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ORIGINAL ARTICLE

Cutaneous sensory and autonomic denervation in progressive supranuclear palsy

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Abstract

Aim: Progressive Supranuclear Palsy (PSP) is a progressive neurodegenerative tauopathy characterised by motor, behavioural and cognitive dysfunction. While in the last decade, sensory and autonomic disturbances as well as peripheral nerve involvement are well-recognised in Parkinson's Disease (PD), little is known in this regard for PSP. Herein, we aim to assess peripheral sensory and autonomic nerve involvement in PSP and to characterise possible differences in morpho-functional pattern compared to PD patients.

Methods: We studied 27 PSP and 33 PD patients without electrophysiological signs of neuropathy, and 33 healthy controls (HC). In addition to motor impairment, evaluated by means of UPDRS-III and the PSP rating scale, all patients underwent clinical, functional and morphological assessment of sensory-autonomic nerves through dedicated questionnaires, sympathetic skin response, dynamic sweat test and skin biopsies. The analysis of cutaneous sensory and autonomic innervation was performed using indirect immunofluorescence and confocal microscopy.

Results: PSP patients displayed a length-dependent loss of sensory and autonomic nerve fibres associated with functional impairment compared to HC and, overall, a more severe picture than in PD patients. The disease severity correlated with the loss of intraepidermal nerve fibre density in the leg of PSP patients ($p < 0.05$).

Conclusion: We demonstrated a length-dependent small fibre pathology in PSP, more severe compared to PD, and paralleling disease severity. Our findings suggest the morphological and functional study of cutaneous nerves as possible biomarkers to monitor disease progression and response to new treatments.

KEYWORDS
skin biopsy, non-motor symptoms, tauopathy, biomarker, epidermal nerve fibres

INTRODUCTION

Progressive Supranuclear Palsy (PSP) is a progressive neurodegenerative disorder comprising a spectrum of clinical phenotypes characterised by motor, behavioural and cognitive dysfunction. PSP is pathologically associated with hyperphosphorylated tau protein in the basal ganglia and brainstem [1]. The clinical picture of PSP is heterogeneous with the recent recognition of several PSP subtypes [1, 2]. However, in general, it is distinguished from Parkinson's

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disease (PD) by the presence of vertical gaze palsy, impaired postural reflexes, leading to frequent falls, greater cognitive dysfunction, a poor response to dopaminergic treatment, and a more rapidly progressive course [2].

Non-motor symptoms, and among them sensory and autonomic disturbances, have been extensively studied in PD patients [3–5] and recently observed also in PSP [5,6]. Indeed, over the last decade, growing evidence has consistently showed a peripheral nerve involvement in PD, involving either autonomic or sensory small fibres [3,7] and the presence of disease-specific markers, such as alpha-synuclein deposits, in dermal nerves [8]. Moreover in another neurodegenerative disorder, Multiple system atrophy (MSA), in which autonomic dysfunction is mostly due to a central nervous system involvement, a postganglionic axonal degeneration has been demonstrated through skin biopsy [9].

Whether there is a peripheral nerve involvement also in PSP, contributing to autonomic and sensory symptoms, is still unknown. To date, neurophysiological evidence of neuropathy in PSP is rare [10]. Analogously, cutaneous innervation has been only anecdotally studied in PSP patients, as disease control group, to test the specificity of alpha-synuclein deposits in synucleinopathies [11, 12] and no systematic description of cutaneous innervation exists in the literature.

Also, although autonomic impairment in PSP patients has been described [13–17], which is supported by autoptic evidence [18, 19], heterogeneous findings have been reported so far [20–24].

Importantly, a recent large retrospective study on PSP patients, showed that the early development of autonomic disturbances, such as constipation and urinary symptoms, was associated with a more rapid disease progression and reduced survival [25]. Sensory disturbances have been also described among the non-motor symptoms in PSP with a recent meta-analysis describing the occurrence of pain in 70% of PSP patients and of neuropathic pain in 7.8% of them [26].

Based on this background, we aimed to assess peripheral sensory and autonomic nerve involvement in PSP and to characterise peculiar morpho-functional patterns of cutaneous innervation compared to PD patients.

METHODS

Twenty-seven patients (18 males, age 69.9 ± 7.4 years) with probable PSP, according to the criteria recently published by the MDS task force [2], were enrolled between January 2017 and June 2019, 23 showed the Richardson's syndrome (PSP-RS) phenotype and four showed the corticobasal syndrome (PSP-CBS) phenotype [27]. In addition, we recruited 33 normal subjects (18 males, age 69.4 ± 3.2 years) as healthy control (HC) group and 33 PD patients (19 male, age 69.4 ± 4.7 years) as disease control group, selected from a larger cohort of PD patients previously published [7] according to age and sex-matched criteria. Disease severity was assessed by means of the Unified Parkinson's Disease Rating Scale part III (UPDRS-III) [27], the Hoehn and Yahr scale [28] and PSP Rating Scale [29]. Before recruitment, all patients underwent neurological examination, dopaminergic responsiveness and neuroimaging evaluation. We excluded patients with abnormal parameters (i.e., latency, velocity and amplitude) of the nerve conduction study in at least two nerves among median, ulnar, sural and peroneal nerves.

In addition, we considered as exclusion criteria concomitant conditions potentially affecting the peripheral nervous system such as glucose intolerance; dysendocrinopathies; vitamin E, vitamin B12 and folic acid deficiency; hepatic or renal failure; HIV; connective tissue disorders; or use of neurotoxic drugs.

All patients underwent clinical assessment of pain and autonomic symptoms using the Small Fiber Neuropathy Symptoms Inventory Questionnaire (SFN-SIQ) [30] and the Scales for Outcomes in Parkinson's Disease–Autonomic Dysfunction (SCOPA-AUT) [31]. The SFN-SIQ consists of 13 items related to the occurrence of autonomic and sensory disturbances. The overall score ranges from 0 (no symptoms) to 39 (all symptoms, always present). The SCOPA-AUT is a 23-item self-administered questionnaire exploring symptoms related to autonomic dysfunction of gastrointestinal, urinary, cardiovascular, thermoregulatory, pupillomotor and sexual domains. The score ranges from 0 (no symptoms) to 69 (all symptoms often occur). We considered a domain involved if at least 1 item obtained a score ≥2. The project was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. As such, the study was approved by the local Ethics Committee and each subject was included upon signature of the informed consent form.

Morphological evaluation

Skin biopsies were taken from hairy (distal leg and distal thigh) and glabrous skin (fifth digit fingertip) with a 3-mm punch in all patients and HC. Skin samples were taken from the most affected side of patients with an asymmetric motor phenotype and from the right side of patients without overt clinical asymmetry. Specimens were processed with indirect immunofluorescence technique [32] using a panel of primary antibodies and an endothelium-binding agglutinin (Ulex europaeus, Vector, CA) to mark nerves and vessels. Confocal (Apotome.2, Zeiss, Jena, Germany) images were analysed by dedicated software. A total of 279 skin samples were analysed (81 from PSP patients, 99 from PD and 99 from HC). In each sample, intraepidermal nerve fibre (IENF) density per linear millimetre was calculated on four non-consecutive sections double-stained with protein gene product (PGP) 9.5 and collagen-IV, according to published rules [33].

In glabrous skin, the density of Meissner corpuscles (MC) and intrapapillary myelinated endings (IME) per mm^2 was calculated following previously described procedures [34]. Sudomotor, pilomotor and vasomotor nerve densities were assessed on sections immunostained with the pan-neuronal marker PGP, vasoactive intestinal peptide (VIP), co-expressed in cholinergic sudomotor axons [35], and dopamine beta hydroxylase (Dβh) as noradrenergic marker, using a semiquantitative method [36] (4 = normal innervation, 3 = mild loss of fibres, 2 = severe loss of fibres, 1 = rare

surviving fibres). A total of 2790 sections were evaluated, 10 for each sample. A mean value for each site was obtained for each nerve subpopulation. A single operator (AS) blinded to diagnosis performed all the quantitative and semiquantitative assessments. A second operator (MN) blindly repeated all the measures on randomly selected 20% of the samples from each group to assess interobserver variability.

Functional evaluation

Autonomic sudomotor function was assessed with the dynamic sweat test (DST) [37], in the distal leg of the most affected side in patients with motor asymmetry and of the right side in patients without asymmetry. Briefly, ten minutes after stimulation with 1% pilocarpine by iontophoresis, the stimulated area was painted with a 2% alcoholic iodine solution and dried. Sweating output was recorded by a digital video camera through a cornstarch-powdered transparent tape used as a contrast-enhancing device. Mean sweat output (nL/min) per gland and per skin area (cm^2) and density of activated sweat gland per cm^2 were evaluated.

Sympathetic skin response (SSR) was evoked by random stimuli of 5 to 20-mA intensity delivered at the volar wrist on the right median nerve and recorded bilaterally at feet sole.

Lastly, we assessed the presence of orthostatic hypotension, defined as a sustained reduction in systolic blood pressure of at least 20 mmHg or in the diastolic blood pressure of at least 10 mmHg occurring within 3 minutes of standing up or sitting (when standing was not possible) from the supine position [38].

Statistical analysis

After checking for normality of the distribution with the Kolmogorov–Smirnov test, differences in variables among the groups were computed with χ^2 or analysis of variance (ANOVA) tests as appropriate. Post hoc comparisons were run with the Bonferroni test.

Pearson or Spearman tests were used as appropriate to explore the relationship between clinical findings (i.e., disease severity, disease duration) and morphological and functional data. $p < 0.05$ were considered significant. Alpha inflation due to multiple comparisons was controlled according to Bonferroni's approach when appropriate. Interobserver variability was assessed by the intraclass correlation coefficient analysis. All statistical analyses used IBM SPSS Statistics software (Version 22 for Windows, New York City, USA). Descriptive statistic is reported as mean ± standard deviation.

Data availability

Anonymised data will be shared by request from any qualified investigator.

RESULTS

Demographic and clinical data

Demographic and clinical data are listed in Table 1 and Table S1. No significant differences of age or gender were observed among the three groups. As expected, even if PSP patients showed a trend for a shorter disease duration compared to PD patients, they had a more severe motor impairment as suggested by the higher scores obtained at UPDRS-III and H&Y (Table 1). All PSP patients, except those with PSP-CBS, exhibited a Richardson's phenotype and reached the degree of certainty of probable PSP [2] (Table S1). In particular, all the 23 PSP-RS patients showed a severe limitation of vertical gaze, moderate to severe postural instability with a history of falls, moderate to severe bradykinesia, and mild to severe dysarthria. Five of 23 patients had severe dysphagia and two of them needed to be fed through a gastrostomy tube. Mild to moderate cognitive/behavioural abnormalities (apathy, depression, irritability, emotional lability) were present in all patients. The four PSP-CBS patients all showed postural instability, slow velocity of vertical saccades, cognitive impairment and orobuccal or limb apraxia. The mean ± SD of PSP Rating Scale score for PSP patients was 43.2 ± 13.3. None of PD and PSP patients showed orthostatic hypotension.

Small fibre symptoms and function

Overall, according to SFN-SIQ, patients complained of moderate autonomic and sensory symptoms related to small fibre impairment with PSP patients showing relatively higher scores compared to PD patients (mean score PSP: 9.2 ± 2.6, PD: 4.5 ± 3.3, $p < 0.001$). Indeed, 100% of PSP and 71% of PD patients reported symptoms of autonomic impairment in at least one domain. Most frequently reported symptoms were micturition disturbances (85% in PSP vs 63% in PD patients) and diarrhoea/constipation (56% in PSP vs 67% in PD patients), see Table S2. Small fibre sensory symptoms of any kind were reported by 40% of PSP and 29% of PD patients, with pain reported by 11% of PSP and 13% of PD patients (Table S2).

The SCOPA-AUT questionnaire confirmed that autonomic complaints were more frequent in PSP compared to PD patients, with urinary domain as the most frequently involved in PSP patients, and gastrointestinal domain in PD patients (Table S2). Average scores were 19.3 ± 10.9 (range 5–40) in PSP and 17.1 ± 16.5 (range 2–69) in PD patients.

Functional evaluation of sudomotor function showed abnormal results in both the patient groups. Specifically, a significant lower SSR amplitude was found in both patient groups compared to HC ($p < 0.001$). A lower SSR amplitude was found in PSP compared to PD patients ($p = 0.018$).

In line with SSR findings, DST disclosed in both patient groups, compared to HC, a marked reduction of activated sweat gland

TABLE 1 Clinical, morphological and functional data.

	HC	PD patients	PSP patients	*p* value (all groups)	*p* value (PD vs HC)	*p* value (PSP vs HC)	*p* value (PD vs PSP)
Participants (M/F), n	(19/14) 33	(18/15) 33	(18/9) 27	0.277			
Age, y	69.4 ± 3.2	69.4 ± 4.7	70 ± 7.4	0.909			
Disease duration, years	NA	5.4 ± 5.2	3.6 ± 2.1		NA	NA	0.052
L-dopa cumulative dose (Kg)	NA	0.85 ± 1.31	0.15 ± 0.14		NA	NA	**0.005**
UPDRS-III	NA	23.7 ± 9.4	41.3 ± 10.4		NA	NA	**<0.001**
PSP Rating Scale	NA	NA	43.2 ± 13.3	NA			
Hoehn and Yahr score	NA	2.8 ± 1	4.1 ± 0.8		NA	NA	**<0.001**
Morphological data							
IENFs leg, ff/mm	12 ± 2.5	9 ± 3.9	5.5 ± 2.8	**<0.001**	**<0.001**	**<0.001**	**<0.001**
IENFs thigh, ff/mm	20.5 ± 3.8	17 ± 5	12.7 ± 5.6	**<0.001**	**0.003**	**<0.001**	**0.001**
Ratio leg/thigh	0.6 ± 0.17	0.57 ± 0.4	0.47 ± 0.2	0.143			
IENFs fingertip, ff/mm	5.9 ± 2.4	2.5 ± 2.1	1.6 ± 1.5	**<0.001**	**<0.001**	**<0.001**	0.184
MC, n/mm^2	25.2 ± 7.3	10.1 ± 8	9.6 ± 7.4	**<0.001**	**<0.001**	**<0.001**	0.837
IME, n/mm	56 ± 18	34.3 ± 22.1	21.4 ± 18.7	**<0.001**	**<0.001**	**<0.001**	**0.042**
Functional data							
SSR amplitude (mV)	5.4 ± 4.9	1.2 ± 1.1	0.3 ± 0.3	**<0.001**	**<0.001**	**<0.001**	**0.018**
DST distal leg							
Sweat gland density	64.6 ± 10.4	49.4 ± 22.1	32.3 ± 20.4	**<0.001**	**0.022**	**<0.001**	**0.023**
Sweat output/ gland	6.8 ± 2.3	4.4 ± 2.4	7.3 ± 4.9	0.061			
Sweat output/cm^2	475.5 ± 133.6	241.8 ± 222.9	209.5 ± 160	**<0.001**	**0.002**	**<0.001**	0.624

Abbreviations: IENF, intraepidermal nerve fibre; IME, intrapapillary myelinated endings; MC, Meissner corpuscles; NA, not applicable; PD, Parkinson disease; PSP, Progressive Supranuclear Palsy.

Significance level was set at $p < 0.05$. In bolds are expressed statistically significant comparisons. Values are expressed as mean ±standard deviation; sweat output is expressed as nL/min; UPDRS-III = Unified Parkinson's Disease Rating Scale–Motor. Sympathetic skin response (SSR). Dynamic Sweat Test (DST). Sweat gland density is expressed as number of glands/cm^2.

density and sweat output/cm^2 (all $p < 0.001$; Table 1). PSP patients with respect to PD patients, displayed a more severe reduction of the number of activated sweat glands ($p = 0.023$).

Skin biopsy findings

Epidermal nerve fibres

Overall, quantitative nerve analysis (Table 1) revealed in the thigh, leg and fingertip of both patient groups a loss of IENFs compared to HC (all $p < 0.001$). PSP patients showed a more severe reduction of IENF in lower limbs with respect to PD patients (all $p < 0.001$).

The percentage of PSP and PD patients showing IENF density below the fifth percentile cut-off was 96% vs 71% at leg/thigh and 96% vs 89% at fingertip.

In HC, we observed a regular distribution of nerve fibres crossing the basement membrane and running through the epidermis until the stratum corneum with a mostly vertically oriented course and occurrence of mild branching (Figure 1 A, D, G). In PD patients (Figure 1 B, E, H), it was evident that an irregular spatial distribution of IENF with clusters alternated with tracts of epidermal denervation and increased horizontally or obliquely oriented branching (Figure 1 H). Often, the subepidermal nerve plexus appeared deranged with several sprouts (Figure 1 E, H). Vascular abnormalities with mega-capillaries and hypertrophy of dermal papillae (Figure 1 B, H) were also common. Overall, aspects of nerve degeneration such as axonal swelling and nerve varicosities were combined with evidence of nerve regeneration (sprouting and clusters). In PSP patients, we observed a severe epidermal and dermal denervation with occurrence of varicosities and axonal swellings and rare IENF clusters and subepidermal nerve sprouting. Overall, the degenerative aspects dominated the morphological picture (Figure 1 C, F, I). Vascular bed as well as capillary loops and dermal papillae were more regular than in PD (Figure 1 C, F, I).

Autonomic nerves

A length-dependent loss of autonomic nerves was found in all patients with a more severe picture of vasomotor (Figure 2 C compared to B), sudomotor (Figure 2 F compared to E) and pilomotor (Figure I compared to H) nerve degeneration in PSP compared to PD. This finding was even more evident analysing Dβh and

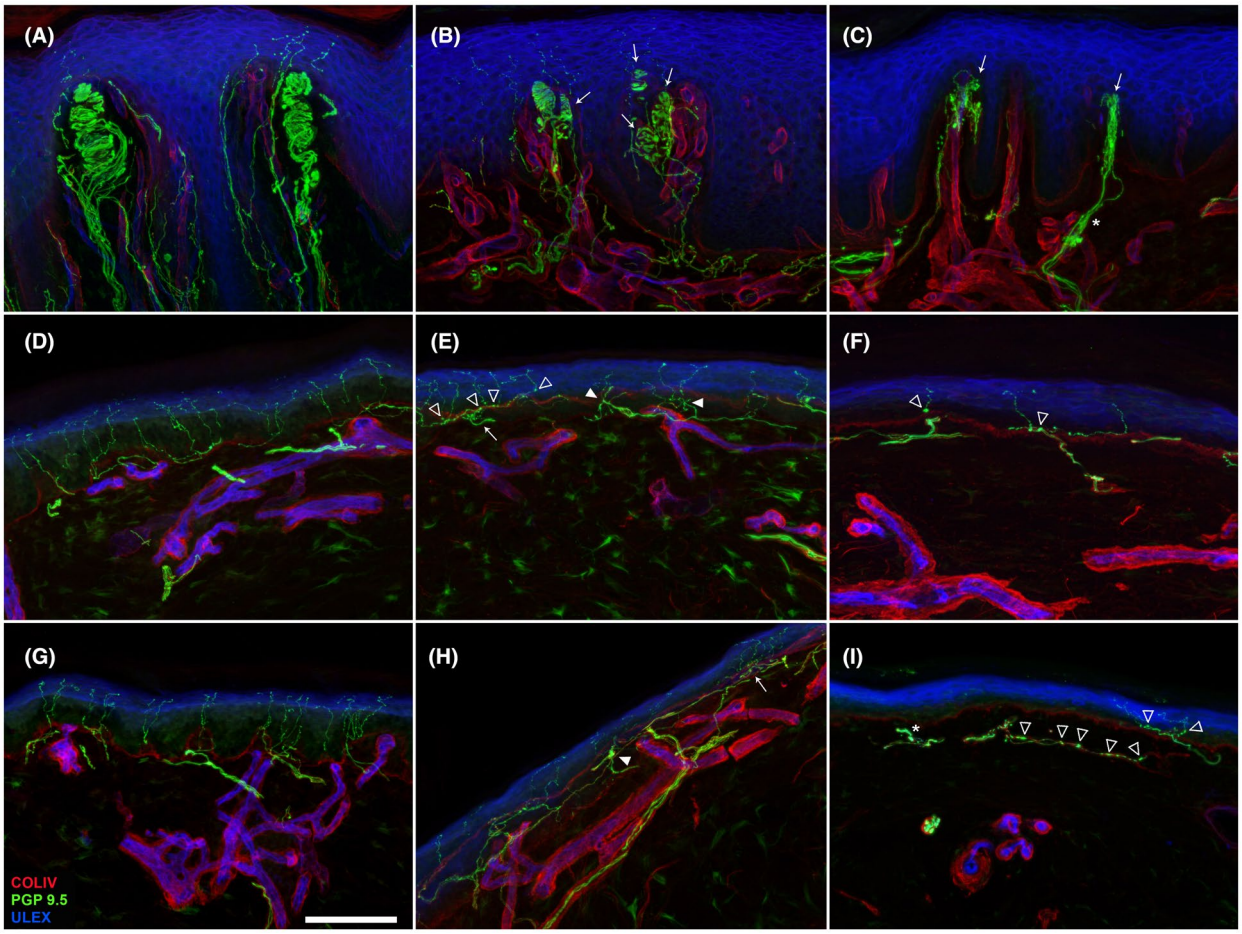

FIGURE 1 Somatic innervation. Confocal images of immunostained skin sections showing a loss of cutaneous nerve endings, moderate in Parkinson disease (PD, B, E and H) and severe in Progressive Supranuclear Palsy (PSP, C, F and I) patients compared to controls (A, D and G). Arrows in B and C indicate morphological abnormalities of the surviving mechanoreceptors in glabrous skin. In PD patients, epidermal fibres show an increased branching and are arranged in clusters (arrows in E, H) alternating with tracts of epidermal denervation. Several sprouts (arrowheads in E, H) of the nerve plexus and vascular abnormalities with mega-capillaries are evident in the papillary dermis. In PSP patients, there is a severe epidermal and dermal denervation with the occurrence of varicosities (empty arrowheads F, I) and axonal swellings (asterisk C, I) and rare IENF clusters and subepidermal nerve sprouting. Scale bar =100 μm; COLIV, collagen IV; PD, Parkinson disease; PSP, Progressive Supranuclear Palsy; ULEX, Ulex Europaeus Agglutinin; PGP=protein gene product 9.5

VIP-immunoreactive nerve fibres (Figure 2, C and I compared to B and H).

Using PGP, PSP patients compared to PD patients, exhibited a lower density score of pilomotor nerves in the thigh (2.3 ± 0.5 vs 2.8 ± 0.8, $p = 0.004$) and leg (1.5 ± 0.4 vs 1.8 ± 0.5, $p = 0.038$) and of sudomotor nerves in the thigh (2.2 ± 0.5 vs 2.7 ± 0.6, $p < 0.001$) and leg (1.4 ± 0.4 vs 1.8 ± 0.5, $p = 0.011$). The same results were evident in the fingertip, with lower sudomotor (2.0 ± 0.4 vs 2.7 ± 0.8, $p = 0.008$) and vasomotor scores (2.0 ± 0.4 vs 3.4 ± 0.7, $p < 0.001$) in the PSP group. In this group a structural derangement of dermal adnexa, and particularly of sweat gland structures (Figure 2, F compared to E and to D), was common in the distal site.

The assessment of autonomic innervation, performed on 56 sections, randomly selected by a second operator (MN) blinded to the origin of the biopsies, showed an excellent repeatability of the procedure (ICC = 0.9, $p = 0.001$).

Large sensory fibres

Paraesthesias were more frequent in PSP (41%) compared to PD patients (13%). These symptoms are caused by small fibres as well as large sensory fibres that were both affected in our patients. Indeed, we observed, in addition to the loss of IENFs, an involvement of large fibre endings with a reduction of MC and IME densities in glabrous skin among patient groups compared to HC (all $p < 0.001$), and a lower IME density in PSP compared to PD patients ($p = 0.042$; see Table 1). The density of MC was below the fifth percentile cut-off in 91% of PSP and 78% of PD patients. The density of IME was below the fifth percentile cut-off in 78% of PSP and 39% of PD patients.

MC showed frequent morphological abnormalities such as elongation and simplification (Figure 1 B, C), and some of them appeared abnormally displaced at the base of the dermal papillae (Figure 3C)

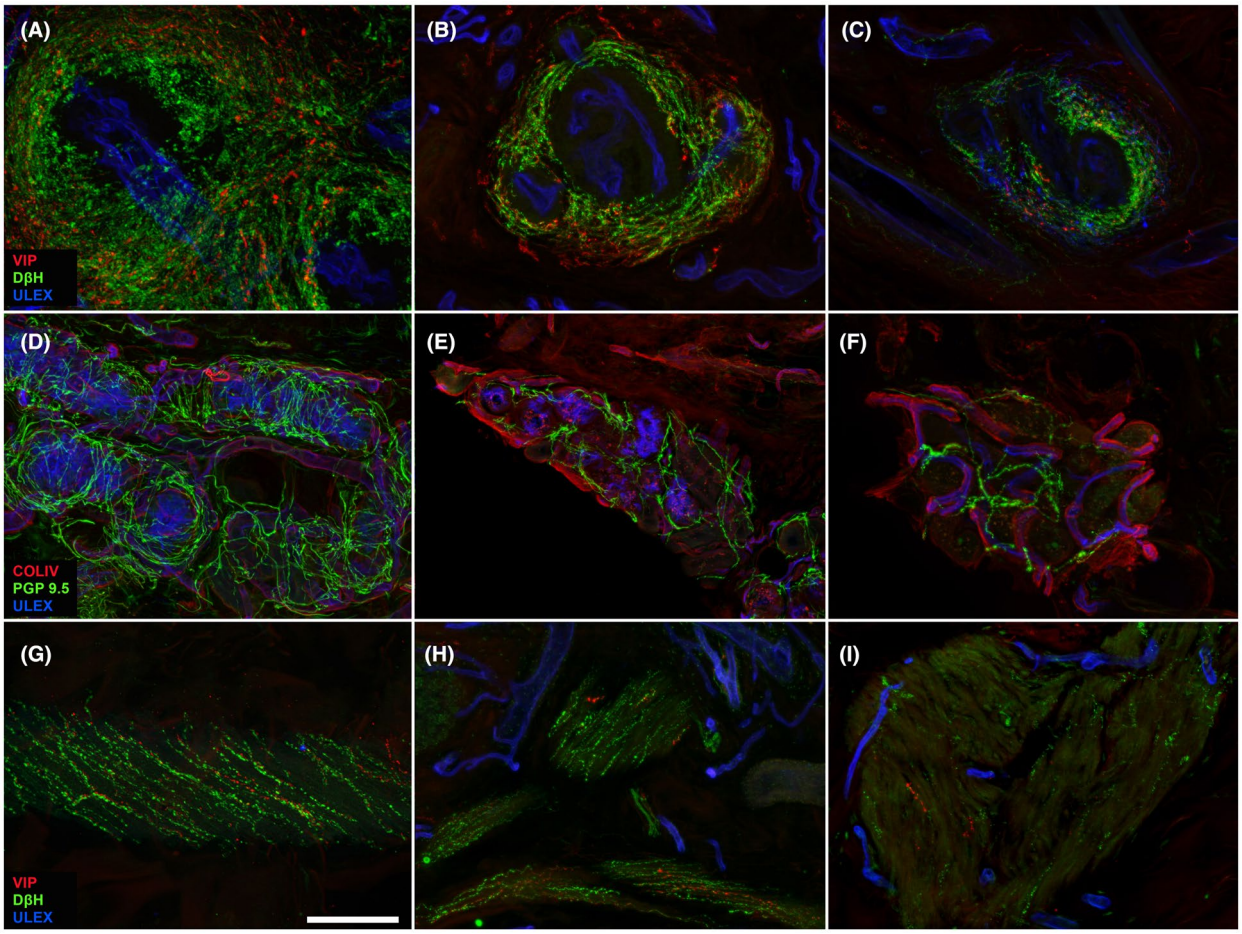

FIGURE 2 Autonomic innervation. Confocal digital images showing the loss of autonomic nerves to arteriovenous anastomosis (B, C), sweat gland (E, F) and errector pili muscles (H, I) in the skin of patients with PD (B, E, H) and PSP (C, F, I) compared to controls (A, D, G). In PSP, the loss of Dβh and VIP-immunoreactive vasomotor (C) and pilomotor (I) nerves is more severe than in PD (B, H). PSP patients also displayed a more severe loss of sudomotor nerves and frequent aspects of sweat gland atrophy (F) respect to PD (E). Scale bar =100 μm; Dβh, dopamine β-hydroxylase; PD, Parkinson disease; PSP, Progressive Supranuclear Palsy; VIP, vasoactive intestinal peptide; PGP, protein gene product 9.5; ULEX, Ulex Europaeus Agglutinin

without clear differences among the disease groups. IME showed signs of axonal degeneration such as thinning, nodal enlargement (Figure 3A), swellings (Figure 3B, E, F).

Dermal papillae and capillary loops were hypertrophic in PD patients whereas they appeared more regular in PSP patients (Figure 1 B and H compared to C and I).

Although sensory nerve conduction studies were within the normal limits for all patients, the amplitudes of sural nerve action potentials of both patient groups (PSP: 5.8 ± 2.7 μV, PD: 9.2 ± 6.4 μV) were significantly lower compared to HC (13.2 ± 5 μV, $p = 0.001$).

Correlation between UPDRS-III score and functional and morphological small fibre parameters

In the PSP group, we found a negative correlation between UPDRS-III score and IENF at leg ($r = -0.533$, $p = 0.004$) and fingertip ($r = -0.562$, $p = 0.005$), and between disease duration and IME ($r = -0.568$, $p = 0.005$). As for the PSP Rating Scale score, the significant correlation still held with the IENF at leg ($r = -0.418$, $p = 0.03$). For the PD patients, the analysis did not show any significant correlation.

DISCUSSION

In this study, we demonstrated a peripheral nerve impairment in PSP through the evidence of a previously undescribed cutaneous denervation involving small and large fibre endings and that, compared to PD patients, was more severe and length-dependent.

The loss of cutaneous sensory nerves may account for the occurrence of sensory symptoms that were present in 60% of PSP and 29% of PD patients, in line with a greater loss of unmyelinated and myelinated nerve endings and with a lower amplitude of sural nerve action potentials in the former compared to the latter group. Moreover, the loss of mechanoreceptors and related myelinated

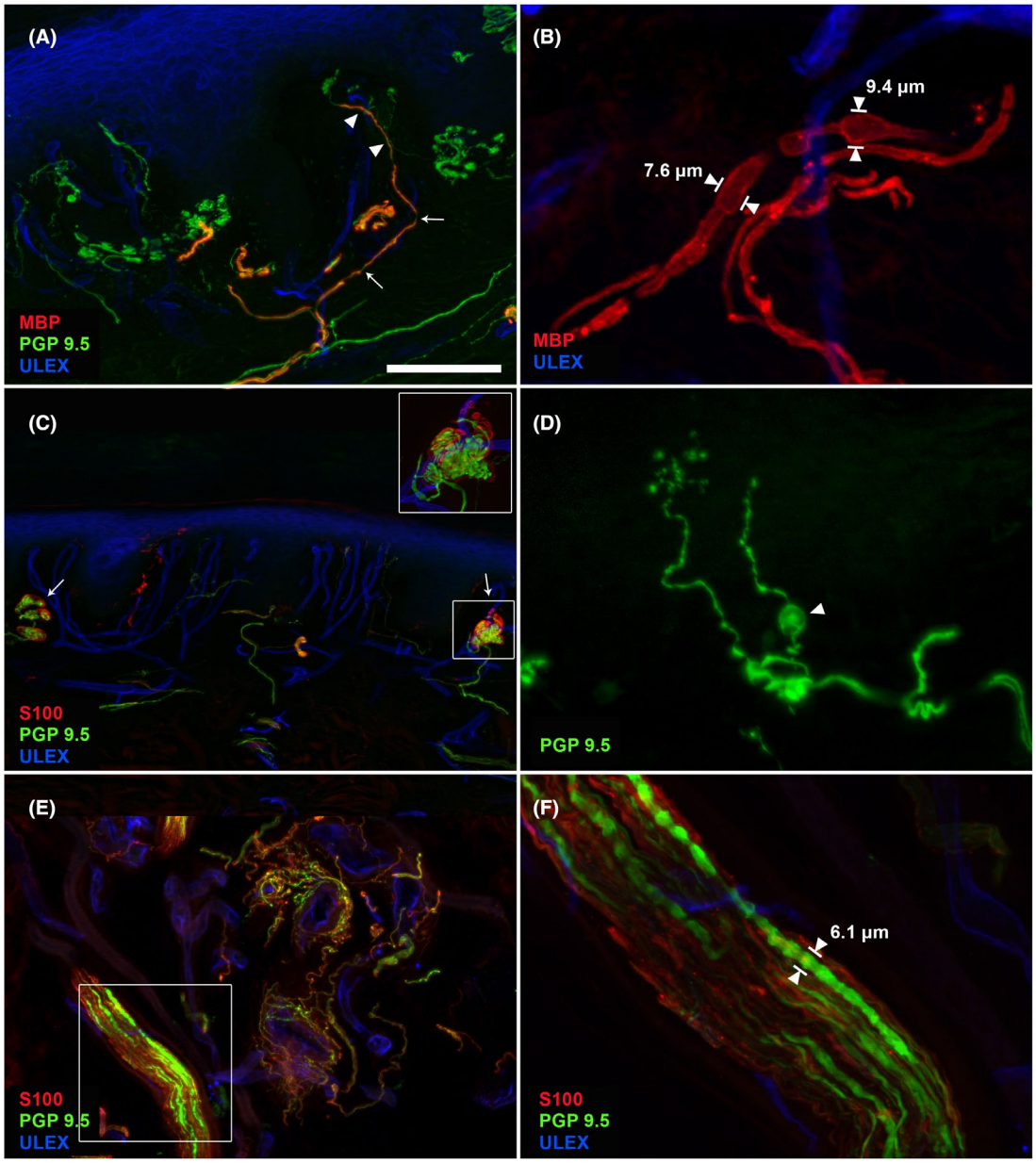

FIGURE 3 Aspects of nerve degeneration in PSP. Irregular calibre of dermal myelinated fibres showing thinning of the last internodes (arrowheads) and nodal enlargement (arrows) in A and axonal swellings (arrowheads) in B, suggesting axonal degeneration. Note that calibre mean value of dermal myelinated fibres of controls is 3.3 ± 0.5 µm [34]. In C, aspects of degeneration of Meissner corpuscles that appear deranged with a reduction of neural material inside the capsule and placed deeper in the dermis (arrows), see the detail in the square at higher magnification. In D, varicosities along the intraepidermal nerve fibres (arrowhead). Severe loss of nerve fibres in an arterio-venous anastomosis (E) and in a dermal nerve fascicle with aspects of axonal degeneration, see the detail at higher magnification in F (arrowheads). The few surviving fibres show swellings and a "string of beads" appearance (F). Scale bar = 100 µm in A, 30 µm in B and D, 200 µm in C and E, 50 µm in F; MBP, myelin basic protein; PGP, protein gene product 9.5; ULEX, Ulex Europaeus Agglutinin.

endings, may contribute to the central mechanisms producing postural instability [39] that is more relevant in PSP.

In PD patients, growing evidence has shown sensory pathway dysfunction, with involvement of both peripheral [3] and central sensory processing [40]. Less is known about sensory involvement in PSP [26] and the few neurophysiological data reported so far, suggest a central sensory pathway dysfunction, showing giant somatosensory evoked potentials that reflect abnormal cortical excitability [41, 42]. More recently a facilitation of nociceptive processing at the spinal level has been demonstrated in subjects with advanced PSP [43].

Autonomic symptoms in our patient populations were reported by all PSP and by most PD patients. Specifically, the urinary domain was the most involved in PSP patients, whereas the gastrointestinal

domain was the most involved in PD patients. This finding is in line with previous studies reporting urinary dysfunction as one of the most common autonomic disturbance in PSP patients [13, 24], and the one associated with a more rapid disease progression, together with constipation [25]. Importantly, post-mortem examination showed pathological changes characterised by severe cell loss, presence of neurofibrillary tangles, and glial inclusions of Onuf's nucleus, which is involved in sphincter control [18].

We did not assess cardiovascular function in our study; however, an overt neurogenic orthostatic hypotension was not present in our PSP and PD patients. Therefore, it may be plausible that the symptom 'dizziness' referred by 17% of PD and 30% of PSP patients could have been partially explained by their postural instability.

We found instead a severe impairment of sudomotor function in almost the totality of our patients although only 44% of PSP and 25% of PD patients reported sweating disturbances. This is not surprising, since it is quite common that patients are not aware of their thermoregulatory dysfunction, especially if overwhelmed by more disabling symptoms. Sweating was more affected in PSP patients, who had a lower number of activated sweat glands after pilocarpine stimulation compared to PD, indicating a greater postganglionic damage to the sudomotor pathway. This was in line with the lower amplitude of SSR and the more severe loss of autonomic sudomotor nerves associated with frequent aspects of sweat gland derangement. Our findings agree with previous works reporting low amplitudes of sympathetic sweat responses [14] and reduced sweat output by means of the Quantitative Sudomotor Axon Reflex Test (QSART) in PSP [20, 24], providing functional and morphological evidence of the postganglionic damage. The loss of autonomic nerves around other dermal adnexa (errector pili muscles, arteriovenous anastomosis, arterioles) was also more severe in PSP compared to PD.

The presence of autonomic dysfunction in PSP has been reported by several authors but remains controversial. The PSP Study Group recently stipulated that "predominant, otherwise unexplained autonomic failure" is an exclusionary criterion for PSP [2]. In fact, adrenergic cardiovascular function seems to be spared in PSP as demonstrated by a recent retrospective study on pathologically confirmed PSP, Lewy Body Disease and MSA patients [24]. However, in the same study the authors showed that other autonomic domains, such as sexual, gastrointestinal, sudomotor and urinary, were impaired without any significant difference between PSP and MSA patients [24].

The conflicting results reported in the literature are mostly due to the clinical heterogeneity of PSP with the frequent occurrence of phenotypes overlapping with other parkinsonian disorders and the lack of specific 'in vivo' disease markers. In fact, a low rate of 'in vivo' diagnostic accuracy has been described [24] and most studies assessing the autonomic nervous system are antemortem.

The pathogenic mechanisms underlying neurodegeneration are linked to the accumulation of phosphorylated alpha synuclein in PD and phosphorylated tau protein in PSP in different regions of the brain [44, 45]. Abnormalities of the spinal cord are uncommon in PSP, however, there have been some neuropathological reports of spinal cord lesions in patients with PSP. In a first study [46] of the spinal cord of five patients with PSP, morphometric analysis revealed 47%, 52% and 32% decreases of cell numbers in motor area (lamina IX) at the three spinal levels, 6th cervical, 7th thoracic and 5th lumbar respectively, and 39% in the intermediolateral column. In a subsequent study enrolling 10 cases with PSP [47], a widespread distribution of neurons with cytoplasmic inclusions and neuropil threads in the spinal grey matter was observed, particularly in the medial division of the anterior horn (motor neurons) and intermediate grey matter, more frequently in the cervical region than in thoracolumbosacral. However, the relationship between the pathological features of spinal cord lesions and clinical symptoms of PSP remains unclear.

Growing evidence shows that this proteinaceous material undergoes patterned propagation with possible prion-like diffusion linked to the neuroanatomical connectome [48]. While phosphorylated alpha-synuclein deposits have been found in the cutaneous nerves of patients with PD [8], which could explain the occurrence of cutaneous nerve degeneration in PD, more controversial are the pathogenic mechanisms underlying peripheral nerve degeneration in PSP. In fact, findings related to the expression of phosphorylated tau immunoreactivity in peripheral nerves and in the skin are contradictory [49–52], and recently it has been even hypothesised that tau pathology may be confined to central nervous system [53]. However, we demonstrated a peripheral nerve involvement in our PSP patients through the morphological evidence of cutaneous denervation supporting the sudomotor postganglionic dysfunction present in our patients, and previously reported in PSP using the QSART [20, 24]. We hypothesise that the mechanisms leading to the degeneration of peripheral nerves can be linked to a prion-like spreading of p-tau pathology through the intermediolateral column of the spinal cord [46], to the paravertebral sympathetic ganglia [49] and then to cutaneous nerves. However, previous studies failed to detect neurofibrillary tangles in the sympathetic or spinal ganglia of PSP patients [50] and contrasting findings have been reported on tau accumulation in the skin [52,53]. This conflicting data requires further studies to clarify the pathophysiological mechanisms underlying the cutaneous denervation in our PSP patients.

Skin is an easily available tissue that allows the visualisation of the last endings of sensory and autonomic nerves providing a unique window to assess peripheral nerve involvement in several neurodegenerative disorders.

An interesting question is if cutaneous denervation reflects motor impairment and therefore central neurodegeneration [8]. We addressed this in a previous cross-sectional study on a larger cohort of PD patients, and we did not find any correlation between epidermal denervation and severity of motor impairment assessed by UPDRS III [7]. The coexistence of regenerative aspects in the skin of PD patients counteracting nerve loss may be a possible explanation for this finding. Therefore, only in a subsequent longitudinal study, could we demonstrate such a correlation, since over time nerve degeneration overcomes regenerative phenomena [54]. Also, in the present cross-sectional study we did not find a significant

correlation between IENF loss and motor impairment in PD patients. Conversely, such correlation was found in PSP patients, and this may be explained by the net predominance of degenerative aspects in the skin, likely driven by a more severe disease progression. Moreover, PSP patients received a lower L-dopa cumulative dose compared to PD patients, making it unlikely that neurotoxic effects of L-Dopa could have contributed to the more severe cutaneous nerve degeneration [7,55].

Finally, most of our PSP patients were PSP-RS, and this may be a limitation since we didn't explore possible differences among the different phenotypes. However this limitation is also a strength for our work, because the high specificity of the clinical characteristics of this classic phenotype [2] reduces the possibility of misdiagnosis even in the absence of pathological confirmation. We did not find any difference in cutaneous innervation between PSP-RS and the four patients with PSP-CBS phenotype. Even if this observation is limited to a few patients, it appears in line with the similar burden of disease shared by PSP-RS and PSP-CBS, while other phenotypes (i.e., PSP with parkinsonism and PSP with progressive gait freezing) have a milder disease progression [56]. Therefore, studies on larger PSP populations, including the complete spectrum of clinical phenotypes are warranted to define possible different morphological patterns.

Moreover, the correlation between peripheral denervation and disease severity found in our cross-sectional study should be verified in future longitudinal studies in order to assess the utility of skin biopsy as a biomarker of disease progression.

In conclusion, we found a severe loss of cutaneous sensory and autonomic nerve fibres in PSP associated with a severe postganglionic impairment of sweating and a mild occurrence of sensory and autonomic symptoms. Our findings showed that the peripheral nervous system is not spared in PSP. Even if cutaneous denervation can explain only in part sensory and autonomic symptoms in PSP, it seems to reflect the severity of disease and therefore, it could be the peripheral expression of neurodegeneration in PSP.

Overall, these results suggest that the morpho-functional analysis of cutaneous sensory and autonomic systems can be useful to investigate pathophysiological mechanisms of neurodegeneration in PSP and may represent a tool to monitor disease progression and to assess treatment efficacy.

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DISCLOSURE

The authors report no disclosures.

ETHICAL APPROVAL

The study was approved by the local ethical committee (Fondazione IRCCS "G. Pascale") and all subjects signed an informed consent.

PEER REVIEW

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SUPPORTING INFORMATION

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