evidence for the possibility of a complex genetic condition involving multiple genes that interact with environmental factors. Recognizing families in which hEDS and HSD run together is central for family-based multi-omics studies that are extremely valuable in understanding the genetic susceptibilities and molecular basis of hEDS/HSD, as the background genetic variation and environmental exposures are controlled to some extent.

Regarding the systemic manifestations included in feature A, our study revealed inconsistent results in patients fulfilling the new hEDS diagnostic criteria versus those that did not, suggesting that many are listed with limitations, also considering that age and sex influence the development of several of those manifestations. In both the entire cohort and in probands, we found that all cutaneous signs, that is, unusually soft/velvety skin, mild skin hyperextensibility, atrophic scarring, abnormal striae, and bilateral piezogenic papules, as well as dental crowding and high or narrow palate and MVP, occurred statistically more frequently among patients who met criteria than those who did not. Despite this, it is worth noting that even in the latter group of patients all these signs were quite common as well, with all except atrophic scarring and MVP showing a prevalence of over 40%. The remaining five items were overall less frequent and showed no statistical difference between hEDS and HSD. These findings must be interpreted with caution due to the design of the criteria, which by requiring at least five such features, intrinsically increases the likelihood of the presence of systemic manifestations in those meeting the criteria. On the other hand, if the criteria for diagnosing hEDS were completely sensitive and specific, most, if not all, disease-defining traits would be expected to be more frequent among those diagnosed,

but this is clearly not the case. In addition, the inclusion of uncommon (arachnodactyly, pelvic floor, rectal, and/or uterine prolapse, and recurrent or multiple abdominal hernias) and sporadic traits (marfanoid habitus and aortic root dilatation) broadens the diagnostic spectrum, further complicating the process of delineating hEDS. Apart from the strictness of the BS, the other main limitations of the 2017 criteria are the low level of specificity associated with many items listed in feature A, along with the subjective nature of their assessment. Indeed, several traits used to define hEDS, especially the cutaneous signs such as soft or velvety skin, mild skin hyperextensibility, atrophic scarring, unusual striae, and piezogenic papules, do not have clear, objective definitions or can vary in personal interpretation. These traits are also commonly observed in other EDS types and related HCTDs that need to be differentiated from hEDS/HSD (Colombi et al., 2015; Ritelli, Rovati, et al., 2020; Ritelli, Venturini, et al., 2020; Tinkle et al., 2017), and are even not unusual in the general population, such as piezogenic papules and striae distensae (Borrelli et al., 2021; Brown & Cook, 2022). Furthermore, different multisystemic manifestations of features A are not appropriate in the evaluation of children and younger people. Atrophic scarring, for example, cannot be detected in a young child who has not yet developed skin tears, dental crowding cannot be determined in a child who has not yet erupted all adult teeth, and stretch marks and recurring hernias do not usually present at a young age. Further hindering hEDS diagnosis at young age, the current criterion of a BS ≥6 might underestimate the presence of gJHM children aged >8 years in the general population (Nicholson et al., 2022; Singh et al., 2017). Some of our concerns about the limited validity of the 2017 criteria for pediatric patients were recently addressed in a paper published by the Pediatric Working Group of the International Consortium on EDS and HSD (Tofts et al., 2023). This publication introduces a specific framework tailored for children from 5 years of age until biological maturity, aiming to provide a more suitable approach for diagnosing pediatric JHM in this population. In particular, the BS cut off at 6/9 is retained, positive family history is eliminated, as well as half of the feature A items, atrophic scars are reduced to 1 site, the musculoskeletal complications are adjusted for the specific age range, and some core comorbidities with established diagnostic definitions (i.e., chronic primary pain, chronic fatigue, functional gastrointestinal and bladder disorders, primary dysautonomia, and anxiety) are identified at the end of the criteria, although they do not influence the diagnosis overall. The framework, which has four main categories, with or without skin involvement which then yield 8 in total, is intended to be fluid, allowing for changes in subtype as a child's JHM and symptoms evolve. Finally, the authors highlight that the framework can be used until adolescents reach skeletal maturity or at their 18th birthday, whichever occurs first. Following that milestone, patients should be assessed by using the 2017 criteria until revised hEDS diagnostic criteria for adults are developed, as announced on the EDS society's website. In this view, our clinical findings from a large cohort of mostly adults may be useful for an evidence-based improvement of these diagnostic criteria.

According to our opinion, criterion 2 is deficient in terms of both objective multisystemic signs and specific symptoms needed to distinguish hEDS/HSD from other HCTDs or complex chronic diseases. We also believe that feature A places too much emphasis on cutaneous signs, and certain items too are rare to be included, that is, arm span-to-height ≥1.05 and aortic root dilatation with Z-score >+2. Moreover, we highlight the need for a more precise description of some current feature A items. For example, the present definition of pedal piezogenic papules is inadequate, which limits the efficiency of the 2017 criteria in establishing a connection between these papules and HCTDs. We agree with other authors that the presence of pain, as well as the number and size of the papules, must be considered in the diagnostic criteria to distinguish between normal and pathological papules (Aubry-Rozier et al., 2021; McGillis et al., 2020). It should be noted that we did not apply these standards in the reclassification of the patients, as we considered this item positive in the presence of at least two papules (including small ones); however, by considering this feature positive only when at least eight painful papules were present, we observed a decrease in prevalence of about 60% in both hEDS and HSD (Additional Table 17). For a more reliable evaluation of skin hyperextensibility, we propose that its assessment should be expanded beyond the volar surface of the non-dominant forearm (Malfait et al., 2017) to include neck, dorsum of hand, chest, abdomen, elbows, and knees, which are the sites that we routinely assess for all patients with an EDS suspicion (Colombi et al., 2017). To consider skin hyperextensible, it should be positively stretched in at least three of these areas, with a minimum of 2 cm for the neck, elbows, and knees, and 1.5 cm for all other sites.

The application of point-score systems to divide the JHS/ht-EDS/hEDS/HSD group into two categories has not been shown to be useful clinically or in research, and our results underline the difficulty in identifying two coherent groups. Although we believe that the path forward will lay in diagnostic laboratory testing rather than phenotype grouping, we will make an attempt here to suggest changes to the existing clinical criteria that remain essential for patients' evaluation and prompting any confirmatory testing. Overall, we recommend a significant restructuring of criterion 2 by implementing a revised cumulative scoring system within an expanded feature A, while removing positive family history, which must be considered outside criterion 2, as detailed above. Additionally, we propose that specific signs and symptoms should carry higher diagnostic importance compared to others. Based on the prevalence of observed signs and symptoms in our cohort, along with cluster analysis and review of relevant literature (Aubry-Rozier et al., 2021; Copetti et al., 2019; Demmler et al., 2019; Gensemer et al., 2021; Kumskova et al., 2023; Malfait et al., 2020; Martinez et al., 2021; McGillis et al., 2020; Morlino et al., 2019; Pietri-Toro et al., 2023; Rashed et al., 2022; Robbins, 2022; Scicluna et al., 2021; Yew et al., 2021), we suggest assigning a diagnostic value of two points to the following items: unusually soft or velvety skin; atypical striae distensae/rubrae; mild skin hyperextensibility in at least three sites; easy bruising; capillary fragility, recurrent epistaxis and/or gingival bleedings; dental crowding and high or narrow palate; genua valga, cubita valga and/or halluces valgi; spine curvature anomalies (scoliosis/kyphosis/lordosis); and temporomandibular joint dysfunction. On the other hand, abnormal

atrophic scarring; bilateral pathogenic piezogenic papules of the heels; recurrent or multiple abdominal hernia(s); bilateral pes planus/pes planovalgus; non-postmenopausal osteopenia (early in men); early osteoarthritis; disc hernias/protrusions; spondylolisthesis; arachnodactyly; recurrent soft-tissue rheumatisms; recurrent myalgias and cramps; MVP; Raynaud's phenomenon, acrocyanosis, and/or livedo reticularis; pelvic floor, rectal, and/or uterine prolapse; and meno/metrorrhagias and/or disabling dysmenorrhea, should be assigned a diagnostic value of 1 point. To meet the new criterion 2, individuals should have a minimum score of 14 points out of 33 for females (13 out of 32 for males) in the updated feature A, along with at least one musculoskeletal manifestation from the current feature C, which can remain unchanged. We recognize that some of the multisystemic signs and symptoms that we propose adding to a new feature A are also seen in other EDS forms (Malfait et al., 2020; Ritelli, Rovati, et al., 2020; Ritelli, Venturini, et al., 2020) and in inherited and acquired CTDs, including rheumatological conditions (Hakim et al., 2021). However, these features are commonly seen both in hEDS and HSD and their inclusion should provide a more comprehensive view of the patients' clinical manifestations and aid in clinical diagnosis. By combining this revised criterion with a less stringent criterion 1, such as wider use of the 5PQ, potential implementation of alternative assessment tools for JHM, and a greater emphasis on family history, a significant reduction in the number of patients currently diagnosed with HSD is expected. To test this hypothesis, we applied our proposed diagnostic criteria to all adult probands of our cohort, which included 78 individuals classified as hEDS and 112 as HSD based on the 2017 criteria (Additional Table 17). By applying the novel weighted cumulative scoring system for feature A and considering patients who scored one-point below the BS cut-off as positive for criterion 1 when they met the 5PQ, we identified 110 individuals who meet the revised criteria, which marks an increase of 33 hEDS diagnoses compared to the 2017 criteria. Upon closer examination, of these 33 newly classified hEDS patients, 12 were positive based on the novel criterion 2 (these individuals correspond to those classified ad gHSD according to the 2017 criteria), 12 were positive for both the novel criterion 2 and the 5PQ-adapted criterion 1, while 9 turned positive due to the less stringent criterion 1. Concerning the 80 patients not fulfilling the proposed updated diagnostic criteria, 9 of them were positive for the new criterion 1 but did not fulfill the novel criterion 2, while 71 patients were negative for the absence of a proper BS (mean 2.2; range 1–3), despite having a positive 5PQ. Notably, among the former patients, there was one male (patient n. 32 in Additional Table 17) who was initially classified as hEDS based on the 2017 criteria. However, according to the new feature A, this patient only scored 11 points, falling short of meeting the revised criteria. It is important to highlight that all the other patients who were classified as hEDS based on the 2017 criteria also satisfied the criteria proposed in the revised framework. Of the 71 patients not fulfilling the new criterion 1, only 10 patients were also negative for the novel criterion 2, while the remaining 61 patients met the revised criterion 2. It is noteworthy that more than 50% of these patients (34/61) were initially negative for criterion 2 of the 2017 nosology. Overall, these findings reinforce our idea that the BS may be insufficient for a thorough assessment of JHM. Therefore, we strongly advocate for further research on alternative methods, such as the ULHAT and the LLAS. It is reasonable to expect that by using these 12-items tests assessing joints in multiple planes of movement, the number of patients fulfilling the hEDS diagnostic criteria will rise even further. This aspect is critical in our opinion since the strictness of the 2017 criteria has already caused considerable confusion among researchers, clinicians, and, most importantly, those who live with these conditions.

Regarding other comorbidities such as chronic fatigue, dysautonomia (e.g., orthostatic intolerance, POTS), psychological distress (e.g., anxiety, sleep disturbances, impaired memory/concentration, depression), functional gastrointestinal and bladder disorders, and allergic/atopic features, we found that, with a few exceptions, the presence of individual comorbid features was no more or less frequent in hEDS and HSD, in line with other reports (Brock et al., 2021; Celletti et al., 2020; Hakim et al., 2021; Lam et al., 2021; Mathias et al., 2021; McGillis et al., 2020; Ruiz Maya et al., 2021; Wasim et al., 2019). In addition, many of these comorbidities are also frequently observed in other EDS forms, such as classical and vascular EDS, as well as rarer types (Malfait et al., 2020; Ritelli, Rovati, et al., 2020; Ritelli, Venturini, et al., 2020), and in other chronic pain and fatigue conditions (Hakim et al., 2021). Although more research is needed to determine whether certain comorbidities are more common in hEDS and HSD compared to these other diseases, these data strongly suggest that the existing criteria do not define a group in which comorbid features are more common. A fundamental challenge when dealing with comorbidities is that they are often interrelated and influence each other, rather than occurring independently. This makes it difficult to isolate the effects of any single condition. For instance, in chronic fatigue syndrome, widespread musculoskeletal pain and fatigue often coexist and may have reciprocal relationships. Pain can exacerbate fatigue, while fatigue can increase pain sensitivity. Dysautonomia is also common and can impact pain and fatigue levels. Autonomic dysfunction may underlie both musculoskeletal pain and fatigue (Mathias et al., 2021; Ruiz Maya et al., 2021). Psychiatric and psychological comorbidities like depression and anxiety also frequently co-occur and can worsen physical symptoms (Baeza-Velasco et al., 2021; Bulbena-Cabré et al., 2021). However, it remains unclear whether they are a cause or consequence of the chronic fatigue. Thus, the high frequency of comorbidities in chronic illnesses like chronic fatigue syndrome or hEDS/HSD present challenges for researchers trying to identify primary causes and effective treatments. Another concern is the variability in presentation. The vague symptomatology results in patients with different phenotypes being grouped together in studies, leading to conclusions drawn in the presence of confounding variables and unclear patients' selection. This represents a crucial gap in current knowledge, particularly in hEDS and HSD, compounded by the past variations in patients' classifications and related published clinical research. Addressing these issues requires large epidemiological studies in homogeneous populations as possible, advancements in biomarkers, and improved theoretical models of comorbidity. Since it is crucial to further raise awareness among both researchers and

clinicians about comorbidities in hEDS and HSD, we recommend that they should all be flagged in any hEDS criteria and must be assessed based on their established diagnostic definitions, in line with the recent published framework for pediatric JHM (Tofts et al., 2023). Notably, while comorbidities do not influence the diagnosis overall, they are highly significant in corroborating the diagnosis and determining the optimal management plan for each individual patient. Indeed, the diverse range of symptoms and comorbidities observed in our patients' cohort highlights the need for personalized multidisciplinary management approaches. A typical multidisciplinary team should include medical geneticists to confirm diagnosis, provide genetic counseling, and identify associated conditions; rheumatologists to evaluate and manage joint pain, instability, and other orthopedic issues; pain specialists to develop pain management strategies; physiotherapists to develop tailored exercise programs that minimize joint strain; neurologists to evaluate neurological symptoms and provide treatment for conditions such as headaches and neuropathic pain; psychiatrists to diagnose and medically manage mental health conditions through pharmacological interventions; psychotherapists and psychologists to develop coping strategies and lifestyle interventions for managing anxiety, depression, and other mental health challenges; gastroenterologists to assess and treat gastrointestinal issues such as dysmotility, nausea, vomiting, and abdominal pain; nutritionists to recommend dietary changes that may alleviate symptoms and promote overall health; hematologists to evaluate and manage easy bruising and excessive bleeding; and cardiologists to monitor for signs of vascular abnormalities, heart valve problems, and rhythm issues. Each specialist contributes unique expertise, perspectives and interventions that collectively aim to improve patients' quality of life by broadly targeting their multiple sources of suffering (Atwell et al., 2021; Demes et al., 2020; Estrella & Frazier, 2023; Spanhove et al., 2023; Yew et al., 2021).

5 | CONCLUSION

hEDS remains a complex and elusive condition that continues to pose diagnostic challenges despite the updated diagnostic criteria introduced in Malfait et al. (2017). Similarly, HSD does not have a definite set of diagnostic criteria and is still in the process of evolving, resulting in a lack of consensus in its diagnosis (Castori et al., 2017). Perspectives from patients living with undiagnosed or misdiagnosed hEDS and HSD highlight the personal and social impact of this diagnostic challenge and point to real-world solutions. Until and unless greater scientific consensus and physician education converge, patients with hEDS and HSD will continue navigating a diagnostic wasteland of uncertainty, disadvantage, and vulnerability. This is especially true for HSD patients living in nations with a public healthcare system, such as Italy, where, unlike hEDS, HSD is not officially recognized and thus exemptions are not provided, creating debilitating care gaps, financial hardship, and injustices leading to psychological distress for patients diagnosed with this condition, further exacerbating their poor quality of life. Based on our clinical experience and cellular and molecular evidence we have gathered on hEDS and HSD patients' fibroblasts (Ritelli et al., 2022; Zoppi et al., 2018) and in ongoing in vivo studies, we propose revising the 2017 diagnostic criteria for hEDS to be more inclusive, using the latest knowledge about clinical findings and laboratory abnormalities. Expanding the diagnostic criteria could officially recognize a wider spectrum of patients who deserve an official diagnosis and appropriate care and support, while constraining the vague HSD umbrella term. Broadening the hEDS diagnostic criteria would have important practical implications as well as benefits for research. Indeed, it would provide patients better access to medical services, resources, and social support tailored to hEDS. It could also enable larger studies that would yield more robust findings, accelerate progress in genomics research for understanding etiology, expedite the development and validation of biological markers for diagnosis and prognosis, and hasten the identification of new treatments. The benefits of this approach could be substantial for moving science and medicine forward to better support patients that is indeed more important than diagnostic labels. Once the genetic basis of hEDS/HSD will be elucidated or specific biomarkers will be validated, the spectrum of the phenotype will undoubtedly evolve, regardless of any particular diagnostic label. The primary goal, however, remains the development of treatment strategies that target patients' symptoms and experiences, and improving their quality of life is of greater importance than debates over diagnostic criteria. While diagnosis provides context, it is the patients' well-being that matters most.

AUTHOR CONTRIBUTIONS

All authors have contributed to this article significantly. Conceptualization, Marco Ritelli and Marina Colombi; formal analysis, Valeria Cinquina, Marika Vezzoli, Nicola Chiarelli, and Marco Ritelli; investigation, Marco Ritelli, Nicola Chiarelli, Valeria Cinquina, Marika Vezzoli, and Marina Colombi; resources, Marina Venturini and Marina Colombi; data curation, Marco Ritelli, Valeria Cinquina, Marika Vezzoli, and Marina Colombi; writing-original draft preparation, Marco Ritelli and Nicola Chiarelli; writing-review and editing, Nicola Chiarelli, Valeria Cinquina, Marika Vezzoli, Marina Venturini, and Marina Colombi; visualization, Valeria Cinquina and Marika Vezzoli; supervision, Marco Ritelli and Marina Colombi; project administration, Marina Colombi All authors have read and agreed to the published version of the manuscript.

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CONFLICT OF INTEREST

All authors declare that there is no conflict of interest concerning this work.

DATA AVAILABILITY STATEMENT

Almost all data generated or analyzed during this study are included in this published article and its Additional files. Additional data and

materials are available from the corresponding author upon reasonable request, subject to compliance with our obligations under human research ethics.

ORCID

Marco Ritelli https://orcid.org/0000-0002-7025-2495
Nicola Chiarelli https://orcid.org/0000-0002-1760-5079
Valeria Cinquina https://orcid.org/0000-0002-1545-4801
Marika Vezzoli https://orcid.org/0000-0002-0424-4235
Marina Venturini https://orcid.org/0000-0001-6800-3695
Marina Colombi https://orcid.org/0000-0002-3105-5990

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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