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ORIGINAL ARTICLE

Skin biopsy reveals generalized small fibre neuropathy in hypermobile Ehlers–Danlos syndromes

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Abstract

Background and purpose: Ehlers–Danlos syndromes are hereditary disorders of connective tissue that are characterized by joint hypermobility, skin hyperextensibility and tissue fragility. The most common subtype is the hypermobile type. In addition to symptoms of small fibre neuropathy (SFN) due to damage to the small peripheral nerve fibres, with degeneration of the distal nerve endings, autonomic disorders such as postural tachycardia syndrome (PoTS) are frequently reported features in patients with hypermobile Ehlers–Danlos syndrome (hEDS). To date, the underlying pathophysiological mechanisms are still not completely understood.

Study Purpose: To better understand pathophysiological mechanisms of small fiber neuropathy and autonomic neuropathy in hypermobile Ehlers-Danlos Syndromes.

Methods: We prospectively investigated 31 patients with hEDS compared to 31 healthy controls by using skin biopsy, quantitative sensory testing, tilt-table testing, the painDetect, Small Fibre Neuropathy Screening List and the COMPASS-31 (Composite Autonomic Symptom Score 31) questionnaire.

Results: Nineteen (61%) patients with hEDS were diagnosed with SFN, and 10 (32%) fulfilled the criteria for PoTS. Patients with hEDS had significantly higher heart rates than controls. According to quantitative sensory testing, these patients had generalized thermal and tactile hypesthesia. Skin biopsy revealed significantly reduced intraepithelial nerve fibre density proximally (thigh) and distally (lower leg) in patients compared to controls. This was consistent with various complaints of pain and sensory disturbances in both the proximal and distal body regions.

Conclusion: These results confirm histologically proven SFN as a common feature in patients with hEDS, revealing a generalized distribution of nerve fibre loss. Regarding the frequently reported autonomic and neuropathic dysfunctions, the findings support SFN as an important, but not the only, underlying pathomechanism.

KEYWORDS
autonomic small fibre neuropathy, Ehlers–Danlos syndrome, neuropathic pain, postural tachycardia syndrome, skin biopsy, Small Fibre Neuropathy Screening List

Trial Registration Information ClinicalTrials.gov Identifier: NCT04310644

[Correction added on 02 February 2023 after first online publication: The 18th reference has been replaced with a new reference: Hoitsma et al. 'Small Fibre Neuropathy Screening List' has been added to the keywords.]

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INTRODUCTION

Ehlers–Danlos syndromes (EDS) comprise a clinically and genetically heterogeneous group of hereditary disorders of the connective tissue [1]. With an estimated prevalence of 1/5000 [2], EDS are defined as rare diseases. Women are predominantly affected [3]. EDS is often diagnosed late, which is associated with long periods of suffering for patients [1].

Ehlers–Danlos syndromes are characterized by three main features: joint hypermobility, skin hyperextensibility and fragility of connective tissue [2, 4]. Since 2017, an international classification has been established, which now includes 13 subtypes of EDS [5]. The most common subtype is the hypermobile type (hEDS), which is diagnosed clinically [5, 6]. Patients with hEDS experience a variety of symptoms, such as general joint pain, joint instability, often accompanied by (sub-)luxations, fatigue, disorders of proprioception and increased bruising [7, 8]. In addition to these symptoms, only a few previous studies suggest that small fibre neuropathy (SFN) may be a common feature of EDS, especially of hEDS [9–11].

Structural damage to small peripheral nerve fibres with degeneration of the distal nerve endings underlies the pathology of the classic, length-dependent SFN [12] (Figure 1). The small, afferent, thinly myelinated Aδ fibres and unmyelinated C fibres are selectively affected [13]. Patients with SFN experience neuropathic and nociceptive pain, characterized by burning sensations, disturbed perception of temperature, numbness, allodynia, hyperalgesia, and paraesthesia [10, 13]. Furthermore, patients with EDS may also show a reduced intraepithelial nerve fibre density (IENFD) [9, 10].

Other frequently described features of hEDS are visceral and especially autonomic dysfunctions such as tachycardia in orthostasis, disorders of the vasomotor (cold hands and feet) and secretomotor (dry eyes, mouth and skin) systems, headache, fatigue, and disorders of the gastrointestinal, urinary and sudomotor systems [8, 14, 15]. Autonomic cardiovascular dysfunctions such as postural tachycardia syndrome (PoTS) or orthostatic hypotension are common features in patients with hEDS and have a great impact on their everyday life [16]. Many autonomic features in hEDS resemble those of autonomic disorders common in the framework of SFN [17–19].

To date, few studies [9–11] have investigated the involvement of SFN in hEDS. However, the underlying causes and mechanisms of neuropathic and nociceptive pain in hEDS are still incompletely understood [4, 9, 10]. It remains unclear whether the symptoms of autonomic neuropathy are attributable to dysfunctional collagen synthesis and hyperlax connective tissue, selective destruction of the autonomic nerve fibres based on SFN, or both dysfunctional collagen synthesis and SFN [4, 8, 10, 14, 20].

The present prospective study was designed (i) to determine the frequency of SFN in hEDS, (ii) to further specify if hEDS patients present a length- versus a non-length-dependent SFN and (iii) to contribute to a better understanding of the underlying pathomechanisms of neuropathic pain and autonomic dysfunctions in hEDS.

METHODS

Standard protocol approvals, registrations, and patient consents

All patients gave their written consent to participate in this study and were able to understand all instructions. This prospective investigation was performed in accordance with the Declaration of Helsinki and good clinical practice. It was approved by the Institutional Review Board (Ethics Committee) of the Medical Faculty of RWTH Aachen University (EK 092/19) and registered

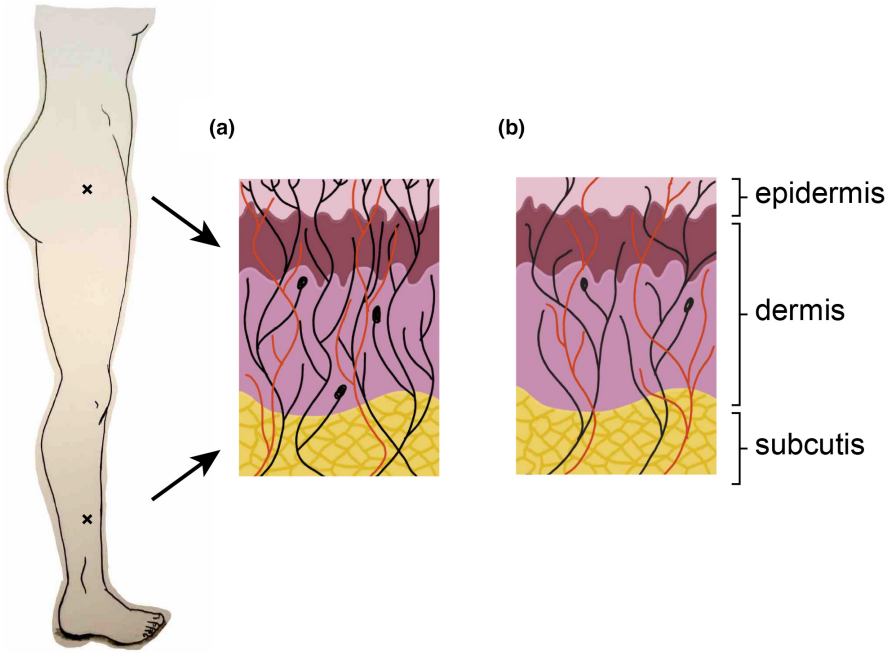


FIGURE 1 Schematic drawing of the extracted skin biopsies. Skin biopsies were taken 15 cm below the spina iliaca anterior superior (proximally marked cross) and 10 cm above the lateral malleolus (distally marked cross). Skin layers are shown with normal (a) and reduced (b) intraepidermal nerve fibre density [Colour figure can be viewed at wileyonlinelibrary.com]

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at ClinicalTrials.gov (NCT04310644). All investigations were performed under standardized conditions in our centre, which is highly specialized in the diagnosis and therapy of hEDS and autonomic neuropathies.

Participants

Thirty-one patients with hEDS (age 35.3 ± 1.7 years, *n* = 3 male) were prospectively examined and compared to 31 age- and sex-matched healthy controls (age 35.1 ± 1.9 years, *n* = 3 male; no presence of connective tissue and autonomic disorders). Adult patients with hEDS were diagnosed by A.M. and D.I. of the Autonomic Nervous System Center, Department of Neurology, RWTH Aachen University Hospital. The diagnosis was based on the 2017 international classification of EDS [5]. According to this classification, the following three criteria must be fulfilled. First, a Beighton score of at least 5/9 is needed. Beighton score assesses generalized joint hypermobility. Second, two out of three features must be present (A: at least five manifestations of a generalized connective tissue disorder; B: hEDS-positive family history; C: at least one musculoskeletal complication). Third, other underlying hereditary connective tissue disorders, including other subtypes of non-hypermobile EDS, must be excluded [5]. In line with the NEURODIAB criteria, patients were assigned to the SFN group if they presented with the combination of length-dependent symptoms (e.g., burning sensations, numbness, paraesthesia) or clinical signs (hypesthesia, hyperalgesia or allodynia) and an abnormal thermal quantitative sensory testing (QST) and/or a reduced IENFD at the distal leg [21].

Relevant pre-existing comorbidities of the patients were obtained by taking medical histories and through medical reports. Comorbidities included: mast cell activation (*n* = 11); Hashimoto's disease (*n* = 3); sleep disorder, such as insomnia (*n* = 5); mild–moderate depression (*n* = 6); and anxiety disorder, not further specified (*n* = 2).

Pregnancy, drug abuse, pacemakers, deep brain stimulation, sensorimotor polyneuropathy, large fibre neuropathy, and other neurodegenerative diseases, such as Parkinson's and Alzheimer's disease, and inflammatory diseases, such as multiple sclerosis, were considered exclusion criteria for all participants.

Questionnaires

The impairment of the autonomic nervous system, pain status and pain characteristics of all participants was assessed using the following questionnaires: painDetect (Cronbach's alpha = 0.71) [22], the Small Fibre Neuropathy Screening List (SFNSL; Cronbach's alpha = 0.88) [18] and the Composite Autonomic Symptom Score 31 (COMPASS-31; Cronbach's alpha = 0.72) [46]. Based on the total score of complaints recorded in painDetect, indications of probable (>18), unclear (13–18), or unlikely (<13) neuropathic involvement can be detected. Additionally, by recording the total score of the frequency and intensity of neuropathic symptoms in SFNSL, a classification into definite (>48), probable (11–48) or no (<11) SFN can be made. According to COMPASS-31, autonomic symptoms are divided into six domains: orthostatic intolerance; vasomotor; secretomotor; gastrointestinal; bladder; and pupillomotor.

Skin biopsy

To identify morphological small fibre changes and to determine the IENFD, a 4-mm skin punch biopsy was taken from the right leg 20 cm below the anterior superior iliac spine and 10 cm above the lateral malleolus using a disposable punch [23]. According to the standard protocol [23], the physicians disinfected the skin and injected a local anaesthetic with 0.5 ml bupivacain 0.5% + epinephrine 0.0005% (1:200,000; JENAPHARM mibe GmbH) near but not directly into the relevant (above-mentioned) areas to prevent artefacts. After carefully inserting the punch, a cylindrical piece of tissue was obtained and transferred into a small sample tube filled with Zamboni solution. The biopsy material was processed using standard PGP9.5 immunofluorescence at the Institute of Neuropathology, RWTH University Hospital Aachen, Germany [24, 25]. The IENFDs of the hEDS patients and of the controls were compared to normative values matched for sex and age [26].

Quantitative sensory testing

To assess the functions of the thinly myelinated Aδ fibres and unmyelinated C fibres, QST was performed according to the DFNS protocol [27]. QST is a noninvasive, standardized and formalized clinical sensitivity investigation evaluating the function of the fibres with their projection paths into the brain [28]. The DFNS QST protocol consists of a battery of thermal and mechanical testing procedures that were performed on the patients' clinically more affected hands and feet [29]. Within the thermal testing, the detection thresholds for cold, warm, paradoxical heat sensations and for pain thresholds for cold and heat pain were tested using an MSA thermal stimulator (Somedic) [29, 30]. During mechanical testing, the tactile perception and mechanical pain thresholds, mechanical pain sensitivity and wind-up ratio for pinprick stimuli, vibration detection and pressure pain thresholds were examined using standardized mechanical stimuli [29, 31, 32].

Tilt-table

To specifically examine autonomic function, standardized tilt-table testing was performed [33]. The participants were ordered not to ingest any food, coffee, tea, nicotine, medications or alcohol at least 12 h before the examination. The participants were secured by belts on the tilt-table. After being in a lying position for 10 min, the participants were tilted at 70° for 12–20 min and then tilted back in a

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lying position for 5–10 min. During the examination, the blood pressure and heart rate were continuously measured and documented both automatically by the tilt-table (WR Medical Electronics Co. Testworks 3) and manually by the blood pressure monitor (Welch Allyn). To diagnose orthostatic hypotension, a systolic drop of 20 mmHg and a diastolic drop of 10 mmHg had to be present during 3 min of standing [8]. PoTS was diagnosed by a persisting heart rate increase of more than 30 bpm within the first 10 min after tilting at 70°, accompanied by orthostatic symptoms for at least 3 months [15, 34]. Furthermore, heart rate and blood pressure values in the supine and upright positions were acquired in terms of the initial (<15 s) and mean heart rate increase after tilting at 70°, the mean systolic/diastolic drop after tilting at 70° and a correlation between proximal/distal IENFD and Beighton score.

Statistical analyses

Statistical analyses were performed with IBM SPSS (Version 27). The normal distribution of the data was screened with the Shapiro–Wilk test. Unpaired two-sided *t*-tests were used to evaluate the age, tilt data, questionnaires and skin biopsy results. An unpaired one-sided *t*-test was used for the mean increase in heart rate. Paired two-sided *t*-tests were used to compare the proximal and distal IENFD within the hEDS group. For the subgroup comparison of controls, a nonparametric Mann–Whitney *U*-test was used based on a nonnormal distribution of the questionnaire total scores. QST data were z-transformed before applying between- and within-subject analysis of variance. To assess a linear relationship between variables, Pearson's correlation analysis was performed. A linear regression analysis was performed to determine the possible influences of Beighton score on heart rate, blood pressure values and proximal/distal IENFD. Data are presented as mean ± standard error of mean. The alpha level of significance was set to $p < 0.05$.

RESULTS

Questionnaires

Regarding the total scores of painDetect and SFNSL, hEDS patients and controls differed significantly (painDetect: $t[49.96] = 9.60$, $p < 0.001$; SFNSL: $t[42.10] = 12.89$, $p < 0.001$). According to the numeric rating scale (NRS) in painDetect, the average pain intensity during the previous 4 weeks was 5.35 ± 0.30. Twenty-six patients (84%) had an NRS score of at least 4/10, corresponding to clinically relevant pain. Analysis of the painDetect scores revealed that seven patients (22.6%) had a probable, 12 (38.7%) an unclear, and 12 (38.7%) an unlikely neuropathic pain component. The SFNSL results indicated that 11 patients (35.5%) had definite SFN, 19 (61.3%) had probable SFN and one (3.2%) had no SFN. hEDS patients had significantly higher COMPASS-31 scores than controls (total score: $t[46.97] = 13.52$, $p < 0.001$; Table 2).

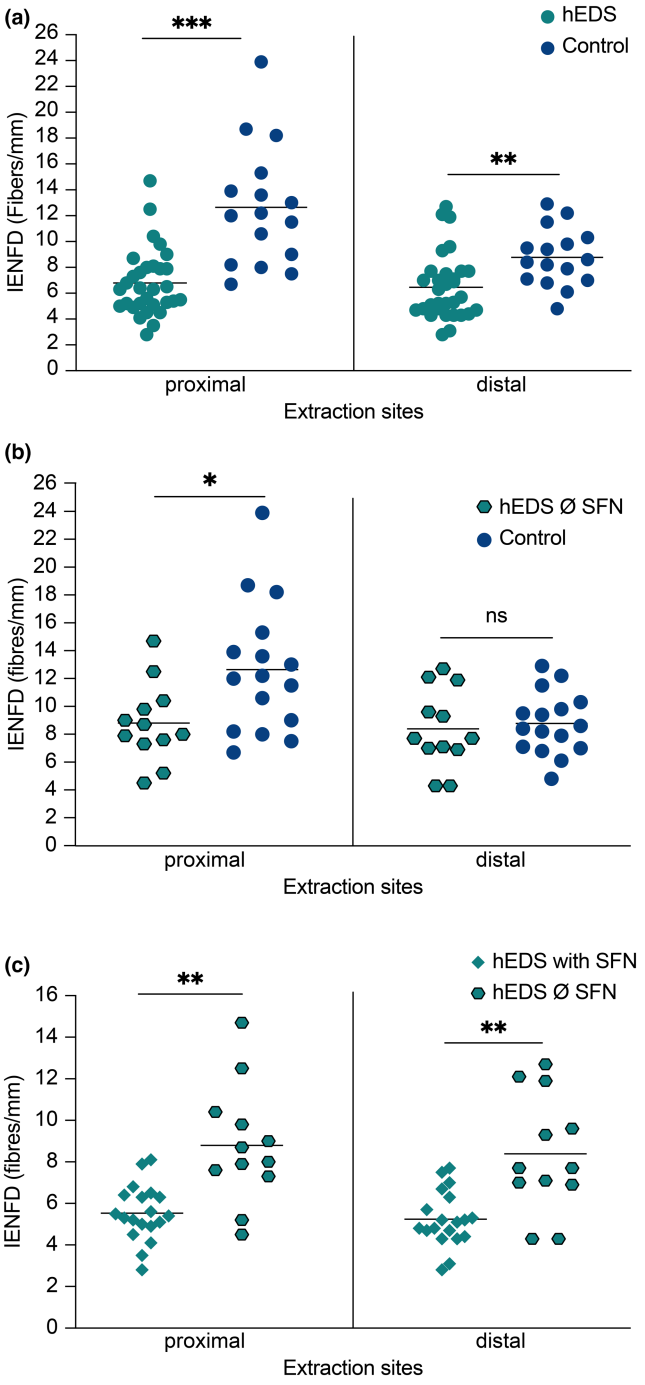


FIGURE 2 Intraepithelial nerve fibre density (IENFD). Hypermobile Ehlers–Danlos syndrome (hEDS) patients differed significantly from controls (a). Compared to controls, hEDS patients without small fibre neuropathy (SFN) demonstrated significantly reduced IENFD proximally but not distally (b). hEDS patients with SFN differed significantly from hEDS patients without SFN (c). *$p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ns, not significant [Colour figure can be viewed at wileyonlinelibrary.com]

Skin biopsy

Patients with hEDS versus controls

Not all controls agreed to a skin biopsy. A comparison between the control subgroup with skin biopsy ($n = 16$, one male) and without

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TABLE 1 Comparison of demographic and clinical data of hypermobile Ehlers–Danlos syndrome patients and healthy controls

	hEDS	Healthy controls
	***n* = 31**	***n* = 31**
Demographics		
Age, years	35.3 ± 1.7 n.s	35.1 ± 1.9 n.s
Sex: female/male, *n* (%)	28 (90)/3 (10)	28 (90)/3 (10)
Comorbid conditions, *n* (%)		
Mast cell activation	11 (35)	0 (0)
Hashimoto's disease	3 (10)	0 (0)
Sleep disorder	5 (16)	0 (0)
Depression	6 (19)	0 (0)
Anxiety disorder	2 (6)	0 (0)
Orthostatic complaints, *n* (%)		
Yes	29 (94)	0 (0)
No	2 (6)	31 (100)
Vegetative complaints, *n* (%)		
Yes	23 (74)	0 (0)
No	8 (26)	31 (100)
Questionnaires: Mean (±SEM) total scores		
painDetect	**17 (±1)****	**3 (±1)****
SFNSL	**41 (±3)****	**5 (±1)****
COMPASS-31	**51.3 (±2.6)****	**10.9 (±1.5)****
Tilt table		
Mean (±SEM) heart rate when supine, beats/min	**71 (±2)****	**60 (±1)****
Mean (±SEM) heart rate when upright, beats/min	**88 (±3)****	**73 (±2)****
Mean (±SEM) increase in heart rate, beats/min	**18 (±2)***	**14 (±1)***
Initial increase in heart rate, beats/min	**16 (±2)***	**9 (±2)***
Circulatory disturbance, *n* (%)		
Postural tachycardia syndrome	10 (32)	0 (0)
Orthostatic hypotonia	0 (0)	0 (0)
SFN-typical symptom localization, *n* (%)		
Face	11 (35)	-
Trunk	9 (29)	-
Arm	8 (26)	-
Hand	15 (48)	-
Leg	18 (58)	-
Foot	22 (71)	-
Small fibre neuropathy		
Proximal IENFD, fibres/mm	**6.80 (± 0.46)****	**12.64 (± 1.17)****
Distal IENFD, fibres/mm	**6.46 (± 0.45)***	**8.78 (± 0.56)***
Criteria[a] fulfilled for diagnosis of SFN, *n* (%)	19 (61)	0 (0)
Pathological findings in		
QST (only), *n* (%)	1 (5)	-
Skin biopsy (only), *n* (%)	2 (11)	-
QST & skin biopsy (combined), *n* (%)	16 (84)	-
Criteria[a] not fulfilled for diagnosis of SFN, *n* (%)	12 (39)	31 (100)
Pathological findings in		
QST (only), *n* (%)	8 (67)	2 (7)
Skin biopsy (only), *n* (%)	0 (0)	1 (3)
QST and skin biopsy normal, *n* (%)	4 (33)	28 (90)
Skin biopsy (if SFN was diagnosed), *n* (%)		
Length–dependent	12 (63)	-
Non-length–dependent	6 (32)	-
Normal	1 (5)	-

Note: $^{*}p < 0.05$; $^{**}p < 0.001$.

Abbreviations: COMPASS-31, Composite Autonomic Symptom Score 31; hEDS, hypermobile Ehlers–Danlos syndrome; IENFD, intraepithelial nerve fibre density; n.s, not significant; QST, quantitative sensory testing; SEM, standard error of the mean; SFN, small fibre neuropathy; SFNSL, small fibre neuropathy screening list.

[a]Criteria for the diagnosis of SFN according to Tesfaye et al [21].

skin biopsy ($n = 15$, two male) revealed no significant differences in age ($p = 0.054$), questionnaire scores (painDetect: $p = 0.617$; SFNSL: $p = 0.459$) and tilt-table test results (heart rate: $p = 0.081$; systolic: $p = 0.077$; diastolic: $p = 0.793$).

The hEDS patients had a lower IENFD, both proximally and distally, than the controls (proximal: $t[19.86] = -4.66$, $p < 0.001$; distal: $t[45] = -3.10$, $p = 0.003$; Figure 2a). A significant difference in hEDS without SFN compared with control values could be detected concerning proximal IENFD ($t[26] = -2.52$ $p = 0.018$), but not for distal IENFD ($p = 0.680$; Figure 2b).

Patients with hEDS with and without SFN

According to the NEURODIAB criteria [21], 19 (61%) of the hEDS patients were diagnosed with SFN. Of these 19 patients, 12 (63%) showed length-dependent SFN, six (32%) showed non-length-dependent SFN, and one (5%) showed SFN based only on pathological QST and clinical symptoms (Table 1). On average, hEDS patients presented a proximal (6.80 ± 0.46) and distal (6.46 ± 0.45) reduced IENFD with distally accentuated SFN ($t[30] = 2.25$, $p = 0.032$). Regarding specifically hEDS patients with ($n = 19$) and those without SFN ($n = 12$), differences were detected in terms of proximal and distal IENFD (proximal:

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TABLE 2 Comparison of autonomic symptoms in hypermobile Ehlers–Danlos syndrome patients with and without small fibre neuropathy based on the domains of COMPASS-31.

	hEDS cohort $n = 31$	Healthy controls $n = 31$	hEDS with SFN $n = 19$	hEDS without SFN $n = 12$	Differences between hEDS subgroups
Total score [0–100]	51.3 (±2.6)**	10.9 (±1.5)**	49.3 (±3.3)	54.3 (±4.3)	n.s.
Orthostatic intolerance score [0–40]	24.6 (±1.5)**	3.9 (±1.1)**	22.9 (±1.9)	27.3 (±2.2)	n.s.
Vasomotor score [0–5]	2.3 (±0.2)**	0.6 (±0.2)**	1.9 (±0.3)	2.8 (±0.2)	n.s.
Secretomotor score [0–15]	6.9 (±0.7)**	1.7 (±0.4)**	7.3 (±0.8)	6.4 (±1.4)	n.s.
Gastrointestinal score [0–24]	11.7 (±0.7)**	3.5 (±0.4)**	11.0 (±1.0)	12.8 (±1.1)	n.s.
Bladder score [0–10]	2.9 (±0.5)**	0.6 (±0.2)**	3.3 (±0.7)	2.4 (±0.6)	n.s.
Pupillomotor score [0–6]	2.6 (±0.1)**	0.7 (±0.1)**	2.7 (±0.2)	2.5 (±0.2)	n.s.

Note: Values are mean (±standard error of the mean) scores.

Abbreviations: COMPASS-31, Composite Autonomic Symptom Score 31; hEDS, hypermobile Ehlers–Danlos syndrome; n.s, not significant; SFN, small fibre neuropathy.

**$p<0.001$.

$t[14.15] = 3.73$, $p = 0.002$; distal: $t[14.14] = 3.62$, $p = 0.003$; Figure 2c). Analysis of a correlation between proximal/distal IENFD and Beighton score, heart rate and blood pressure values showed no significant correlations (Table S1). The hEDS subgroups did not differ regarding autonomic symptoms based on COMPASS-31; (Table 2).

Quantitative sensory testing

Hypermobile EDS patients had a lower cold detection threshold (foot: $F[1, 60] = 4.90$, $p < 0.05$), warm detection threshold (hand: $F[1, 60] = 4.80$, $p < 0{,}05$), thermal sensory limen (foot: $F[1, 60] = 3.80$, $p < 0.05$) and mechanical detection threshold (hand and foot: $F[1, 60] = 20.80$, $p < 0.001$) than controls (Figure 3). When comparing hand versus foot within the hEDS group, only pressure pain threshold testing for deep pain sensitivity differed significantly ($F[1, 60] = 5.20$, $p < 0.05$). For results of the other QST parameters see Table S2.

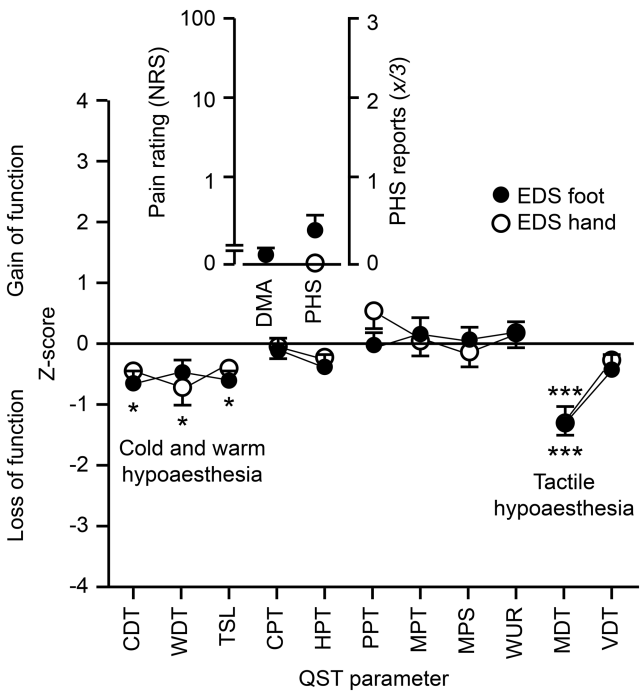


FIGURE 3 Quantitative sensory testing (QST) parameters. Compared to controls, hypermobile Ehlers–Danlos syndrome (hEDS) patients showed an epicritic deficit for small-fibre and large-fibre performance for nonpainful stimuli. QST findings were consistent with generalized SFN over the hands and feet in hEDS. *$p < 0.05$; *** $p < 0.001$. CDT, cold detection threshold; CPT, cold pain threshold; DMA, dynamic mechanical allodynia; HPT, heat pain threshold; MDT, mechanical detection threshold; MPS, mechanical pain sensitivity; MPT, mechanical pain threshold; PHS, paradoxical heat sensation; PPT, pressure pain threshold; TSL, thermal sensory limen; VDT, vibration detection threshold; WDT, warm detection threshold; WUR, wind-up ratio

Tilt-table

Of the 31 hEDS patients, 10 (32%) fulfilled the criteria for PoTS. There was a difference between all hEDS patients and controls with regard to the heart rate in the supine and upright positions (supine: $t[60] = 4.78$, $p < 0.001$; upright: $t[60] = 4.65$, $p < 0.001$; Figure 4a,d). In addition, hEDS patients had a higher mean increase in heart rate than controls ($t[52.27] = 1.78$, $p = 0.040$; Figure 4e). The two groups differed regarding the initial (<15 s) increase in heart rate ($t[60] = 2.09$, $p = 0.041$; Figure 4f). In contrast, the mean systolic and diastolic drops did not differ between the groups (systolic: $p = 0.514$; diastolic: $p = 0.944$; Figure 4b,c).

The linear regression analysis in the hEDS group revealed a significant effect of Beighton score on the mean increase in heart rate ($R^2 = 0.152$; $F[1, 29] = 5.20$, $p = 0.030$), with a higher score indicating a higher mean orthostatic heart rate.

DISCUSSION

The results of this study confirm previous study findings [9–11] that histologically proven SFN is a common feature in patients with

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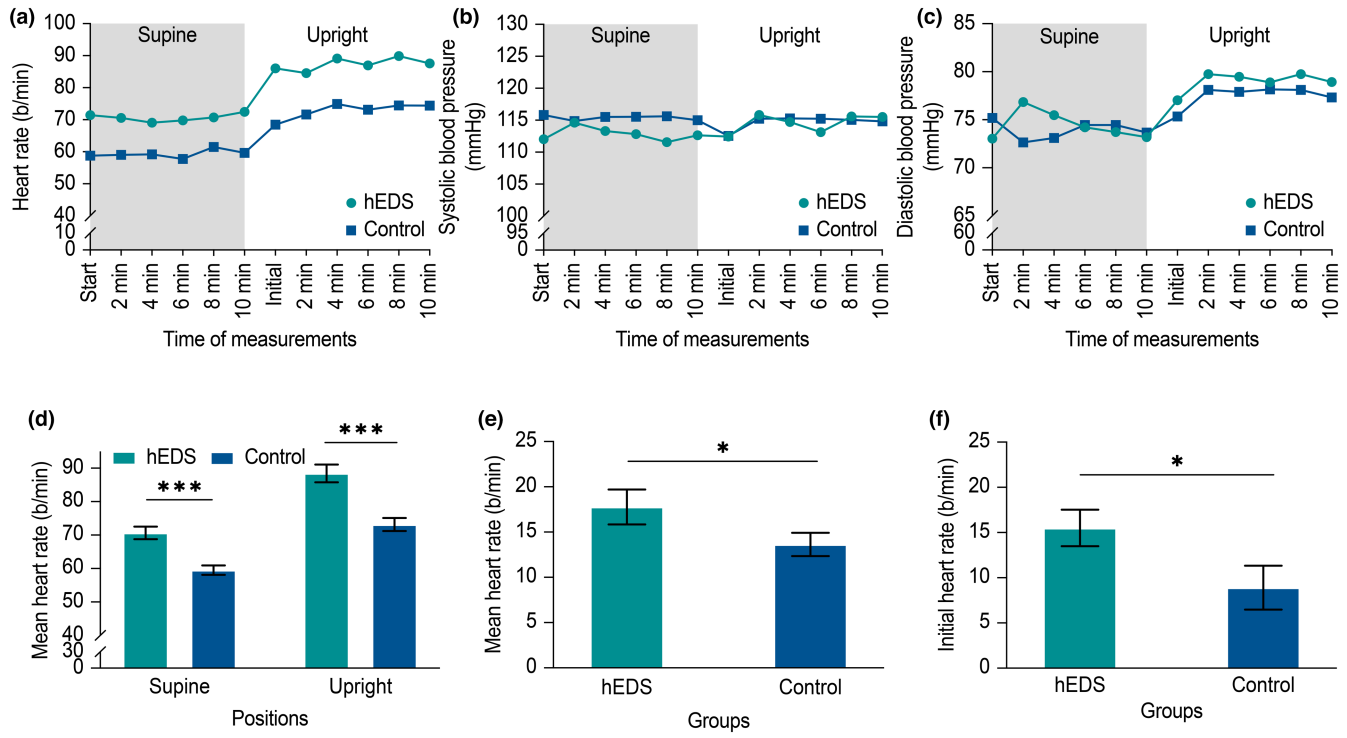


FIGURE 4 Tilt-table parameters. Compared to controls, hypermobile Ehlers–Danlos syndrome (hEDS) patients demonstrated a higher heart rate (a) but no significant difference in systolic (b) and diastolic (c) blood pressure values. Compared to controls, hEDS patients showed a higher mean heart rate in the supine and upright positions (d) and a higher mean (e) and initial (<15 s) (f) increase in heart rate after tilting; $^{*}p < 0.05$; $^{***}p < 0.001$ [Colour figure can be viewed at wileyonlinelibrary.com]

hEDS. As a new finding, by taking proximal and distal skin biopsies, our results show that SFN in hEDS includes a generalized distribution of nerve fibre loss consistent with the generalized complaints of such patients.

Nineteen (61%) of the hEDS patients in this prospective study had SFN. Of these 19 patients, 63% showed length-dependent SFN, and 32% showed non-length-dependent SFN (Table 1). Length dependency is defined when the distal IENFD is more abnormal than the proximal IENFD [35]. Even though a length-dependent reduction was the most prevalent form in these skin biopsies (63% vs. 32%), 89% of the hEDS patients also had a reduced proximal IENFD. This is consistent with the fact that hEDS patients frequently reported neuropathic pain in proximal regions, such as the face, neck, trunk, arms and hands (Table 1). The observation that the distal IENFD is additionally abnormal in patients with proximally reported pain was also confirmed by Saperstein [35]. Despite normal proximal IENFD in our hEDS patients without SFN (Figure 2b), they not only significantly differed from controls but also still reported typical SFN symptoms. These facts could indicate that nerve fibre damage may have already been in progress but that this damage had only manifested clinically, but not significantly in quantitative terms. To what extent manifest SFN will be developed later is currently not possible to determine. To date, no standardized normal ranges of proximal IENFD have been established. A normal range for proximal IENFD could enable investigators to see in which range the proximal values of the patients are located. Thus, these results must be interpreted as preliminary. Since only the proximal IENFD in the hEDS group without SFN was significantly different from controls, autoimmune in addition to degenerative processes may underly the pathology of SFN, as previously described [19, 45] and as also seen in patients with autoimmune autonomic neuropathy [47]. Further studies with long-term follow-up are needed to better understand the pathophysiological mechanisms and biological presentation of SFN in hEDS.

In terms of function, the QST analysis was also consistent with a more generalized SFN distribution. The QST results showed decreased function of small Aδ and C fibres as well as largely myelinated Aβ fibres, in line with prior data [1, 11]. As mentioned above, hEDS patients often experience (sub)luxations due to hypermobility that might damage proprioceptive receptors in the joints [7, 8]. As proprioceptive functions are also partly mediated by Aβ fibres, an epicritic deficit may further intensify an already existing impaired proprioception, with more possible luxations as a consequence. A vicious cycle of instability could thus be reinforced and sustained. Neither proximal nor distal IENFD correlated with Beighton score, indicating that IENFD is not induced and biased by the lax and fragile connective tissue of hEDS. To date, there is no supplementary literature regarding this aspect.

Autonomic dysfunctions

In line with others [8, 14, 36], the results of this study showed that PoTS is the most common orthostatic dysfunction in patients with hEDS. Even though only 32% of the hEDS patients fulfilled the

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criteria for PoTS, more than 90% reported orthostatic complaints (Table 1). Furthermore, De Wandele et al [14]. found that hEDS patients have a significantly higher mean increase in heart rate than controls, which is also supported by our results.

Connective tissue laxity, SFN and deconditioning are possible autonomic underlying mechanisms frequently discussed in the literature [8, 14, 20, 37, 38]. Regarding the question of to what extent autonomic regulation is influenced by connective tissue laxity, our analysis revealed that Beighton score positively correlated with mean increase in heart rate in hEDS patients. This positive correlation has also been demonstrated by De Wandele et al [14]. Blood vessels and connective tissue of the joint and the skin mainly contain collagen types I and III [39]. Defects in these collagen types not only result in hyperlaxity in the affected areas but also destabilize the blood vessels, thus enabling excessive venous blood pooling in the lower limbs and a compensatory, higher heart rate rise [14, 37, 40]. Based on Porges Body Perception Questionnaire, which proposes a model depending on the myelinated vagal nerve fibres [49], higher scores for interoceptive sensitivity were obtained for hypermobile patients by Eccles et al. [50] Increased interoceptive perception could be associated with more autonomic arousals [50]. Due to their hypermobility and impaired proprioception in the joints, patients with hEDS do not always notice when they are in hyperextended positions [8]. Additionally, hEDS patients often cross their physical limits because they have difficulties in estimating their muscle strength and endurance [8]. As a result, a process of deconditioning might be initiated, which is furthered by long recovery periods with restricted or avoided movements after painful episodes [7, 8, 14, 37].

Autonomic dysfunctions can also be caused by SFN [8, 20, 38]. Impaired autonomic innervation can result in a loss of compensatory vasoconstriction after tilting. Consequently, autonomic cardiac sympathetic nerve fibres induce tachycardia to maintain sufficient cardiac output [41]. Particularly in neuropathic PoTS, SFN is considered to play an active role in the underlying pathophysiology [42, 43]. A comparison of the autonomic symptoms between hEDS with SFN and hEDS without SFN yielded no significant differences (Table 2). Only 61% of the hEDS patients met the criteria for SFN, which is consistent with similar findings of previous studies in patients with hEDS and patients with autonomic neuropathies [11, 42, 43, 47, 48], Thus, it must be mentioned that, even though small fibres control the autonomic nervous system regarding the neuropathic and autonomic components, SFN may not be the only pathophysiology for the autonomic dysfunctions frequently reported in hEDS.

Strengths and limitations

To date, only few studies [9–11] have investigated the involvement of SFN in hEDS. According to a case report [9] ($n = 1$), skin biopsies were taken from the fingertip, and the lower and upper leg, while in another study of 20 EDS patients [10] and a study of 79 hEDS/hypermobility spectum disorder (HSD) patients [11], biopsies were obtained exclusively from the distal leg. To the best of our knowledge, this prospective study is the first to obtain proximal and distal skin biopsies in patients with hEDS compared to healthy controls. This enabled us not only to investigate the involvement of SFN in hEDS but also to address the issue of length or non-length dependency in SFN. Nevertheless, performing skin biopsies in the upper limbs should be considered in future for a more detailed characterization of SFN in hEDS and a better comparison with the QST data from the upper limb. In addition to skin biopsies as a quantitative investigation, this study included functional investigations of nerve fibres using QST and autonomic testing [9, 10]. A limitation of this study is the sample size. Furthermore, as only 10% of the hEDS patients were male, sex differences cannot be excluded. Given that hEDS patients were selectively recruited from an autonomic centre, it is important to mention that particular focus was set on further characterizing SFN, which has already been identified in previous studies as a common feature in hEDS [9–11], and on contributing to a better understanding of the underlying pathomechanisms of neuropathic pain and autonomic dysfunctions in patients with hEDS.

To further differentiate SFN, proximally and distally performed skin biopsy is highly recommended [35, 44]. Furthermore, a comparison between hEDS patients and patients with SFN as primary diagnosis should be conducted in the future and could extend our knowledge of the underlying mechanisms.

CONCLUSIONS

This study confirms that SFN is a common feature in patients with hEDS [9, 10]. Based on our results, SFN in hEDS patients is not purely distally located but is often presented with a generalized distribution. In terms of the various autonomic and neuropathic dysfunctions frequently reported in hEDS, our study supports the role of SFN as an important underlying pathomechanism in addition to the connective tissue laxity.

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CONFLICT OF INTEREST

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DATA AVAILABILITY STATEMENT

Anonymized data will be shared upon reasonable request to the corresponding author.

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REFERENCES

1. Hamonet C, Brock I, Pommeret S, et al. Ehlers-Danlos syndrome type III (hypermobile): clinical somatosensory scale (SSCS-62) validation, about 626 patients. *Bull Acad Natl Med*. 2017;201(1–3):405-415.
2. Malfait F, De Paepe A. Ehlers-Danlos syndrome. *Brenner's Encyclopedia of Genetics: Second Edition*. Academic Press; 2013:456-459.
3. Malfait F, De Paepe A. The Ehlers-Danlos syndrome. Progress in heritable soft connective tissue diseases. In: Halper J, ed. *Advances in Experimental Medicine and Biology*. Springer Science+Business Media Dordrecht; 2014:129-143.
4. Castori M. Ehlers-Danlos syndrome, hypermobility type: an underdiagnosed hereditary connective tissue disorder with mucocutaneous, articular, and systemic manifestations. *ISRN Dermatol*. 2012;2012:751768.
5. Malfait F, Francomano C, Byers P, et al. The 2017 international classification of the Ehlers-Danlos syndromes. *Am J Med Genet C Semin Med Genet*. 2017;175(1):8-26.
6. Beighton P, De Paepe A, Danks D, et al. International nosology of heritable disorders of connective tissue, Berlin, 1986. *Am J Med Genet*. 1988;29(3):581-594.
7. Hamonet C, Brissot R, Gompel A, et al. Ehlers-Danlos syndrome (EDS)-contribution to clinical diagnosis-a prospective study of 853 patients. *EC Neurol*. 2018;10(6):428-439.
8. Celletti C, Camerota F, Castori M, et al. Orthostatic intolerance and postural orthostatic tachycardia syndrome in joint hypermobility syndrome/Ehlers-Danlos syndrome, hypermobility type: neurovegetative dysregulation or autonomic failure? *Biomed Res Int*. 2017;2017:9161865.
9. Pascarella A, Provitera V, Lullo F, et al. Evidence of small fiber neuropathy in a patient with Ehlers-Danlos syndrome, hypermobility-type. *Clin Neurophysiol*. 2016;127(3):1914-1916.
10. Cazzato D, Castori M, Lombardi R, et al. Small fiber neuropathy is a common feature of Ehlers-Danlos syndromes. *Neurology*. 2016;87(2):155-159.
11. Fernandez A, Rozier BA, Vautey M, Berna C, Suter MR. Small fiber neuropathy in in hypermobile Ehlers Danlos syndrome/hypermobility spectrum disorder. *J Intern Med*. 2022;292:957-960.
12. Stewart JD, Low PA, Fealey RD. Distal small fiber neuropathy: results of tests of sweating and autonomic cardiovascular reflexes. *Muscle Nerve*. 1992;15(6):661-665.
13. Hovaguimian A, Gibbons CH. Diagnosis and treatment of pain in small-fiber neuropathy. *Curr Pain Headache Rep*. 2011;15(3):193-200.
14. De Wandele I, Rombaut L, Leybaert L, et al. Dysautonomia and its underlying mechanisms in the hypermobility type of Ehlers-Danlos syndrome. *Semin Arthritis Rheum*. 2014;44(1):93-100.
15. Diehl RR. Posturales tachykardiesyndrom in Deutschland bislang zu selten diagnostiziert. *Dtsch Arztebl*. 2003;100(43):2185-2190.
16. McDonald C, Koshi S, Busner L, Kavi L, Newton JL. Postural tachycardia syndrome is associated with significant symptoms and functional impairment predominantly affecting young women: a UK perspective. *BMJ Open*. 2014;4(6):e004127.
17. Chan AC, Wilder-Smith EP. Small fiber neuropathy: getting bigger! *Muscle Nerve*. 2016;53(5):671-682.
18. Hoitsma E, De Vries J, Drent M. The small fiber neuropathy screening list: Construction and cross-validation in sarcoidosis. *Respir Med*. 2011;105(1):95-100.
19. Sène D. Small fiber neuropathy: diagnosis, causes, and treatment. *Joint Bone Spine*. 2018;85(5):553-559.
20. Fedorowski A. Postural orthostatic tachycardia syndrome: clinical presentation, aetiology and management. *J Intern Med*. 2019;285(4):352-366.
21. Tesfaye S, Boulton AJ, Dyck PJ, et al. Diabetic neuropathies: update on definitions, diagnostic criteria, estimation of severity, and treatments. *Diabetes Care*. 2010;33(10):2285-2293.
22. Freynhagen R, Baron R, Gockel U, Tölle TR. painDETECT: a new screening questionnaire to identify neuropathic components in patients with back pain. *Curr Med Res Opin*. 2006;22(10):1911-1920.
23. Devigili G, Tugnoli V, Penza P, et al. The diagnostic criteria for small fibre neuropathy: from symptoms to neuropathology. *Brain*. 2008;131(Pt 7):1912-1925.
24. Weis J, Katona I, Müller-Newen G, et al. Small-fiber neuropathy in patients with ALS. *Neurology*. 2011;76(23):2024-2029.
25. Schrempf W, Katona I, Dogan I, et al. Reduced intraepidermal nerve fiber density in patients with REM sleep behavior disorder. *Parkinsonism Relat Disord*. 2016;29:10-16.
26. Lauria G, Hsieh ST, Johansson O, et al. European federation of neurological societies/peripheral nerve society guideline on the use of skin biopsy in the diagnosis of small fiber neuropathy. Report of a joint task force of the European federation of neurological societies and the peripheral nerve society. *Eur J Neurol*. 2010;17(7):903-912.
27. Rolke R, Baron R, Maier C, et al. Quantitative sensory testing in the German research network on neuropathic pain (DFNS): standardized protocol and reference values. *Pain*. 2006;123(3):231-243.
28. Mücke M, Cuhls H, Radbruch L, et al. Quantitative sensory testing. *Schmerz*. 2014;28(6):635-646; quiz 647–638.
29. Rolke R, Magerl W, Campbell KA, et al. Quantitative sensory testing: a comprehensive protocol for clinical trials. *Eur J Pain*. 2006;10(1):77-88.
30. Claus D, Hilz MJ, Neundörfer B. Thermal discrimination thresholds: a comparison of different methods. *Acta Neurol Scand*. 1990;81(6):533-540.
31. Baumgärtner U, Magerl W, Klein T, Hopf HC, Treede RD. Neurogenic hyperalgesia versus painful hypoalgesia: two distinct mechanisms of neuropathic pain. *Pain*. 2002;96(1–2):141-151.
32. Chan AW, MacFarlane IA, Bowsher D, Campbell JA. Weighted needle pinprick sensory thresholds: a simple test of sensory function in diabetic peripheral neuropathy. *J Neurol Neurosurg Psychiatry*. 1992;55(1):56-59.
33. Novak P. Quantitative autonomic testing. *J Vis Exp*. 2011;53:2502. doi:10.3791/2502
34. Jones PK, Shaw BH, Raj SR. Clinical challenges in the diagnosis and management of postural tachycardia syndrome. *Pract Neurol*. 2016;16(6):431-438.
35. Saperstein DS. Small fiber neuropathy. *Neurol Clin*. 2020;38(3):607-618.
36. Roma M, Marden CL, De Wandele I, Francomano CA, Rowe PC. Postural tachycardia syndrome and other forms of

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orthostatic intolerance in Ehlers-Danlos syndrome. *Auton Neurosci*. 2018;215:89-96.
37. Gazit Y, Nahir AM, Grahame R, Jacob G. Dysautonomia in the joint hypermobility syndrome. *Am J Med*. 2003;115(1):33-40.
38. Garland EM, Celedonio JE, Raj SR. Postural tachycardia syndrome: beyond orthostatic intolerance. *Curr Neurol Neurosci Rep*. 2015;15(9):60.
39. Gelse K, Pöschl E, Aigner T. Collagens-structure, function, and biosynthesis. *Adv Drug Deliv Rev*. 2003;55(12):1531-1546.
40. Rowe PC, Barron DF, Calkins H, Maumenee IH, Tong PY, Geraghty MT. Orthostatic intolerance and chronic fatigue syndrome associated with Ehlers-Danlos syndrome. *J Pediatr*. 1999;135(4):494-499.
41. Sheldon RS, Grubb BP, Olshansky B, et al. 2015 heart rhythm society expert consensus statement on the diagnosis and treatment of postural tachycardia syndrome, inappropriate sinus tachycardia, and vasovagal syncope. *Heart Rhythm*. 2015;12(6):e41-e63.
42. Gibbons CH, Bonyhay I, Benson A, Wang N, Freeman R. Structural and functional small fiber abnormalities in the neuropathic postural tachycardia syndrome. *PLoS One*. 2013;8(12):e84716.
43. Haensch CA, Tosch M, Katona I, Weis J, Isenmann S. Small-fiber neuropathy with cardiac denervation in postural tachycardia syndrome. *Muscle Nerve*. 2014;50(6):956-961.
44. Khan S, Zhou L. Characterization of non-length-dependent small-fiber sensory neuropathy. *Muscle Nerve*. 2012;45(1):86-91.
45. Oaklander AL. Immunotherapy prospects for painful small-fiber sensory neuropathies and ganglionopathies. *Neurotherapeutics*. 2016;13(1):108-117.
46. Sletten DM, Suarez GA, Low PA, Mandrekar J, Singer W. COMPASS 31: a refined and abbreviated composite autonomic symptom score. *Mayo Clin Proc*. 2012;87(12):1196-1201.
47. Maier A, Kapfenberger R, Katona I, Weis J, Schulz JB, Rolke R. Nonregional small fibre neuropathy in cases of autoimmune autonomic neuropathy. *J Neurol*. 2022;269:6648-6654.
48. Billig SCI, Schauermann JC, Rolke R, Katona I, Schulz JB, Maier A. Quantitative sensory testing predicts histological small fiber neuropathy in postural tachycardia syndrome. *Neurol Clin Pract*. 2020;10(5):428-434.
49. Poli A, Maremmani AGI, Chiorri C, et al. Item reduction, psychometric and biometric properties of the Italian version of the body perception questionnaire-short form (BPQ-SF): the BPQ-22. *Int J Environ Res Public Health*. 2021;18(7):3835.
50. Eccles JA, Beacher FD, Gray MA, et al. Brain structure and joint hypermobility: relevance to the expression of psychiatric symptoms. *Br J Psychiatry*. 2012;200(6):508-509.

SUPPORTING INFORMATION

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