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ARTICLE

Spectrum of Mucocutaneous Manifestations in 277 Patients with Joint Hypermobility Syndrome/Ehlers-Danlos Syndrome, Hypermobility Type

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Cutaneous manifestations are a diagnostic criterion of Ehlers–Danlos syndrome, hypermobility type (EDS-HT) and joint hypermobility syndrome (JHS). These two conditions, originally considered different disorders, are now accepted as clinically indistinguishable and often segregate as a single-familial trait. EDS-HT and JHS are still exclusion diagnoses not supported by any specific laboratory test. Accuracy of clinical diagnosis is, therefore, crucial for appropriate patients' classification and management, but it is actually hampered by the low

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consistency of many applied criteria including the cutaneous one. We report on mucocutaneous findings in 277 patients with JHS/EDS-HT with both sexes and various ages. Sixteen objective and five anamnestic items were selected and ascertained in two specialized outpatient clinics. Feature rates were compared by sex and age by a series of statistical tools. Data were also used for a multivariate correspondence analysis with the attempt to identify non-causal associations of features depicting recognizable phenotypic clusters. Our findings identified a few differences between sexes and thus indicated an attenuated sexual dimorphism for mucocutaneous features in JHS/EDS-HT. Ten features showed significantly distinct rates at different ages and this evidence corroborated the concept of an evolving phenotype in JHS/EDS-HT also affecting the skin. Multivariate correspondence analysis identified three relatively discrete phenotypic profiles, which may represent the cutaneous counterparts of the three disease phases previously proposed for JHS/EDS-HT. These findings could be used for revising the cutaneous criterion in a future consensus for the clinical diagnosis of JHS/EDS-HT.

KEY WORDS: Ehlers–Danlos syndrome hypermobility type; diagnostic criteria; atrophic scar; skin hyperextensibility; lingual and oral frenula

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INTRODUCTION

Skin involvement represents one of the best known manifestations of Ehlers–Danlos syndrome (EDS). Hyperextensible skin, papyraceous-atrophic scars at sites of repetitive damage, molluscoid pseudotumors at the extensor surfaces of elbows and knees, and subcutaneous nodules best appreciable on the limbs are the pathognomonic cutaneous tetrad of classic EDS according to Villefranche nosology [Beighton et al., 1998]. However, taken in isolation, none of these features may be considered constant in classic EDS and the growing number of reported patients with a definite mutation in either *COL5A1* or *COL5A2* suggests the need of updating the actual approach of classifying patients and selecting them for subsequent laboratory investigations [Ritelli et al., 2013]. This is expected also for the other EDS variants with an available confirmatory molecular test. Such an issue is equally or, perhaps, more relevant for the EDS hypermobility type (EDS-HT), which is the sole major variant lacking a known genetic defect [Mayer et al., 2013]. In the Villefranche criteria for EDS-HT, skin involvement is defined as "hyperextensibility and/or smooth, velvety skin" and considered a major item [Beighton et al., 1998]. In such terms, the chance to attribute a positive cutaneous sign in EDS-HT is entirely left to the practitioner's experience. The same is expected for the joint hypermobility syndrome (JHS), a clinically overlapping condition that many experts now consider indistinguishable from EDS-HT [Tinkle et al., 2009]. In the Brighton criteria for JHS, skin involvement is identified with "skin *striae*, hyperextensibility, or scarring" and considered a minor feature [Grahame et al., 2000]. There is an emerging need to review the Villefranche criteria for EDS-HT and the Brighton criteria for improving the agreement among practitioners [Remvig et al., 2011], as well as for promoting the advancement of knowledge in both molecular basis and therapeutic resources [Castori et al., 2013].

In the recent past, the efforts of a few research groups have been focused on trying to standardize and, then, more objectively measure skin hyperextensibility and consistency. In particular, the corrected skin extensibility score was identified as a useful and reproducible measure of skin extensibility in 250 healthy volunteers [Farmer et al., 2010]. The use of suction cup, soft tissue stiffness meter and soft tissue ultrasonography was applied to measure skin hyperextensibility, consistency and thickness in JHS/EDS-HT patients [Remvig et al., 2009, 2010]. Although promising and more objective than simple palpation and skin stretching, these tools need validation in larger samples and actually are not used in the routine clinical evaluation of JHS/EDS-HT patients in many specialized clinics. The clear-cut evidence of a lack of consensus among 15 expert centers from various countries on tests and criteria for generalized joint hypermobility, EDS-HT and JHS stands for a general inconsistency for most of the available "diagnostic criteria", also including the "skin signs" [Remvig et al., 2014] as actually defined. Furthermore, clinical practice suggests an unexpectedly wide range of mucocutaneous features in JHS/EDS-HT [Castori, 2012a, 2013].

This cross-sectional work presents cutaneous and mucosal findings in 277 patients with JHS/EDS-HT attending two Italian centers for the diagnosis and management of heritable connective tissue disorders (HCTDs). Collected data were used for delineating a more comprehensive picture of the mucocutaneous manifestations of JHS/EDS-HT, and offering clues for future revision of the cutaneous criterion of this condition.

PATIENTS AND METHODS

Patients 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). Between January 2009 and May 2014, these two services collected more than 400 patients with a "confirmed" clinical diagnosis of JHS/EDS-HT, particularly, with hyperextensibility and/or smooth, velvety skin. Only 277 patients with additional mucocutaneous observational and historical features were included in this study. In a subset of 135 patients, data on the oral features were available.

The diagnosis of JHS and EDS-HT was established by applying published diagnostic criteria. In particular, generalized joint hypermobility was assessed by the Beighton score (BS) with a maximum score of nine and a cut-off of four for JHS and five for EDS-HT [Beighton et al., 1973]. In specific circumstances (e.g., patients in wheelchair and toddlers), the maximum score was reduced to eight by subtracting the maneuver of anterior bending of the trunk, while the cut-offs remained unchanged. Then, Villefranche criteria were used for EDS-HT [Beighton et al., 1998], whereas Brighton score was applied for JHS [Grahame et al., 2000]. Although a consensus is still lacking on the correct procedure of performing a satisfactory differential diagnosis, partially overlapping HCTDs were excluded on clinical grounds as detailed elsewhere by us [Castori et al., 2014]. In selected cases showing marked hyperextensibility and/or widened atrophic scars in addition to soft/velvety skin, the differential with classic EDS was carried out by molecular testing for *COL5A1* and *COL5A2* [Ritelli et al., 2013]. In our experience, JHS and EDS-HT can be often diagnosed in the same pedigree in which they seem to segregate as a single genetic trait. In a few cases, both disorders can also occur in the same individual, who meets both Villefranche and Brighton criteria. Hence, we were persuaded to use Villefranche criteria for EDS-HT and Brighton criteria as complementary tools during the assessment procedure with three possible diagnostic outcomes: JHS, EDS-HT and JHS + EDS-HT [for more details see Castori et al., 2014].

A set of objective and historical mucocutaneous features was selected independently by both groups. Before and during data gathering, comparisons and adjustments were needed for standardizations. Selected features comprised cutaneous (i.e., skin hyperextensibility, soft/velvety/silky skin, visible ecchymoses, history of easy bruising, atrophic non-papyraceous scars, piezogenic papules, *striae rubrae* and/or *distensae*, inguinal/umbilical hernia, *keratosis pilaris*/hyperkeratosis of extensor surfaces, and acquired *cutis laxa*/premature skin aging), mucosal (i.e., gingival/mucosal fragility, gingival recessions/chronic gingivitis, hypoplastic lingual frenulum, hypoplastic inferior labial frenulum, Gorlin's sign, and blue *sclerae*) and surgical (i.e., delayed wound healing, post-surgical dermatologic complications, and resistance to local anesthetics) manifestations. Some of them need further clarification, as the entire study was carried out in a clinical setting, during the outpatient evaluation of affected individuals.

Concerning skin hyperextensibility, a single reproducible method of assessing this feature was described by using two dots applied to the dorsum of hand and an electronic caliper [Farmer et al., 2010], but this is not yet considered a valid substitute of clinical observation. Hence, skin hyperextensibility was assessed qualitatively by pinching the dermis and stretching the skin. Sites of skin extensibility testing were dorsum of hand, dorsal aspect of the forearm, lateral aspect of the neck or thorax. Presence of skin hyperextensibility was arbitrarily registered for a stretching above 3 cm [Remvig et al., 2010] (Fig. 1). Variation in the range of skin extensibility was registered in a subgroup of patients and a home-made graduation was applied (i.e., moderate and marked hyperextensibility). Soft/velvety/doughy skin was an entirely subjective feeling developed during clinical practice. Atrophic non-papyraceous scars were considered those with a resulting atrophic texture but with a minor extend, so that atrophy can be best appreciated by a gentle stretching of the patient's skin between the observer's index finger and thumb (Fig. 2). Presence of true papyraceous scars lead to the exclusion from the study according to Villefranche nosology [Beighton et al., 1998]. The term "widened post-surgical scars" referred to mildly defective scar formation after surgery (usually, orthopedic interventions) (Fig. 3). Acquired *cutis laxa*/premature skin aging was considered present when the skin remained lifted up without immediately recovering its original appearance after gentle stretching. The term "acquired" was used because this feature was never observed congenitally and appeared in early adulthood. Similarly to the general population, this finding was usually appreciable at the dorsum of hands and upper eyelids. Focal hyperkeratosis at extensor surfaces (Fig. 4A–C) and *keratosis pilaris* (Fig. 4D), the latter undistinguishable from the common trait in the general population, often run together in the same individual. *Striae* were linear lesions of the skin at sites of maximum tension during growth. We annotated only the presence of those with a background persistent red halo (i.e., *rubrae*) or those appearing widened with atrophic skin (i.e., *distensae*) (Fig. 4E). Piezogenic papules were small round lesions appearing at heels in standing position and likely resulting from subcutaneous fat herniations through a mildly deficient dermis (Fig. 4F).

Blue *sclerae* is a typical sign of *osteogenesis imperfecta* and their presence and graduation are usually assessed subjectively. In an early study, grading of blue *sclerae* was assessed with dilutions of a custom-made ink [Sillence et al., 1993]. More recently, blue *sclerae* was established semi-quantitatively with the Munsell color system in *osteogenesis imperfecta* [Zack et al., 2007]. In our sample, absence of blue *sclerae* (i.e., normally pigmented *sclerae*) was fixed for a hue of 10B while a lower hue score indicated their presence. The hue range

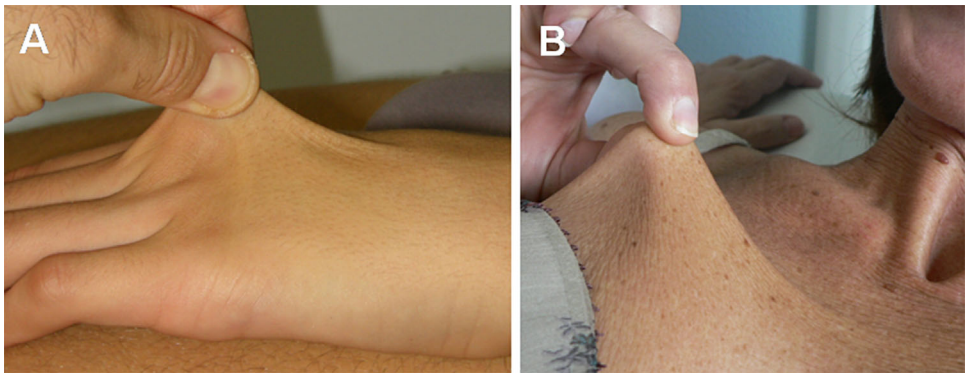

Figure 1. Skin hyperextensibility. Moderate hyperextensibility at dorsum of hand of a boy (A). Accentuated hyperextensibility in the supramammary region in an adult female (B).

in JHS/EDS-HT is more limited and "lighter" than *osteogenesis imperfecta*. Irregularities of scleral pigmentation were also annotated (Fig. 5A–D). Hypoplastic lingual frenulum was used when the frenulum was not visible at the anterior view and absent (i.e., absence) or only mildly visible (i.e., hypoplasia) at the lateral view (Fig. 5E and F). Hypoplastic inferior labial frenulum defined the lack of visualization of the inferior labial frenulum/a at gentle stretching of the patient's lower lip by the observer (Fig. 5H). Gingival anomalies were attributed in presence of recessions

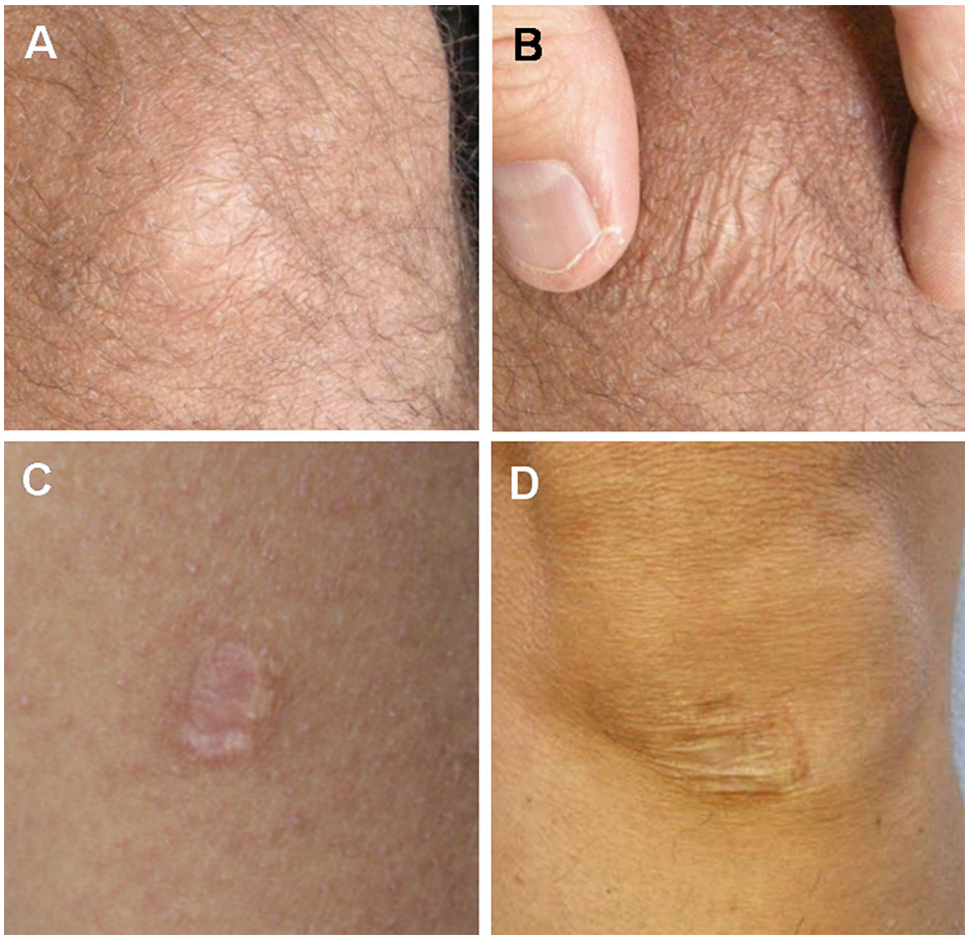

Figure 2. Small atrophic, non-papyraceous scars. A small scar (A) showing dermal atrophy at observer's gentle compression between fingers (B). Two similar scars in a boy (C) and adult female (D).

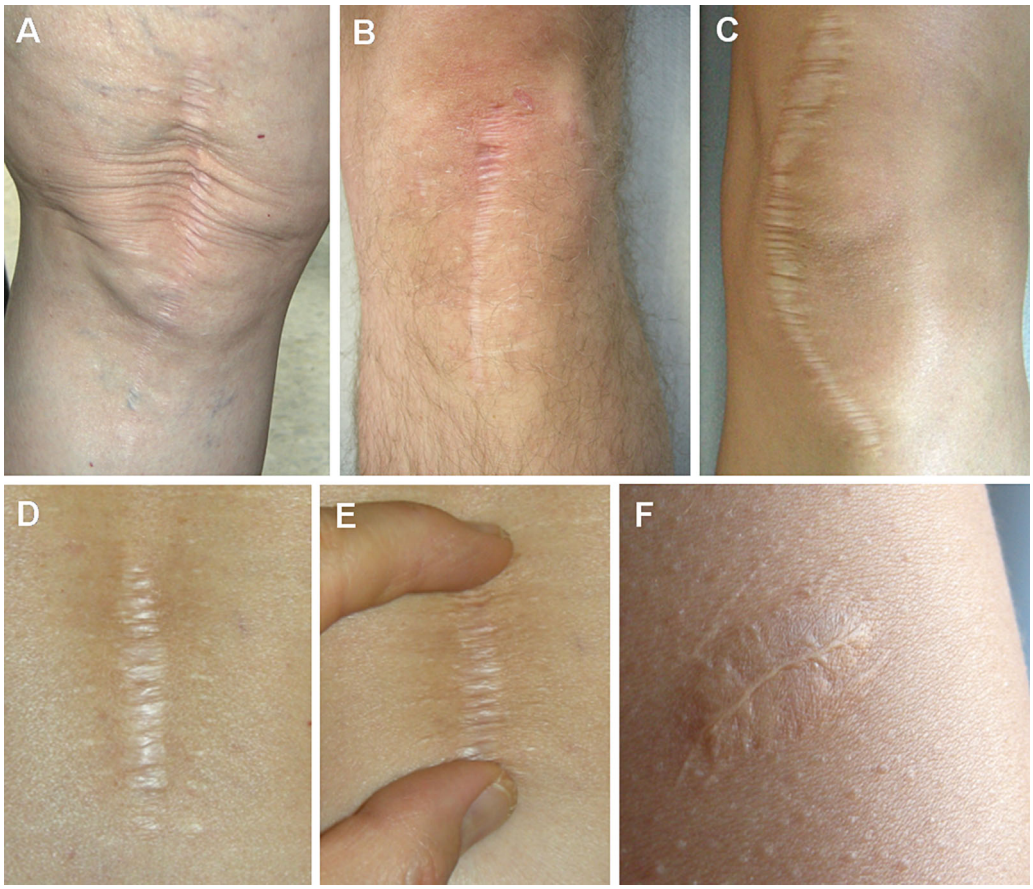

Figure 3. Post-surgical scars. In a subset of patients post-surgical, orthopedic scars were specifically annotated. Surgical scars of the knee in an adult female (A), adult male (B) and young adult female (C). Note widening and dermal atrophy distinguishable from the typical papyraceous scars in classic EDS. A back post-surgical scar for disc hernia in an adult female (D) showing the typical dermal atrophy more evident at examinator's gentle compression (E). A widened, slightly hypertrophic scar is a young female with *keratosis pilaris* (F).

and/or chronic swelling (Fig. 5G). Post-surgical dermatologic complications included hemorrhages and/or the need of additional stitches after skin closure. Resistance to local anesthetics was considered in presence of three or more such episodes.

A series of descriptive statistics were used to summarize pertinent study information. Chi-square and Fisher's exact test was performed for the comparison of categorical variables. The Pearson's correlation was used to investigate possible relationship between age at examination and BS in the entire population, as well as in patients belonging to the two sexes, separately. Comparison between the presence/absence of selected features and continuous variables (i.e., age at examination and BS) was performed using the Student *t* test. All *P* values were reported as 2-sided and *P* values less than 0.05 denotes statistically significant association. Multiple correspondence analysis (MCA), a descriptive and exploratory technique designed to analyze simple two-way and multi-way tables, was used to evaluate the possible relation among selected variables and identify specific profiles. Correlations were performed considering sex, BS (i.e., $\geq$5 vs. <5), age category (i.e., 0–10 years, 11–20 years, 21–30 years, 31–40 years, >40 years) and those features represented in the entire samples (i.e., 277 individuals). Associations between features are represented graphically in the MCA, which represents a graphic representation of the statistical relationships between distinct features, whose position in the graphic is exclusively informative. SPSS software (SPSS version 21.0, SPSS Inc., Chicago, IL) and MedCalc1 (10.0.1) statistical programs were used for all analyses.

RESULTS

Table I summarizes demographic data of the patients' cohort. Frequencies of all selected features were reported in Table II. Data were presented for the entire population and by sex. Statistically significant differences were annotated for acquired *cutis laxa*/premature skin aging, *striae rubrae* and/or *distensae*, enamel discolorations, and resistance to local anesthetics, occurring more frequently in females, while all others showed similar frequencies in both sexes. Common features (rough rate >50%) included soft/velvety/silky skin (80.9%), hypoplastic lingual frenulum (65.9%), blue *sclerae* (61.4%) and piezogenic papules (59.6%); other

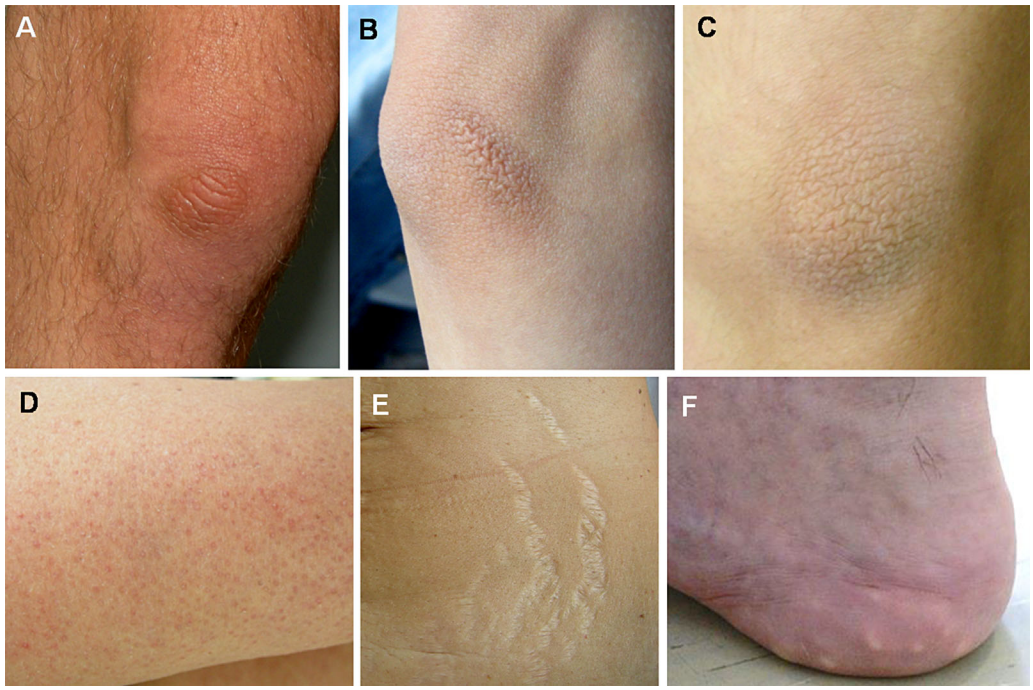

Figure 4. Hyperkeratosis of the extensor surfaces at knee in an adult male (A) and at elbow in a young female (B) and young male (C). *Keratosis pilaris* (D). *Striae distensae* in an adult female (E). Piezogenic papules (F).

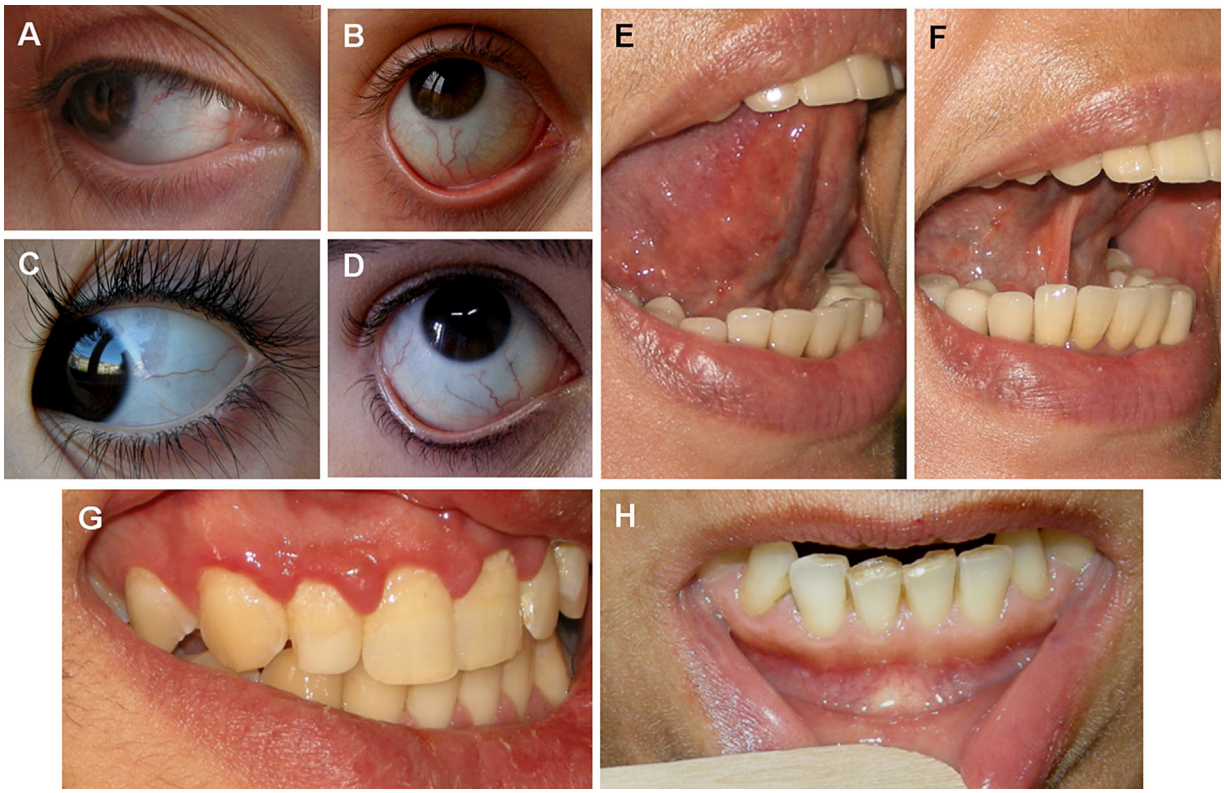

Figure 5. Mucosal findings. Variable degrees of blue *sclerae* in an adult male (A), a young female (B), a female toddler (C) and a second young female (D). Note focal accentuation of sclera pigmentation in C. Apparent absence of the lingual frenulum at the lateral view (E). The frenulum appears but remains small and short when the patient places the tongue adherent to the hard palate (F). Gingival inflammation (G). Absence of the lower lip frenulum (H).

TABLE I. Selected Demographic Data of the Patients' Sample

Characteristic	Rate
Number of patients	
Total	277 (100%)
Females	229 (82.7%)
Males	48 (17.3%)
Age range at examination	
Total (mean; SD)	2–73 yrs (31.22; 16.28)
Females (mean; SD)	2–73 yrs (33.44; 15.72)
Males (mean; SD)	3–56 yrs (20.65; 14.807)
0–10 years	34 (12.3%)
11–20 years	48 (17.3%)
21–30 years	45 (16.2%)
31–40 years	64 (23.1%)
>40 years	86 (31.1%)
Beighton score range (mean; SD)	
Total	0–9 (5.17; 2.153)
Females	0–9 (5.08; 2.185)
Males	1–9 (5.6; 1.954)

%, percentage; SD, standard deviation; yrs, years.

consistently ancillary features (rough rate ≤50% but >30%) were easy bruising (41.5%), gingival/mucosal fragility (37.5%), *striae rubrae* and/or *distensae* (35.7%), hyperextensible skin (33.2%) and hypoplastic inferior labial frenulum (30.4%). Correlation between BS and age was investigated and a statistically significant inverse relationship was noted in the entire population (N = 277) with a Pearson's correlation of 0.448 and a *P* value of <0.001. Similar results were obtained separately in females (N = 229) with a Pearson's correlation of 0.431 and a *P* value of <0.001, and males (N = 48) with a Pearson's correlation of 0.511 and a *P* value of <0.001. Table III shows the relationship between presence/absence of the selected features and age at examination and BS as continuous variables, separately. Statistically significant results were obtained for soft/velvety/silky skin, acquired *cutis laxa/*

TABLE II. Frequencies of Investigated Features in the Entire Cohort and by Sex

	Total			Females			Males			
Feature	N	Total	%	N	Total	%	N	Total	%	*P* value
Observational features										
Soft/velvety/silky skin	224	277	80.9	186	229	81.2	38	48	79.2	0.74
Hyperextensible skin	92	277	33.2	76	229	33.2	16	48	33.3	0.98
Atrophic non-papyraceous scars	70	277	25.3	56	229	24.5	14	48	29.2	0.49
Post-surgical atrophic scars	34	142	23.9	26	114	22.8	8	28	28.6	0.52
Ecchymosis	62	277	22.4	53	229	23.1	9	48	18.8	0.51
Acquired *cutis laxa*/premature skin aging	23	277	8.3	23	229	10.0	0	48	0.0	0.02
Piezogenic papules	165	277	59.6	139	229	60.7	26	48	54.2	0.40
Striae rubrae and/or *distensae*	99	277	35.7	90	229	39.3	9	48	18.8	0.007
Keratosis pilaris/hyperkeratosis of the extensor surfaces	38	277	13.7	29	229	12.7	9	48	18.8	0.26
Gingival recessions/inflammation	32	135	23.7	30	115	26.1	2	20	10.0	0.12
Enamel discolorations	20	135	14.8	20	115	17.4	0	20	0.0	0.04
Gorlin's sign	9	135	6.7	8	115	7.0	1	20	5.0	0.75
Hypoplastic lingual frenulum	89	135	65.9	77	115	67.0	12	20	60.0	0.54
Hypoplastic inferior labial frenulum	41	135	30.4	37	115	32.2	4	20	20.0	0.27
Inguinal/umbilical hernia	24	277	8.7	21	229	9.2	3	48	6.3	0.51
Blue *sclerae*	170	277	61.4	137	229	59.8	33	48	68.8	0.25
Historical features										
Easy bruising	115	277	41.5	101	229	44.1	14	48	29.2	0.06
Gingival/mucosal fragility	104	277	37.5	88	229	38.4	16	48	33.3	0.51
Delayed wound healing	60	277	21.7	52	229	22.7	8	48	16.7	0.36
Dermatological surgical complications	23	135	17.0	21	115	18.3	2	20	10.0	0.36
Resistance to local anesthetics	46	277	16.6	45	229	19.7	1	48	2.1	0.003

TABLE III. Correlation by Age and Beighton Score of Investigated Features

	Age (years)			Beighton score		
Feature	Mean (SD) in *presence* of	Mean (SD) in *absence* of	*P* value	Mean (SD) in *presence* of	Mean (SD) in *absence* of	*P* value
Observational features						
Soft/velvety/silky skin	29.64 (16.267)	37.91 (14.682)	**0.001**	5.48 (1.989)	3.85 (2.332)	**<0.0001**
Hyperextensible skin	32.85 (13.657)	30.42 (17.416)	0.21	5.36 (2.036)	5.08 (2.208)	0.30
Atrophic non-papyraceous scars	31.39 (13.341)	31.17 (17.189)	0.91	4.93 (2.066)	5.25 (2.180)	0.28
Post-surgical atrophic scars	34.53 (13.020)	29.82 (17.135)	0.09	5.50 (2.219)	5.05 (2.034)	0.27
Ecchymosis	29.58 (15.494)	31.70 (16.504)	0.37	5.44 (2.062)	5.09 (2.177)	0.27
Acquired *cutis laxa*/premature skin aging	45.74 (9.358)	29.91 (16.143)	**<0.0001**	4.96 (1.942)	5.19 (2.173)	0.62
Piezogenic papules	31.39 (16.338)	30.98 (16.263)	0.84	5.16 (2.064)	5.18 (2.287)	0.96
Striae rubrae and/or *distensae*	35.38 (13.675)	28.91 (17.166)	**0.001**	4.94 (2.231)	5.30 (2.104)	0.18
Keratosis pilaris/hyperkeratosis of the extensor surfaces	23.55 (13.476)	32.44 (16.377)	**0.002**	5.55 (2.089)	5.11 (2.161)	0.24
Gingival recessions/inflammation	44.44 (10.364)	27.50 (15.719)	**<0.0001**	4.19 (2.481)	5.50 (2.067)	**0.003**
Enamel discolorations	45.3 (12.144)	29.11 (15.749)	**<0.0001**	4.5 (2.328)	5.3 (2.205)	0.14
Gorlin's sign	37.33 (18.042)	31.10 (16.152)	0.27	4.67 (2.784)	5.22 (2.198)	0.47
Hypoplastic lingual frenulum	32.64 (15.903)	29.33 (16.967)	0.26	5.12 (2.016)	5.30 (2.624)	0.68
Hypoplastic inferior labial frenulum	34.05 (14.864)	30.40 (16.824)	0.23	5.05 (2.280)	5.24 (2.222)	0.64
Inguinal/umbilical hernia	34.71 (14.208)	30.89 (16.449)	0.27	5.92 (1.976)	5.10 (2.159)	0.08
Blue *sclerae*	29.62 (15.841)	33.77 (16.714)	**0.04**	5.23 (2.081)	5.07 (2.268)	0.56
Historical features						
Easy bruising	34.46 (15.694)	28.93 (16.344)	**0.005**	4.93 (2.312)	5.34 (2.022)	0.12
Gingival/mucosal fragility	34.40 (15.183)	29.31 (16.656)	0.01	4.57 (2.293)	5.53 (1.984)	**<0.0001**
Delayed wound healing	32.60 (14.565)	30.84 (16.734)	0.46	4.72 (2.351)	5.29 (2.083)	0.07
Dermatological surgical complications	35.04 (13.656)	30.79 (16.737)	0.25	4.57 (2.063)	5.31 (2.254)	0.14
Resistance to local anesthetics	38.26 (12.817)	29.82 (16.553)	**<0.0001**	4.61 (2.081)	5.28 (2.154)	**0.05**

Significant *P* values are in bold.

premature skin aging, *striae rubrae* and/or *distensae*, *keratosis pilaris*/hyperkeratosis of extensor surfaces, gingival recessions/inflammation, enamel discolorations, blue *sclerae*, easy bruising, gingival/mucosal fragility and resistance to local anesthetics in relation to age at examination, and for soft/velvety/silky skin, gingival recessions/inflammation, gingival/mucosal fragility and resistance to local anesthetics in relation to BS value. Results of MCA show a clustering of features in specific circumstances. In particular, MCA suggests the existence of three relatively well defined associations of features (Fig. 6). Briefly, the first one, visualized in the lower left quadrant of the diagram, shows an association among male sex, age $\leq$20 years, BS $\geq$5 and presence of *keratosis pilaris*. The second one, visualized in the upper right quadrant, demonstrates that age >30 years, BS <5, the presence of acquired *cutis laxa*/premature skin aging and the absence of soft/velvety/silky skin tend to run together as well as, with a lower statistical strength, female sex, and the presence of abdominal hernias, gingival/mucosal fragility and resistance to local anesthetics. The third one, visualized in the upper left quadrant, associates age between 21 and 30 years with the presence of atrophic non-papyraceous scars, piezogenic papules, *striae rubrae* and/or *distensae*, as well as, with a lower statistical strength, blue *sclerae* and hyperextensible skin.

Molecular testing of *COL5A1* and *COL5A2* by direct sequencing of both genes and the exclusion of genomic rearrangements of *COL5A1* by multiple ligation-dependent probe amplification was carried out in 36 patients with negative results in all.

DISCUSSION

This work presents data on selected mucocutaneous features in 277 JHS/EDS-HT patients of both sexes and various ages. The study was carried out in a clinical setting by using generally accepted tools/parameters and intended as purely observational, with the primary aim of depicting an incompletely defined aspect of JHS/EDS-HT. Besides age, sex and BS, we selected a set of 21 features, 16 identifiable at examination and five anamnestic. Available diagnostic criteria (i.e., Villefranche criteria for EDS-HT and Brighton criteria) identify the four main cutaneous features of JHS/EDS-HT in soft/velvety skin, hyperextensible skin, *striae* and "scarring" [Beighton et al., 1998; Grahame et al., 2000]. In their original form and semantics, the two partially overlapping

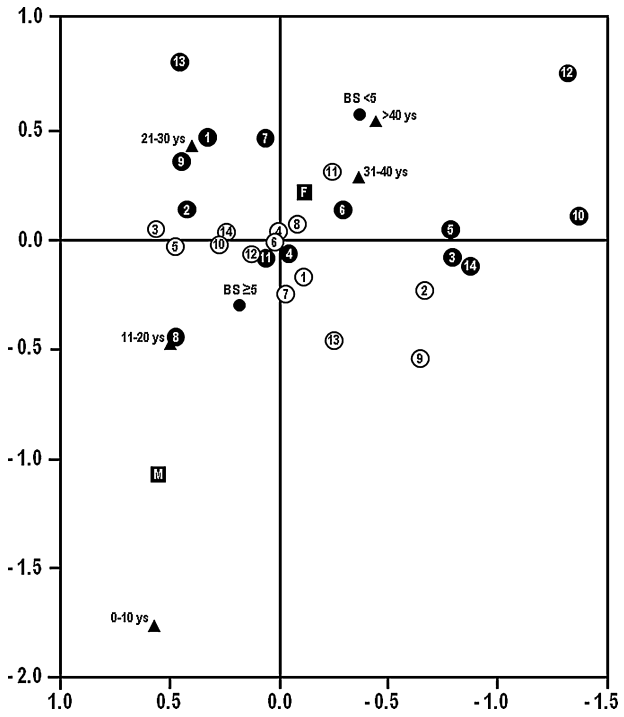

Figure 6. Results of multiple correspondence analysis considering sex, age by category, Beighton score (<5 vs. ≥5) and the fourteen features assessed in the entire sample (i.e., 277 individuals). Sex is indicated by a black square with M indicating males and F indicating females. Age categories are indicated by a black triangle. Beighton score is indicated by a small black circle. For the fourteen features, their presence is indicated by a black circle, while their absence by a white circle. 1, atrophic non-papyraceous scars; 2, blue *sclerae*; 3, capillary fragility; 4, ecchymoses; 5, gingival/mucosal fragility; 6, abdominal/inguinal hernias; 7, hyperextensible skin; 8, *keratosis pilaris*/hyperkeratosis of the extensor surfaces; 9, piezogenic papules; 10, resistance to local anesthetics; 11, soft/velvety/silky skin; 12, premature skin aging/acquired *cutis laxa*; 13, *striae rubrae* and/or *distensae*; 14, delayed wound healing.

cutaneous criteria (i.e., "hyperextensibility and/or smooth, velvety skin" in the Villefranche criteria, and "skin *striae*, hyperextensibility, or scarring" in the Brighton criteria) are likely inclusive but not sufficiently specific, as they are shared by other HCTDs. Accordingly, EDS-HT and JHS are still two exclusion diagnoses. The lack of reliable, widely used tools/procedures for assessing skin texture (i.e., extensibility and consistency) represents a further major hurdle for the acceptance of available cutaneous criteria. In our clinical practice, we also identified a further point of concern in the concept of defective scar formation in JHS/EDS-HT. Villefranche criteria tells us that the presence of papyraceous scars is indicative, when not pathognomonic, of classic EDS [Beighton et al., 1998]. On the other hand, specialized literature testifies for the possibility of mildly widened and atrophic scars also in JHS/EDS-HT [Grahame, 2010]. We completely agree with this last concept, but the difference in the extent of defective soft tissue repair between classic EDS and JHS/EDS-HT has not been sufficiently emphasized in the past. This could lead to inaccurate patients' classification in primary care setting with consequences in their subsequent referrals and management. Among the four previously labeled cutaneous characteristics, soft/velvety/silky skin was the most common feature occurring in ~81%, while *striae* (*rubrae* and/or *distensae*), skin hyperextensibility and atrophic, non-papyraceous scars occurred in 1/4–1/3 of the patients in our sample. Available criteria apparently pose the same diagnostic significance to these four features. Our findings may represent a bias reflecting the subjectivity of involved clinicians and/or the skewed ethnicity of the sample (i.e., all patients are Italian in origin). Conversely, these results could stand for a different (and clinically distinguishable) degree of skin fragility in JHS/EDS-HT compared to classic EDS and other EDS variants with marked cutaneous involvement. In JHS/EDS-HT, skin resistance appears roughly conserved with absence of a true increase in wound formation and exaggerate response to physical traumas. In fact, recurrent wound opening at trauma-prone sites was never registered. In JHS/EDS-HT, the presumed dermal defect manifests nearly exclusively during normal or post-surgical wound healing with a long persistence of the resulting scar, which is often mildly widened and atrophic. This phenomenon was particularly evident after orthopedic surgery. Possible reasons include: (i) because orthopedics is the most common type of surgery referral in these patients, and/or (ii) because the skin overlaying joints is particularly subject to tractions during wound healing.

According to accumulated evidence [Remvig et al., 2007], we found an excess of females with a sex ratio >4:1. Similarly to other works, our sample was directly extracted by patients referred to our clinical centers and we did not distinguish between sporadic and familial cases. Previously, our group suggested the possibility to observe a reduction of the sex skewing by evaluating the extended family [Castori et al., 2010a, 2014]. In other words, the number of "affected" males tends to increase by direct evaluation of apparently non-affected relatives of a (female) proband. At the moment, the reasons are not well understood, but the hypothesis of a sex-influenced autosomal dominant trait is likely [Castori et al., 2010a]. The extent of sexual dimorphism in single manifestations of JHS/EDS-HT is actually under investigation by various research groups. In this work, we found minor differences between sexes in mucocutaneous manifestations with an excess of acquired *cutis*

laxa/premature skin aging, *striae rubrae* and/or *distensae*, enamel discolorations, and resistance to local anesthetics in females (Table II). This implies that, in JHS/EDS-HT, the effect of the disease on skin and *mucosae* is less influenced by sex than other tissues and, then, could represent a "biomarker" for tracing the intrafamilial inheritance of the underlying molecular defect.

In this sample, we also confirmed an inverse correlation between age and BS in the entire patients' cohort, as well as in both sexes separately. By comparing feature occurrence with age and BS, we found interesting differences. The correlation is stronger with age. In particular, soft/velvety/silky skin, *keratosis pilaris*/hyperkeratosis of the extensor surfaces and blue *sclerae* tend to be found in younger patients, while acquired *cutis laxa*/premature skin aging, *striae rubrae* and/or *distensae*, gingival recessions/inflammation, enamel discolorations and resistance to local anesthetics occur more commonly in older individuals (Table III). This finding supports the concept of an evolving phenotype in JHS/EDS-HT [Castori et al., 2013], which seems extending also to skin and mucosal manifestations. MCA results are in line with this hypothesis. In fact, three clusters of features were identified which may correspond to the three prototypical presentations of JHS/EDS-HT in terms of mucocutaneous manifestations. The first profile is that of young or very young patients (i.e., 0–20 years) who are more frequently men and present a high BS and *keratosis pilaris*/hyperkeratosis of the extensor surfaces as the most typical mucocutaneous finding. The second profile corresponds to young adults (i.e., 21–30 years) of both sexes in whom mucocutaneous involvement is more commonly represented by atrophic non-papyraceous scars, piezogenic papules and *striae rubrae*/*distensae*, and, less significantly, blue *sclerae* and hyperextensible skin. The third (last) profile labels older patients (i.e., age >30 years) who tend to be females with a lower BS and more commonly show acquired *cutis laxa*/premature skin aging and, less consistently, abdominal hernias, gingival/mucosal fragility and resistance to local anesthetics. These clusters could represent the mucocutaneous counterparts of the three disease phases previously proposed for the JHS/EDS-HT [Castori et al., 2010b, 2011, 2013]. The cross-sectional nature of this study limits such an assumption and the preliminary identification of these profiles may, in turn, reflect selection biases, genetic heterogeneity or other (more complex) variables leading to different but recurrent (and, then, recognizable) consequences on skin and *mucosae*.

In this work, we also reported data on intraoral frenula in a large sample of JHS/EDS-HT patients. Assessment methods were the same as reported in Celletti et al. [2011]. The issue on the possible increased rate of absence or hypoplasia of the intraoral frenula was first raised by De Felice et al. [2001]; who proposed this feature as suggestive of EDS-HT and classic EDS. Subsequent studies were confirmatory [Perrinaud et al., 2007; Machet et al., 2010; Celletti et al., 2011], while others failed to support this finding [Böhm et al., 2001; Shankar et al., 2006]. All these works are limited by the relatively small patients' sample and clinical heterogeneity of selected subjects (i.e., different EDS subtypes). Here, we report data on 135 patients with a "strict" diagnosis of JHS/EDS-HT and found hypoplasia of lingual frenulum and inferior labial frenulum in ~2/3 and ~1/3 of the patients, respectively. Concerning hypoplasia of the lingual frenulum, we speculated on its origin. Although not accurately registered and, then, reported in this study, we often found an abnormal lingual motility, incompetence to completely adhere the tongue to the hard palate and atypical deglutition in association with hypoplasia of the lingual frenulum. These features, in combination with the visual impression (Fig. 4E and F) and the unexpectedly low rate of positive Gorlin's sign (6.7%), are compatible with a short, instead of hypoplastic or absent, lingual frenulum, thus suggesting the existence of a particular form of "posterior tongue tie" in JHS/EDS-HT. This preliminary finding, if functionally investigated in future works, may contribute in understanding the pathophysiology of other cervicofacial features of JHS/EDS-HT, such as oropharyngeal dysphagia and headache.

Finally, our findings may be of some relevance for the identification of a more reliable "mucocutaneous criterion" for JHS/EDS-HT. Previous studies highlighted the low reproducibility and lack of consensus of soft/velvety skin and skin hyperextensibility [Remvig et al., 2014]. Furthermore, the identification of reliable measurement tools for these characteristics seems hampered by a presumed marked variability by age, sex and ethnicity. Accordingly, we need to select a set of features which can be more easily spotted by gestalt or standardized measurements. In our experience, atrophic non-papyraceous scars (also including post-surgical atrophic scars), piezogenic papules, *striae rubrae* and/or *distensae,* blue *sclerae*, chronic gingival inflammation, and hypoplastic (short) lingual frenulum seem sufficiently common and clear-cut to be considered as good candidates. Given their presumed occurrence in the general population in isolation, it is likely the need of identifying a specific combination of features for attributing a "positive" mucocutaneous criterion. The observation of delayed wound healing in 1/5 of the patients, as well as dermatological surgical complications and resistance to local anesthetics in >1/6 indicates that, though not contraindicated, surgical and anesthetic procedures should be performed by care, by following standard recommendations for EDS [Burcharth and Rosenberg, 2012], which has been customized for JHS/EDS-HT [Castori, 2012b].

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