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Nosology and Inheritance Pattern(s) of Joint Hypermobility Syndrome and Ehlers-Danlos Syndrome, Hypermobility Type: A Study of Intrafamilial and Interfamilial Variability in 23 Italian Pedigrees

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Joint hypermobility syndrome (JHS) and Ehlers-Danlos syndrome, hypermobility type (EDS-HT) are two markedly overlapping heritable connective tissue disorders. The cumulative frequency of JHS and EDS-HT seems high, but their recognition remains an exclusion diagnosis based on different sets of diagnostic criteria. Although proposed by a panel of experts, clinical identity between JHS and EDS-HT is still a matter of debate due to unknown molecular basis. We present 23 families with three or more individuals with a diagnosis of JHS and/or EDS-HT. Rough data from the 82 individuals were used to assess the frequency of major and minor criteria, as well as selected additional features. A series of statistical tools were applied to assess intrafamilial and interfamilial variability, emphasizing intergenerational, and intersex differences. This study demonstrates marked heterogeneity within and between families in terms of agreement of available diagnostic criteria. In 21 pedigrees affected individuals belong to two or three phenotypic sub-categories among JHS, EDS-HT, and JHS + EDS-HT overlap. Intergenerational analysis depicts a progressive shifting, also within the same pedigree, from EDS-HT in childhood, to JHS + EDS-HT in early adulthood and JHS later in life. Female-male ratio is 2.1:1, which results lower than previously observed in unselected patients' cohorts. In these pedigrees, JHS, EDS-HT, and JHS + EDS-HT segregate as a single dominant trait with complete penetrance, variable expressivity, and a markedly evolving phenotype. This study represents a formal demonstration that EDS-HT and JHS contitute the same clinical entity, and likely share the same genetic background, at least, in familial cases. © 2014 Wiley Periodicals, Inc.

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INTRODUCTION

Ehlers-Danlos syndrome (EDS) groups together an increasing number of heritable connective tissue disorders (HCTDs) chiefly characterized by joint hypermobility and instability, dermal dysplasia variably manifesting with hyperextensible, fragile, smooth, thin and velvety skin, and internal organ and vascular fragility [Callewaert et al., 2008]. The last EDS classification identifies six major variants, among which the hypermobility type (EDS-HT) is emerging as one of the most common [Castori, 2012, 2013]. Though recognizable on the basis of specific criteria (i.e. Villefranche criteria for the EDS-HT) [Beighton et al., 1998], EDS-HT is an elusive diagnosis due the lack of highly specific clinical signs and laboratory confirmatory tests [Mayer et al., 2013]. Further confusion is added by the presumed clinical identity with the joint hypermobility syndrome (JHS) [Tinkle et al., 2009], which is outlined as a separate condition by the Brighton criteria [Grahame et al., 2000]. The existence of two distinct sets of diagnostic criteria for EDS-HT and JHS probably reflects the protean natural history within a single condition, which may have different clinical presentations at various ages [Tinkle et al., 2009]. In fact, the Villefranche criteria are typically used by pediatricians and clinical geneticists during the assessment of "double-jointed" toddlers and children, while the Brighton criteria are best known by rheumatologists managing adults with various chronic pain conditions. A few recent publications aimed at describing the evolution of JHS and EDS-HT as a whole (i.e. JHS/EDS-HT) trace a predictable but changing natural history with distinct clinical consequences at different ages [Castori et al., 2010a, 2011, 2013a]. Nevertheless, the phenotypic overlap between EDS-HT and JHS is not accepted by all clinical and research groups due to the actual lack of a molecular proof for this lumping [De Paepe and Malfait, 2012].

The debate between "lumpers" and "splitters" culminates with the recent demonstration of a lack of consensus in the application of Beighton score (BS) for joint hypermobility (JHM), Brighton criteria for JHS and Villefranche criteria for EDS-HT within a small group of "experts" in the field [Remvig et al., 2014]. Further considerations on this result point out a wide range of likely contributors to the low level of reproducibility for the use of maneuvers assessing the presence of "excessive" joint motion, as well as the low specificity of the additional features considered for the syndromic diagnosis of JHS and EDS-HT [Castori et al., 2014]. This implies the urgent need of identifying accurate methods for patients' and pedigrees' stratification in order to carry out a reasonable dissection of the underlying molecular defect. Extended family study may represent a privileged perspective for investigating the biological counterpart of the apparent phenotypic continuum ranging from isolated JHM to symptomatic JHM, either in form of JHS or EDS-HT. A recent report describes a multiplex family with various affected members alternatively fitting the criteria of JHS and EDS-HT, and sharing the same dermal ultrastructural anomaly [Hermanns-Lê et al., 2012]. Nevertheless, the question whether JHS and EDS-HT are or not the two sides of the same coin is still open. Classification of patients with symptomatic JHM is further complicated by the ongoing widening of the clinical spectrum purportedly associated with JHS and EDS-HT and extending, but not limited to chronic fatigue [Voermans et al., 2010], functional gastrointestinal disorders [Fikree et al., 2013], sleep disorders [Guilleminault et al., 2013] and dysautonomia [De Wandele et al., 2013], which are not considered in the pre-existing Villefranche and Brighton criteria.

We report, for the first time, on the phenotypic and segregation analysis of a consistent number of highly characterized pedigrees in which the so-called JHS/EDS-HT trait segregates in at least two generations and affects multiple family members. Families were selected for their high explicatory value, and accuracy of gathered clinical and pedigree data. Intrafamilial and interfamilial comparisons were carried out with the primary aim of testing the consistency of the dichotomy between Brighton and Villefranche criteria for both patients' classification and molecular research.

PATIENTS AND METHODS

This study was aimed at analyzing intrafamilial and interfamilial variability within the broad and still poorly defined spectrum of JHS/EDS-HT. Informative pedigrees were selected from the routine clinical activity of two Italian specialized outpatient clinics for the diagnosis and management of HCTDs (i.e. "Ehlers-Danlos Syndrome and Inherited Connective Tissue Disorders" Clinic at the "Spedali Civili" University Hospital of Brescia, and the jointed service of the Medical/Clinical Genetics Outpatient Clinic at the San Camillo-Forlanini Hospital and the Division of Physical Medicine and Rehabilitation at the Policlinico Umberto I University Hospital in Rome). Since 2009, we collected 393 patients with a "confirmed" clinical diagnosis of JHS/EDS-HT. For this study, we considered only those pedigrees for which we were able to directly evaluate three or more affected individuals.

For the clinical assessment of JHS/EDS-HT index patients and their relatives, we first applied available tools, including the 5-point questionnaire for JHM [Hakim and Grahame, 2003], BS [Beighton et al., 1973], Brighton criteria for JHS [Grahame et al., 2000] and Villefranche criteria for EDS-HT [Beighton et al., 1998] (Table I). The 5-point questionnaire for JHM was slightly modified for the purpose to investigate historical JHM. In fact, objective JHM was directly assessed with the BS, irrespectively from the results of the questionnaire. BS is a nine-point evaluation with attribution of one point in the presence of any of the following: (a) passive apposition of the thumb to the flexor aspect of the forearm (one point for each hand), (b) passive dorsiflexion of the V finger beyond 90° (one point for each hand), (c) hyperextension of the elbow beyond 10° (one point for each arm), (d) hyperextension of the knees beyond 10° (one point for each leg), (e) forward flexion of the trunk with the knees extended and the palms resting flat on the floor. When possible, range of motion was measured with an orthopedic goniometer. In non-collaborative subjects (such as, toddlers and adults in wheelchair), the upper end of the sum was reduced by 1 point by excluding the maneuver for forward flexion of the trunk. In this case, the highest score was 8.

In patients with BS $\geq$5, we applied the Villefranche criteria and a clinical diagnosis of EDS-HT was established by the additional presence of hyperextensible and/or smooth, velvety skin (two major criteria). The presence of one or more minor criteria was considered ancillary as in the original paper by Beighton et al. [1998] not any discrete diagnostic value was attributed to these criteria. Hence, presence or absence of one or more minor Villefranche criteria were

TABLE I. Summary of the Available Questionnaire and Set of Diagnostic Criteria for Assessing (Historical) JHM, JHS, and EDS-HT

5-point questionnaire for (historical) JHM	Brighton criteria for JHS	Villefranche criteria for EDS-HT
1. Could you ever place your hands flat on the floor without bending your knees?	*Major criteria*	*Major criteria*
2. Could you ever bend your thumb to touch your forearm?	Beighton score $\geq$4	Hyperextensible and/or smooth, velvety skin
3. As a child did you amuse your friends by contorting your body into strange shapes OR could you do the splits?	Arthralgia for >3 months in >4 joints	Generalized joint hypermobility (Beighton score $\geq$5)
4. As a child or teenager did your shoulder or kneecap dislocate on more than one occasion?	*Minor criteria*	*Minor criteria*
5. As a child or teenager did you consider yourself double-jointed?	Beighton score 1–3	Recurring joint dislocations
Agreement: Affirmative answer for two or more questions.	Arthralgia in 1–3 joints	Chronic joint/limb pain
Source: Modified from Hakim and Grahame [2003].	History of joint dislocations	Positive family history
	Soft tissue lesions >3	
	Marfan-like habitus	
	Skin striae, hyperextensibility, or scarring	
	Downslanting palpebral fissures, lid laxity, myopia	
	History of varicose veins, hernia, visceral prolapse	
	Agreement: Both major, or 1 major and 2 minor, or 4 minor criteria. Criteria major 1 and minor 1 are mutually exclusive as are major 2 and minor 2.	**Agreement:** Both major criteria (irrespectively of the presence/absence of minor criteria which are "simply" considered supportive).
	Source: Grahame et al. [2000] and subsequent modifications (see, for example, Tinkle et al. [2009].	**Source:** Beighton et al. [1998].

considered not relevant for diagnosis establishment of EDS-HT. Several studies tried to identify a more objective approach to evaluate skin texture abnormalities in JHS and EDS [Grahame and Beighton, 1969; Farmer et al., 2010; Remvig et al., 2010]. However, they were carried out in small populations and were not yet validated as standard method(s) of evaluation in these conditions. In this study, skin/superficial connective tissue features were assessed qualitatively by palpation and gentle stretching of the skin at the volar aspect of the palm (at the IV metacarpal) and/or forearm. All patients were also screened for the Brighton criteria. A diagnosis of JHS was established in the presence of both major, or one major and two minor, or four minor criteria, according to Grahame et al. [2000] and subsequent comments to the Brighton criteria [Tinkle et al., 2009]. In the most recent literature, it is stated that "two minor (Brighton) criteria will suffice (to establish a diagnosis of JHS) where there is an unequivocally affected first degree relative" [Ross and Grahame, 2011]. Hence, for family selection, we adhered to this recommendation. Nevertheless, for further data interpretation, the isolated presence of one-to-three minor Brighton criteria and/or borderline/generalized JHM without meeting the original clinical definition of JHS [Grahame et al., 2000] was not considered sufficient for the status of "affected". Hence, the presence of family members with only two minor Brighton criteria were exclusively used for selecting pedigrees and all data were evaluated pooling patients strictly meeting the original JHS criteria [Grahame et al., 2000].

For practical reasons, the Villefranche major item "hyperextensible and/or smooth, velvety skin" and the Brighton minor item "skin *striae*, hyperextensibility, or scarring" were considered the same. In other words, a patient with skin features was considered having both the criteria. In this setting, abnormal scar formation and/or skin *striae* (i.e. *striae distensae* and *striae rubrae*) was considered only if coexistent with minor abnormalities of the skin texture (i.e. midly hyperextensible, soft and/or velvety skin). Similarly, the Brighton minor item "history of joint dislocations" and the Villefranche minor item "recurrent joint dislocations" were considered the same and counted as "positive" in presence of three or more dislocations in two or more joints, congenital hip dysplasia and/or other congenital joint dislocations. The Brighton minor item "Marfan-like *habitus*" was considered met in the presence of a tall and slim *habitus* with dolichostenomelia (i.e. arm span/height ratio $\geq$1.03–according to Grahame et al., [2000] and/or arachnodactyly (assessed with the presence of bilateral positive thumb and wrist sign, and/or middle finger/total hand ratio >97th centile by age). This combination of features was established following the original definition of the corresponding minor Brighton item "tall and slim *habitus*, arm span/height ratio >1.03, upper/lower segment ratio less than 0.89, positive thumb/wrist signs", in which how many of these sub-features are needed was not further defined. The "middle finger/total hand ratio >97th centile by age" was used as a quantitative surrogate of arachnodactyly in patients with limited finger joint motions due to advanced disease progression.

As the Villefranche and Brighton criteria are not mutually exclusive, we were expected to detect patients who met both the set of diagnostic criteria. Hence, in our sample, the JHS/EDS-HT clinical spectrum included patients fulfilling Brighton criteria only (JHS), Villefranche criteria only (EDS-HT) and subjects meeting both sets (JHS + EDS-HT) (Table II). In addition to accurate medical history and physical examination, most patients underwent a set of baseline investigations/consultations, including cardiologic evaluation with electrocardiogram and standard heart ultrasound [McDonnell et al., 2006], complete ophthalmologic

survey [(Gharbiya et al., 2012], baseline coagulation tests [Jackson et al., 2013] and bone mineral density test [Gulbahar et al., 2006]. A few patients also needed a head-up tilt-test [Mathias et al., 2011]. Both the Villefranche and Brighton criteria consider mandatory the exclusion of other (partially) overlapping HCTDs, but, at the moment, an international consensus on investigations necessary for carrying out such a differential is lacking. Tofts et al. [2009] present an accurate procedure for differential diagnosis of children with joint hypermobility and we tried to adhere to their recommendations also for adults. In very selected cases, the differential diagnosis procedure extended to epiaortic vessels and abdominal aorta ultrasound, repeated exertion-free serum creatine kinase dosages, electromyo- and electroneurography of the four limbs, as well as molecular testing of the *COL5A1* and *COL5A2* genes.

We also annotated the presence/absence of some additional recurrent findings not included in the above mentioned sets of diagnostic criteria. Most of them were historical simple features. Among them, we included two further complex/syndromic diagnoses, i.e. chronic fatigue syndrome and developmental coordination disorder. The attribution of these co-morbidities were established according to Fukuda et al. [1994] and the DSM IV-TR, respectively. In selected cases, we were able to report the presence of attention deficit/hyperactivity disorder according to previous evaluation by a child neurologist.

A series of descriptive statistics were used to summarize pertinent study information. Chi-square, Chi square for trend or Fisher's exact test were performed for the comparison of categorical variables. Comparison between groups of continuous variables was performed using the Mann-Whitney *U*-test or Student *t* test. The Spearman rho or Pearson correlation were used to investigate possible relationship between continuous variables. Univariate and multivariate linear regression model was used to investigate the relationship between variables. All *P* values corresponded to two-sided tests, and significance was set at $P < 0.05$. Statistical analyses were conducted using SPSS® (21.0).

RESULTS

Review of collected data identifies 23 families with multiple affected members with JHS/EDS-HT according to the Villefranche criteria for EDS-HT and the Brighton criteria. After subtraction of relatives not meeting the "original" Brighton criteria, the patients' cohort is composed of 82 subjects. Overall demographic and major clinical data of these subjects are summarized in Table III. Females exceed males with a 2.1:1 ratio and adults (i.e. age ≥18 years) account for approx. 60% of the sample. JHS + EDS-HT is the most common phenotype (40.2%), followed by JHS (35.4%) and then EDS-HT (24.4%). The mean age at diagnosis is different among the distinct phenotypes. In particular, EDS-HT has the youngest mean age at diagnosis (8 years [SD = 9 years]), JHS + EDS-HT has an intermediate mean age at diagnosis (30 years [SD = 16 years]) and JHS the oldest mean age at diagnosis (42 years [SD = 19 years]). Rate of single Brighton and Villefranche criteria, as well as a selected additional features not yet included in any diagnostic set is reported in Table III (features by single individual are reported in the Supplementary Materials file—see supporting information online).

Selected pedigrees have three and two affected generations in five and 18 instances, respectively. Three pedigrees have two affected individuals, 14 have three affected individuals, four have four affected individuals, one have eight affected individuals, and one have ten affected individuals (Fig. 1). In 21 (91.3%) families there is intrafamilial phenotypic discordance with affected members belonging to two or all three phenotypic sub-categories. Two (8.7%) families have affected members belonging to a single sub-category, i.e. JHS for Family 5 and JHS + EDS-HT for Family 15. Disease is transmitted 48 times from 26 affected mothers and four affected fathers, who have 19 (39.6%) affected sons and 29 (60.4%) affected daughters. Intergenerational phenotypic discordance (concerning phenotypic sub-category) is registered in 35 (72.9%) cases. Male-to-male transmission is observed in two pedigrees (i.e., Families 8 and 12). Table IV shows differences concerning major features (i.e., sub-category, major clinical features, mean age at diagnosis and instances of transmission of the disease) between affected males and females. Not any major statistically relevant difference is noted, except for mean age at diagnosis in JHS and JHS + EDS-HT. Females tend to receive the diagnosis of JHS and JHS + EDS-HT according to Grahame et al. [2000] in a more advanced age than males. A positive, but not statistically significant trend between sexes is noted for BS ≥4 and ≥5 (dichotomic feature), chronic musculoskeletal pain and Villefranche criteria, with the latter being more common in males and the remaining in females.

TABLE II. Phenotype Stratification by Criteria Satisfaction

Phenotype[a]	5-point questionnaire	Beighton score	Brighton criteria	Villefranche criteria	Other HCTDs "excluded"
Isolated historical JHM	+	−	−	−	+
Isolated borderline JHM	+/−	+ (4)	−	−	+
Isolated generalized JHM	+/−	+ (≥5)	−	−	+
JHS	+/−	+/−	+	−	+
EDS-HT	+/−	+	+/−	+	+
JHS+EDS-HT	+/−	+	+	+	+

EDS-HT, Ehlers-Danlos syndrome, hypermobility type; JHM, joint hypermobility; JHS, joint hypermobility syndrome.
Note: family members with historical JHM only were not further considered for pedigree studying.
[a]At the time of examination.

TABLE III. Summary of Major Demographic and Clinical Features of the Sample

Characteristic	Value	Percentage
Number of affected individuals		
Total	82	100.0
Females	56	68.3
Males	26	31.7
Children (<18 years)	34	41.5
Adults (≥18 years)	48	58.5
EDS-HT = a	20	24.4
JHS+EDS-HT = b	33	40.2
JHS = c	29	35.4
Positive Villefranche criteria = a+b	53	64.6
Positive Beighton criteria = b+c	62	73.8
Female/male ratio	2.1:1	NA
Mean age at diagnosis (standard deviation)		
Total [years]	29 (±20)	NA
EDS-HT [years]	8 (±9)	NA
JHS+EDS-HT [years]	30 (±16)	NA
JHS [years]	42 (±19)	NA
Mean Beighton score (standard deviation)	5 (±2.5)	NA
Brighton criteria		
Major criteria		
Beighton score ≥4	64	78.0
Arthralgias for >3 months at >4 joints	28	34.1
Minor criteria		
Beighton score 1–3[a]	14	77.8
Arthralgias at 1–3 joints[b]	27	50.0
History of joint dislocation	41	50.0
Soft tissue lesions >3	22	26.8
Marfan-like *habitus*	16	19.5
Skin *striae* and/or hyperextensibility, or abnormal scarring	70	85.4
Myopia of mild degree, lid laxity, and/or antimongoloid slants	14	17.1
History of varicose veins, hernia(s), and/or visceral prolapse	25	30.5
Villefranche criteria for EDS-HT		
Major criteria		
Beighton score ≥5	57	69.5
Hyperextensibility and/or smooth, velvety skin	70	85.4
Minor criteria		
Recurring joint dislocations	43	52.4
Chronic joint/limb pain	49	59.7
Positive family history	59	71.9
Chronic fatigue/easy fatigability	48	58.5
Chronic fatigue syndrome	19	23.2
Memory/concentration troubles	25	30.5
Motor delay	19	23.2
Clumsiness	31	37.8
Developmental coordination disorder[c]	11	55.0
Attention deficit/hyperactivity disorder[d]	8	34.8
Orthostatic intolerance[e]	22	52.4
Recurrent tachycardias/palpitations	17	20.7
Raynaud's phenomenon/acrocyanosis/livedo reticularis	14	17.1
Cardiac valve prolapse/insufficiency	23	28.0
Recurrent unexplained abdominal pain	16	19.5
Chronic gastritis	19	23.2
Gastroesophageal reflux	28	34.1
Chronic constipation/*alvus alternus*	31	27.8
Symptoms of stress incontinence[f]	15	37.5

TABLE III. (*Continued*)

Characteristic	Value	Percentage
Dysmenorrhea[f]	19	45.2
Meno/metrorrhagias[f]	20	47.6

[a]Excluding individuals with a Beighton score ≥ 4.
[b]Excluding individuals with arthralgias for >3 months at >4 joints.
[c]Data available on 20 subjects only.
[d]Data available on 23 subjects only.
[e]Including four cases of confirmed postural orthostatic tachycardia syndrome and two cases of neuro-mediated hypotension. Data available on 42 subjects only.
[f]Data available on 42 subjects only.

Table V shows the distribution of major features by generation. Arbitrarily, affected family members were grouped according to the generation nomenclature of Figure 1. Most features reported in Table V show statistically significant differences. The natural excess of affected females in JHS/EDS-HT [Castori et al., 2010b] is mostly represented in the oldest generation (i.e. first generation). In fact, the number of affected males and females is similar in the middle generation (i.e. second generation) and nearly overlapping in the youngest one (i.e. third generation). The oldest generation consists of JHS in 63.4% of the patients and JHS + EDS-HT in the remaining ones (no case with EDS-HT), the middle generation shows an approximately equal number of EDS-HT and JHS + EDS-HT, and JHS + EDS-HT is the most common diagnosis in the youngest generation. Both Villefranche and Brighton criteria are prevalent in all generations. However, Villefranche criteria has the highest rate in the youngest generation (81.8%), whereas Brighton criteria are always met in the oldest generation only (100.0%). All three jointed Brighton and Villefranche major criteria (i.e. BS rate, skin signs and generalized arthralgias) are influenced by generation. In particular, the rate of a BS ≥ 5 and of positive skin sign is lowest in the oldest generation (36.4% and 63.4%) and highest in the youngest generation (81.8% and 100.0%). Conversely, the frequency of generalized

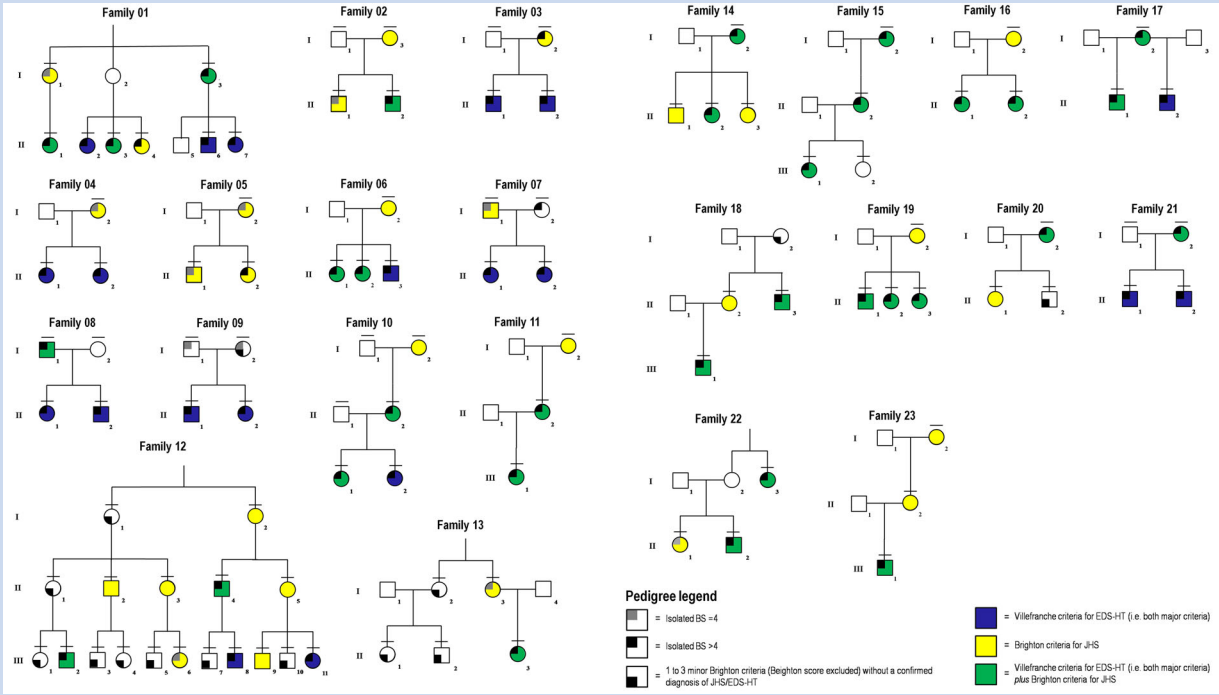

FIG. 1. Summary of reported families with JHS/EDS-HT. Clinical data were simplified by indicating the presence of Villefranche criteria (blue), Brighton criteria (yellow) and both sets (green) with different colors. A Beighton score of 4 and >4 were indicated by a grey and black square, respectively. The presence of single minor Brighton criteria in relatives without a diagnosis of JHS/EDS-HT was also reported. Directly investigated subjected were marked by a horizontal bar.

TABLE IV. Differences Between Males and Females in Familial Cases of JHS/EDS-HT

Characteristic	Males	Females	*P* value
Beighton score (standard deviation)	5 (± 2)	5 (± 2)	0.99
Number of affected individuals (%)			
Total	26 (100)	56 (100)	
EDS-HT = a	10 (38.5)	10 (17.8)	0.09
JHS+EDS-HT = b	10 (38.5)	23 (41.1)	
JHS = c	6 (23.0)	23 (41.1)	
Positive Villefranche criteria = a+b	20 (76.9)	31 (55.3)	0.09
Positive Brighton criteria = b+c	16 (61.5)	45 (80.3)	0.99
Major clinical features (%)			
Beighton score ≥5 (Villefranche criteria)	20 (76.9)	31 (55.3)	0.09
Positive skin sign(s) (Villefranche criteria)	22 (84.6)	43 (76.8)	0.56
Beighton score ≥4 (Brighton criteria)	22 (84.6)	36 (64.3)	0.07
Chronic musculoskeletal pain (Brighton criteria)	5 (19.2)	22 (39.3)	0.08
Mean age at diagnosis (standard deviation)			
Total [years]	15 (± 19)	21 (± 33)	0.39
EDS-HT [years]	8 (± 10)	8 (± 9)	0.99
JHS+EDS-HT [years]	24 (± 13)	33 (± 16)	**0.01**
JHS [years]	31 (± 16)	45 (± 19)	**0.002**
Instances of transmission (%)			
Total	5 (100)	43 (100)	
Affected sons	2 (40.0)	17 (39.5)	0.99
Affected daughters	3 (60.0)	26 (60.4)	
Intergenerational phenotypic concordance[1]	0 (0.0)	13 (30.2)	0.30
Intergenerational phenotypic discordance[1]	5 (100)	30 (69.8)	

Significant P values are in bold.
EDS-HT, Ehlers-Danlos syndrome hypermobility type; JHS, joint hypermobility syndrome.
1: intergenerational phenotypic concordance was considered in case of phenotypic homogeneity between the affected son/daughter and transmitting parent (i.e. JHS → JHS; EDS-HT → EDS-HT; JHS+EDS-HT → JHS+EDS-HT). On the contrary, intergenerational phenotypic discordance was considered in presence of different clinical diagnoses between subsequent generations (i.e. JHS → EDS-HT, JHS+EDS-HT; EDS-HT→ JHS, JHS+EDS-HT; JHS+EDS-HT→JHS, EDS-HT).

arthralgias (= major Brighton item) is lowest in the youngest generation (18.2%) and highest in the oldest generation (59.1%).

Figure 2 illustrates the relationship between age at evaluation and BS for the 82 patients. Multivariate analysis was carried out considering BS as dependent variable and sex, diagnosis and age at ascertainment as independent variables. BS resulted significantly associated with age at ascertainment ($P = 0.004$) and diagnosis ($P < 0.0001$). In particular, BS reduces with age (linear regression) and is generally higher in EDS-HT and JHS + EDS-HT compared to JHS. Not any correlation with sex is found.

DISCUSSION

In this work, we studied intrafamilial and interfamilial variability in 23 pedigrees comprising multiple members with the JHS/EDS-HT spectrum, for a total of 82 individuals. This study was focused on analyzing the intergenerational distribution of Brighton and Villefranche criteria in order to test whether JHS and EDS-HT may be considered or not the same genetic trait. We observed a striking intrafamilial and interfamilial discordance for the two available sets of diagnostic criteria without a clear separation between JHS and EDS-HT in terms of genetic transmission. In line with published data [Castori et al., 2010b], females are generally more represented in our patients' cohort. In particular, females were two times more affected than males. Conversely, females and males patients seemed to do not display significant differences in terms of distribution of BS, and Villefranche and Brighton major criteria, according to our previous findings [Castori et al., 2011]. However, symptom severity and related disability were not assessed in this study; hence, we were not able to differentiate sexes in terms of quality of life.

Nosology

In the reported families, JHS and EDS-HT are diagnosed in multiple members within the same pedigree and, then, can be considered a single genetic trait. This hypothesis is further testified by the high number of individuals meeting both sets of diagnostic criteria (i.e. JHS + EDS-HT). In these pedigrees, the JHS/EDS-HT spectrum (now including JHS, EDS-HT and JHS + EDS-HT) is homogeneously transmitted in a vertical fashion with variable expressivity, and marked age-dependence for all Villefranche and Brighton major criteria, and range of BS. This work represents a formal demonstration for the inconsistency of the use of different sets of diagnostic criteria for JHS and EDS-HT, which are, indeed, undistinguishable on clinical grounds [Tinkle et al., 2009]. One of the pillars of such an inconsistency seems the BS itself, which offers a

TABLE V. Phenotype Comparison by Generation

Characteristic	First generation(%)	Second generation(%)	Third generation(%)	*P* value
# of affected (total)	22 (100.0)	49 (100.0)	11 (100.0)	
# of affected (males)	2 (9.0)	19 (38.8)	5 (45.5)	**0.01**
# of affected (females)	20 (90.9)	30 (61.2)	6 (54.5)	
# of EDS-HT = a	0 (0.0)	17 (36.7)	3 (27.8)	**0.004**
# of JHS/EDS-HT = b	8 (36.4)	19 (38.8)	6 (54.5)	
# of JHS = c	14 (63.4)	13 (26.5)	2 (18.2)	
# of positive Villefranche criteria = a+b	8 (36.4)	36 (73.5)	9 (81.8)	**0.003**
# of positive Brighton criteria = b+c	22 (100.0)	32 (65.3)	8 (72.7)	**0.02**
Major clinical features				
Beighton score ≥5 (Villefranche criteria)	8 (36.4)	36 (73.5)	9 (81.8)	**0.003**
Positive skin sign(s) (Villefranche criteria)	14 (63.4)	40 (81.6)	11 (100.0)	**0.01**
Beighton score ≥4 (Brighton criteria)	14 (63.4)	38 (77.5)	10 (90.9)	0.07
Chronic musculoskeletal pain (Brighton criteria)	13 (59.1)	12 (24.5)	2 (18.2)	**0.005**

Significant *P* values are in bold.

punctual evaluation of generalized JHM without accounting for age, gender, past injuries, ethnicity and training. The opposed approach of considering JHS and EDS-HT separate conditions for genetic/research purposes [De Paepe and Malfait, 2012] is not supported by our findings. However, the presence of two families with all affected members belonging to a single phenotypic sub-category stands for the possible co-existence of both models under the umbrella term of JHS/EDS-HT, which still remains without a well-defined molecular basis. In fact, assuming genetic heterogeneity, it is still possible that while in many families the genetic trait segregates with a wide range of phenotypic outcomes, selected families may show more stringent genotype-phenotype correlations and, then, a more homogeneous clinical picture.

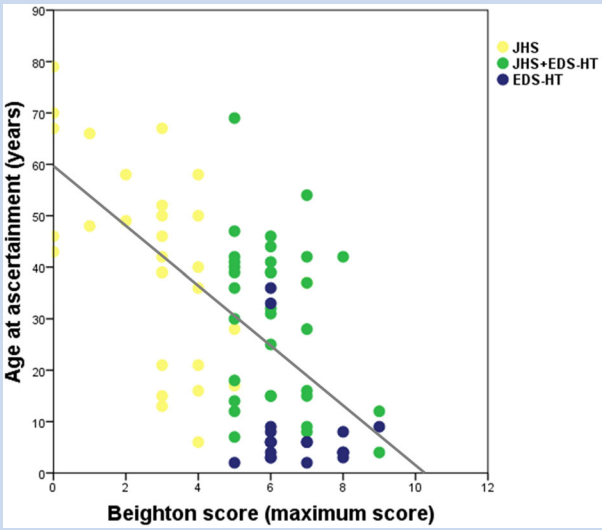

FIG. 2. Scatter plot showing inverse linear correlation between age at examination and Beighton score. Linear R2 score refers to the whole patients' group. Patients are identified with different colors (JHS = yellow, JHS + EDS-HT = green, EDS-HT = blue). JHS patients cluster in the upper left area of the chart (lower Beighton score, higher age at ascertainment). JHS + EDS-HT patients are more heterogeneously scattered in the right half of the chart, while EDS-HT patients cluster in the lower right area (higher Beighton score, lower age at ascertainment).

Actually, EDS-HT is considered an autosomal dominant trait with complete penetrance [Levy, 2004]. Nevertheless, our work shows that EDS-HT *strictu sensu* is a trait with incomplete penetrance, once excluded individuals meeting Brighton criteria but not Villefranche criteria (i.e. subjects in "yellow" in Fig. 1). Coalescing JHS and EDS-HT in a single trait, we can define JHS/EDS-HT a dominant condition with nearly complete penetrance, variable expressivity within and between families, and marked age-dependent variability. A nearly complete penetrance is possible only after the inclusion of older and/or more severely affected individuals who have lost their congenital joint laxity due to a progressive chronic musculoskeletal pain and the resulting stiffness of joints. Variable expressivity is testified by the heterogeneity in sub-category attribution among affected individuals belonging to the same pedigree, as well as the inter-individual discordance for the wide range of associated features still not included in any set of diagnostic criteria (Table III and Supplementary Materials in supporting information online).

Previously, we introduced the term "metatropism" to define the protean natural history and the differential distribution of diagnostic criteria among generations in pedigrees with JHS/EDS-HT spectrum [Castori et al., 2013a]. It has been used for the same purpose in other connective tissue disorders [Nishimura et al., 2004; Castori et al., 2013b]. Although our data are not longitudinal, repeated evidence in multiple families prompts us to infer a trend of progressive shifting, possibly also in the same individual, from Villefranche criteria (EDS-HT) in childhood, to both Villefranche and Brighton criteria (JHS + EDS-HT) in early adulthood, to Brighton criteria (JHS) later in life. This is well

illustrated in Table V and Figure 2. In fact, in symptomatic patients, both BS and rate of skin signs reduce by age, whereas there is a general increase in frequency of generalized arthralgias by advancing age. This testifies for a presumed progression from EDS-HT to JHS during the life of an affected individual with a midlife transition in which both sets are met (i.e. JHS + EDS-HT). However, this model cannot be fully applied in all families, as in only a minority of them (4/23 = 17.4%) we observed all three phenotypic sub-categories. Hence, in JHS/EDS-HT, clinical expressivity is markedly variable with a dynamic and age-dependent attribute, which may manifest also in the same individual who skips from a set of diagnostic criteria to the other at different ages. A list of possible factors influencing phenotypic outcome and then determining age-dependent variability has been previously proposed [Castori et al., 2013a]. Nevertheless, at the moment, not any robust experimental proof is available for confirming the weight of these factors in disease manifestation and evolution.

Inheritance Pattern(s)

The debate on the more likely transmission model for JHM, JHS and EDS-HT is still unsolved and lays on the unclear relationships among these apparently distinct conditions. As previously stated, in the past, EDS-HT has been considered an autosomal dominant trait [Levy, 2004]. Vertical transmission of the disease with an overt excess of affected women is accepted for JHS [Remvig et al., 2007], whereas apparently isolated JHM is better explained by the multifactorial model [Wood, 1971; Grahame, 1999]. In our families, in addition to the acceptance of the inconsistency of assuming JHS and EDS-HT distinct genetic entities, we observed a discrete number of additional family members with isolated historical or objective JHM, as well as a few Brighton criteria not sufficient for diagnosing JHS. Therefore, in at least some pedigrees, these three entities may well represent the different consequences of the same genetic trait under the influences of various, still largely unknown modifier factors. Verticalization of genetic transmission is clear in our families with a very few instances of generation skipping.

Concerning Mendelism, we registered two instances of male-to-male transmission, an indirect proof for autosomal inheritance. However, this value is much lower than a priori expected in presence of 48 registered instances of disease transmission. At the moment, the excess of affected females remains without a formal proof. Sexual dimorphism, as well as different steroid hormones metabolism and pain thresholds between sexes are all possible contributors to the skewed sex ratio for the presumed autosomal transmission of JHS/EDS-HT [Castori et al., 2010b]. Hence, the gender bias characterizing JHS/EDS-HT can be best interpreted as the result of a sex-influenced autosomal trait.

Complementary and assuming genetic heterogeneity, the ~2:1 ratio between affected females and males observed in this study cannot exclude the existence of, at least, one form with X-linked dominant inheritance. The absence of an overt sex bias in terms of disease manifestations with respect to diagnostic criteria and BS may reflect a "true" dominant trait, although subtler phenotypic discordances could be emphasized in the future, as presumed by the trend of significance registered by us for BS and chronic musculoskeletal pain. The higher excess of affected females in sporadic cases compared to familial ones [Castori et al., 2010b], also confirmed by the present study, may be explained by either ascertainment bias due to a lower chance for an affected male to request medical attention, or a higher rate of asymptomatic or oligo-symptomatic male carriers. The latter hypothesis, in the case of a X-linked dominant inheritance, can be in turn explained by cellular or metabolic interference, as proposed for other X-linked dominant traits such as cranio-fronto-nasal dysplasia [Johnson, 1980; Wieland et al., 2004]. Nosologic confusion, a too simplistic approach in inheritance model design, as well as the likely underlying locus heterogeneity are all good reasons for the missing knowledge concerning JHS/EDS-HT molecular basis.

Procedural diagnostics

At the moment, both JHS and EDS-HT are considered "exclusion" diagnoses due to the lack of any consistent confirmatory test [Beighton et al., 1998; Grahame et al., 2000]. By comparing Villefranche criteria for EDS-HT and Brighton criteria, the formal distinction between these two disorders most lie on the value attributed to BS, skin features and generalized *vs* localized arthralgias in terms of major and minor criteria. JHS may be further diagnosed in presence of four minor criteria, which also include a set of additional features not considered in the Villefranche criteria. In addition, there are a series of analogies among selected criteria from the two sets. The lack of a strict correspondence in their formal definition does not help the unexperienced practitioner in assessing and attributing JHS and EDS-HT (see, for example, the Villefranche major criteria and the Brighton minor criteria for skin features – Table I). Accumulated experience on nearly 400 patients with a clinical confirmed diagnosis of JHS/EDS-HT prompted us to consider one and the same various couples of criteria, as reported in "Patients and Methods". Once assumed JHS and EDS-HT the same genetic and nosologic entity, the practitioner could not separate JHS and EDS-HT *before* diagnosis attribution. Instead, she/he could use these two terms for patient's subclassification under the same clinical entity. We also highlight the need for a better definition of "exclusion" diagnosis in the broad field of HCTDs. In fact, the mandatory feature of "excluding any other partially overlapping HCTD" for JHS is not mirrored by a shared procedural diagnostics. In the near future and in parallel with molecular research, it is expected that an international consensus will draw a comprehensive and unified method of patients' assessment including more reproducible tools and the involvement of structures/functions actually not considered in the Villefranche and Brighton criteria [Castori et al., 2013a; 2014].

Limitations and Future Directions

This study suffers of major limitations. In particular, we were able to conduct this study in a relatively small number of families all belonging to the same genetic background (i.e. Italy). Although this work was carried out in two centers localized in two distant regions of the same country and then attracting patients from various areas of Italy, we do not have a full picture representative of the entire country. A further limit is represented by the cross-sectional design of the study. In fact, our considerations in terms of

natural history of the disease is entirely based on intrafamilial and interfamilial comparisons. We hope that in the following years we will be able to gather a sufficient amount of data from the long-term clinical histories of a representative number of patients in order to formally confirm our generalizations.

The primary aim of this work is to present a clear picture for the apparent inconsistency of considering JHS and EDS-HT different disorders. Accordingly, we demonstrated that, in at least most presented pedigrees, this distinction is not applicable. Once our experience will meet that of other research groups and, hopefully, will be included in a wider international consensus, we will still need of debates and research. In particular, unraveling the molecular basis of JHS/EDS-HT is crucial for differentiating it from other HCTDs, as well as planning future molecular therapies. We also need of reproducible severity scores for more evidence-based treatment strategies under the increasingly rigid laws of the various Healthcare Systems. Finally, in the actual absence of treatments successfully impacting quality of life of patients, we should work for identifying precise tools for prognostication and, then, to design more tailored prevention plans.

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