RESEARCH ARTICLE

Skin Biopsy May Help to Distinguish Multiple System Atrophy–Parkinsonism from Parkinson's Disease with Orthostatic Hypotension

Vincenzo Donadio MD, PhD,[1*] Alex Incensi BSc,[1] Giovanni Rizzo MD,[1,2] Rosa De Micco MD, PhD,[3] Alessandro Tessitore MD, PhD,[3] Grazia Devigili MD,[4] Francesca Del Sorbo MD,[5] Salvatore Bonvegna MD,[5] Rossella Infante MD,[1] Martina Magnani BSc,[1] Corrado Zenesini MSc,[1] Luca Vignatelli MD, PhD,[1] Roberto Cilia MD,[5] Roberto Eleopra MD,[4] Gioacchino Tedeschi MD,[3] and Rocco Liguori MD[1,2]

[1]*IRCCS Istituto delle Scienze Neurologiche di Bologna, UOC Clinica Neurologica, Bologna, Italy*
[2]*Dipartimento di Scienze Biomediche e Neuromotorie, Università di Bologna, Italia*
[3]*Department of Advanced Medical and Surgery Sciences, Università della Campania Luigi Vanvitelli, Napoli, Italia*
[4]*Fondazione IRCCS Istituto Neurologico Carlo Besta, Milano, Italia*
[5]*Parkinson Institute ASST Gaetano Pini-CTO, Milano, Italia*

ABSTRACT: Background: The differential diagnosis between multiple system atrophy parkinsonism type (MSA-P) and Parkinson's disease with orthostatic hypotension (PD+OH) is difficult because the 2 diseases have a similar clinical picture. The aim of this study is to distinguish MSA-P from PD+OH by immunostaining for abnormal phosphorylated α-synuclein at serine 129 (p-syn) in cutaneous nerves.

Method: We recruited 50 patients with parkinsonism and chronic orthostatic hypotension: 25 patients fulfilled the diagnostic criteria for MSA-P and 25 patients for PD+OH. The patients underwent a skin biopsy from the cervical area, thigh, and leg to analyze somatic and autonomic skin innervation and p-syn in skin nerves.

Results: Intraneural p-syn positivity was found in 72% of patients with MSA-P, mainly in distal skin sites. More important, p-syn deposits in MSA-P differed from PD +OH because they were mainly found in somatic fibers of subepidermal plexi, whereas scant autonomic fiber involvement was found in only 3 patients. All patients with PD+OH displayed widely distributed p-syn deposits in the autonomic skin fibers of proximal and distal skin sites, whereas somatic fibers were affected only slightly in 4 patients with PD+OH. Skin innervation mirrored p-syn deposits because somatic innervation was mainly reduced in MSA-P. Sympathetic innervation was damaged in PD+OH but fairly preserved in MSA-P.

Conclusions: The p-syn in cutaneous nerves allows the differentiation of MSA-P from PD+OH; MSA-P mainly shows somatic fiber involvement with relatively preserved autonomic innervation; and by contrast, PD+OH displays prevalent abnormal p-syn deposits and denervation in autonomic postganglionic nerves. © 2020 International Parkinson and Movement Disorder Society

Key Words: orthostatic hypotension; parkinsonism; skin biopsy; misfolded alpha-synuclein; multiple system atrophy

Multiple system atrophy parkinsonism type (MSA-P) and Parkinson's disease (PD) with orthostatic hypotension (PD+OH) are 2 synucleinopathies characterized by similar clinical presentations, particularly during the early disease stages.[1] Accordingly, patients with MSA with parkinsonism symptoms may be misdiagnosed as PD,[1] but also

***Correspondence to:** Dr. Vincenzo Donadio, IRCCS Istituto delle Scienze Neurologiche, Bologna, Italia, UOC Clinica Neurologica, via Altura 3, 40139 Bologna, Italy; E-mail: vincenzo.donadio@unibo.it

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approximately 20% of patients with a clinical diagnosis of MSA were reclassified as PD or dementia with Lewy bodies at autopsy.[2] This implies clear difficulty for clinicians in distinguishing these 2 disorders, which are characterized by a different prognosis and levels of disability.[1,3] In fact, MSA-P is a rapidly progressive disorder in the majority of patients affected[1]; by contrast, PD+OH shows a longer disease duration.[1,4] Furthermore, misdiagnosis can lead to inappropriate treatments increasing the risk of adverse events and compromising eligibility for clinical trials.

Few diagnostic tests are available to help make the differential diagnosis of these 2 conditions. In fact, cardiovascular autonomic reflexes are useful in identifying neurogenic OH underlying these 2 disorders, but they do not provide enough information to distinguish between them.[1,5] Cardiac sympathetic innervation evaluated with positron emission tomography (PET)[6] or MIBG single-photon emission computed tomography[7] can clearly distinguish MSA from PD+OH because of preserved cardiac sympathetic innervation in the first disorder.[8-10] Nevertheless, abnormal cardiac sympathetic innervation does not exclude MSA because a minority of MSA patients may have cardiac sympathetic denervation.[11]

Skin biopsy is a recently available in vivo diagnostic tool for synucleinopathies that can be used to identify misfoldedα-synuclein (α-syn) in cutaneous nerves.[12-16] Preliminary data suggest the possibility of distinguishing MSA from the remaining synucleinopathies by revealing abnormal α-syn deposits in cutaneous nerves because these abnormal deposits are located mainly in the somatic terminals in MSA, but in autonomic fibers in PD and other synucleinopathies.[17,18] If confirmed in a larger cohort of patients, this is important information that may be used to differentiate MSA in vivo, particularly the parkinsonism type, from different clinical variants of synucleinopathy, especially PD.

Therefore, the aim of this study was to verify whether misfolded α-syn in cutaneous nerves differs in a large sample of patients with MSA-P and PD+OH, matched for motor and autonomic involvement and with similar disease durations. We also measured the diagnostic accuracy of misfolded α-syn deposits in somatic and autonomic cutaneous innervation for distinguishing MSA-P from PD-OH.

Methods

We screened 60 consecutive patients with parkinsonism and OH. Only 50 who fulfilled the current clinical and instrumental diagnostic criteria for idiopathic probable MSA-P[19] and PD+OH[20] were recruited. They included 25 patients with MSA-P and 25 patients with PD+OH (Table 1). None of the patients had a positive family history for parkinsonism or autonomic disorders. A total of 10 patients with PD+OH were reported in a previous study[21] and were selected for this study to match the MSA-P group in terms of clinical involvement and disease duration. Neurogenic OH was objectively defined in all patients by a 3-minute head-up tilt at 65° (18 patients with MSA-P and 19 patients with PD+OH) or standing (7 patients with MSA-P and 6 patients with PD+OH). OH was considered to be present with a blood pressure drop of at least 20/10 mmHg (systolic/diastolic)[22] without a significant change in heart rate (Table 1). OH was also confirmed in most patients by a need for specific treatments (eg, fludrocortisone, midodrine), including 21 patients with MSA-P and 19 patients with PD+OH.

The clinical diagnosis was then supported by specific diagnostic tests on all patients (Table 1). Nigrostriatal dopamine transporter imaging with [123I] ioflupane single-photon emission computed tomography was abnormal in all patients. Cardiac uptake of [123-I])-MIBG single-photon emission computed tomography was found in a minority of patients, and it revealed cardiac postganglionic sympathetic denervation in all patients with PD+OH (13 patients); findings were normal in all patients with MSA-P (8 patients), except for 1 patient who had slightly decreased cardiac sympathetic innervation. However, this patient displayed stridor during sleep and the "hot-cross bun" sign on brain magnetic resonance imaging, along with genitourinary dysfunction, which strongly supported the MSA diagnosis (Table 1).[23] In addition, brain magnetic resonance imaging was performed on almost all patients, revealing normal findings in the patients with PD+OH, but typical abnormalities that support the clinical diagnosis in most patients with MSA-P (ie, putaminal atrophy and hyperintense putaminal rim, hot-cross bun sign, and brainstem atrophy).[23,24] Clinical involvement was quantified with specific questionnaires for autonomic (ie, COMPASS-31[25]), motor (Unified Parkinson's Disease Rating Scale Part III and Hoehn and Yahr scores) and nonmotor (Non-Motor Symptoms Scale[26]) dysfunction, and a focused scale for MSA (Unified MSA Rating Scale [UMSARS-I and UMSARS-II[27]]; Table 1). The presence of rapid eye movement sleep behavior disorder was reported by bed partners frequent enacted dreams. In addition, in 4 patients who reported rapid eye movement sleep behavior disorder (2 MSA-P and 2 PD+OH), a polysomnographic study confirmed rapid eye movement sleep without atonia.

Pharmacological treatment for parkinsonism included levodopa (L-dopa) with/without dopamine agonists (Table 1). Serum screening for predisposing causes for peripheral neuropathy (diabetes, microbiological, autoimmune, paraneoplastic, and thyroid disorders and vitamin B12 deficiency) was performed, and 4 male patients (3 MSA-P and 1 PD+OH) who were found to have diabetes and vitamin B12 deficiency were excluded from the innervation analysis. A total of 23 age-matched healthy subjects without neurological deficits served as controls for the skin biopsy findings. The proced-

TABLE 1. Demographic and clinical data of recruited subjects

	MSA-P, N = 25	PD+OH, N = 25	*P* Value
Age, mean (SE)	66 (6.1)	74 (6.7)	<0.001
Sex, male, n (%)	20 (80)	18 (72)	0.74
Disease duration, mean (SE)	4.9 (2.6)	6.8 (4.1)	0.061
Levodopa dose, mean (SE)	310 (191)	518 (250)	0.003
Dopamine agonists, n (%)	2 (8.7)	18 (72.0)	<0.001
RBD, n (%)	19 (76)	17 (68)	0.75
Compass–31, mean (SE)	34.9 (10.9)	35.4 (11.2)	0.89
NMSS, mean (SE)	100.9 (33.9)	80.5 (27.0)	0.033
U-I, mean (SE)	23.1 (5.0)	16.5 (4.5)	<0.001
U-II, mean (SE)	25.6 (5.0)	23.6 (8.1)	0.33
UPDRS Part III, mean (SE)	35.9 (9.7)	32.9 (12.1)	0.37
H&Y, mean (SE)	2.5 (0.8)	2.2 (0.8)	0.19
Nigro-striatal dopamine transporter–Abn, n (%)	25 (100)	25 (100)	1.00
Brain MR–Abn, n (%)	17 (71)	1 (8)	<0.001
Cardiac MIBG–Abn, n (%)	0 (0)	13 (100)	<0.001
Tilt table test–SPB, mmHg, mean (SE)	46.6 (16.9)	44.7 (19.9)	0.78
Tilt table test–DBP, mmHg, mean (SE)	18.0 (5.1)	19.1 (7.6)	0.64
Tilt table test–HR, mmHg, mean (SE)	3.0 (4.9)	4.3 (5.3)	0.20

MSA-P, multiple system atrophy parkinsonism type; PD+OH, Parkinson's disease with orthostatic hypotension; SD, standard deviation; RBD, rapid eye movement sleep behavior disorder; SE, standard error; NMSS, Non-Motor Symptoms Scale total score; U-I= Unified MSA Rating Scale–I; U-II, Unified MSA Rating Scale–II; UPDRS, Unified Parkinson's Disease Rating Scale; H&Y, Hoehn and Yahr scores; Abn, abnormal; MR, magnetic resonance; MIBG, metaiodobenzylguanidine; SBP, systolic blood pressure change; DBP, diastolic blood pressure change; HR, heart rate change.

ures used were approved by the local human ethics committee and followed the Helsinki Declaration regarding international clinical research involving humans. All subjects gave written informed consent for the study.

Skin Biopsy

Punch biopsies of 3 mm were taken from proximal and distal hairy skin sites. The proximal site was the cervical C7 paravertebral area, and distal sites were located in the thigh (15 cm above the patella) and distal leg (10 cm above the lateral malleolus). A second skin biopsy was taken 3 to 4 cm from the first sample to assess for the distribution of abnormal phosphorylated α-syn at serine 129 (p-syn).[13,21] According to previously published procedures,[28] the skin samples were immediately fixed in cold Zamboni fixative and kept at 4°C overnight.

p-syn Deposits

The 10-μm sections were obtained using a cryostat (CM 1950; Leica, Deerfield, IL) to evaluate α-syn deposits.[13,21] They were double immunostained overnight with a panel of primary antibodies, including rabbit monoclonal p-syn (1:500; Abcam, Cambridge, UK; cat. no. ab-51253), mouse pan-neuronal marker protein gene product 9.5 (PGP; 1:750; Abcam; catalog no. ab72911), and specific autonomic markers such as rabbit tyrosine-hydroxylase (1:1000; Novus Biologicals, Littleton, CO; catalog no. NB300-109) to identify noradrenergic fibers and rabbit vasoactive intestinal peptide (1:1000; Incstar, Stillwater, MN; catalog no. NB300-109) colocalized in sudomotor cholinergic fibers.[28,29] The sections were then washed, and secondary antibodies were added for an incubation period of 1 hour. Anantimouse Alexa Fluor 488 (1:400; Jackson ImmunoResearch, West Grove, PA; catalog no. 715-545-150) and rabbit cyanine dye fluorophores 3.18 (1:200; Jackson ImmunoResearch; catalog no. 711-165-152) were used as secondary antibodies. A microscope analysis and relevant criteria were followed to define p-syn positivity, as described previously.[12,13,18,21] The sections were analyzed in a blinded fashion using a confocal laser scanningmicroscope (Nikon Eclipse Ti A1 [Tokyo, Japan] confocal microscopy) by 2 authors with expertise in immunofluorescent analysis (D.V. and I.A.). Our recent analysis revealed that intralaboratory reproducibility of our technique was high, with 100% concordance of classification in all patients (K = 1).[30] Digital images were collected in successive frames of 1-μm to 2-μm increments on a Z-stack plan at the appropriate wavelengths for secondary antibodies with a ×20 or ×40 plan apochromat objective and were subsequently projected to obtain a double-stained digital image with a computerized system. The correspondence between the rabbit p-syn and the mouse PGP staining helped to verify the intraneuronal deposits, excluding possible nonspecific staining arising from the background. The analysis of p-syn staining was rated in each skin site as the percentage of autonomic structures or nerve bundles that showed positive staining at high magnification (×40).

Skin Innervation

Additional 50-μm sections from the same skin sample were obtained during the cryostat session. A total of

12 free-floating sections were incubated overnight with a panel of primary antibodies, including the rabbit PGP 9.5 (1:500; Abcam; catalog no. ab108986) and mouse collagen IV (1:800; Chemicon, Temecula, CA; catalog no. MAB1910). The sections were then washed, and secondary antibodies labeled with mouse Alexa Fluor 488 (see above) and rabbit cyanine dye fluorophores 3.18 (1:800 see above) were added for an overnight incubation. Autonomic innervation density was quantified under a Nikon fluorescent microscope using the previously described automated method that has high interobserver and intraobserver reliability.[28] Briefly, this method is based on a technique known as the "unsharp mask filter," which creates a composite image by subtracting the background color in the out-of-focus image from the base image, expressing the autonomic innervation staining (Image Pro Plus, Media Media Cybernetics, Inc., Rockville Pike, Rockville, USA). Target autonomic structures included muscle arrector pilori and the sweat gland. The corresponding innervations were quantified as PGP percentage area, providing stronger staining that was easier to quantify than the specific autonomic markers, but that was correlated with the innervation quantified by the specific autonomic markers.[28] The autonomic innervation score in each skin site usually represented the mean of 2 or 3 different target structures identified by the collagen IV staining. Epidermal nerve fiber densities (ENFs; number of unmyelinated fibers per linear millimeter of epidermis) was calculated by considering a single ENF marked by PGP 9.5 crossing of the dermal–epidermal junction. The confocal laser-scanning microscope specified previously was also used in this case to study the innervation pattern.

Statistical Analysis

Statistical analyses were performed using SPSS 25.0 for Windows (IBM Corp., Armonk, NY) and Stata SE 14.2 (StataCorp, College Station, TX). For the analysis of continuous variables, we used the Kolmogorov-Smirnov test to verify the normal data distribution. To compare the normally distributed data, we used the *t* test, 1-way analysis of variance followed by a post hoc Bonferroni test depending on the data. When the variables were not normally distributed, we used the Mann-Whitney *U* test. To compare categorical variables, we used the χ^2 test, and the results are shown as absolute and relative frequency. The correlations between variables was evaluated with the Pearson *r* coefficient. We used multivariate linear regression to adjust the results as age, gender, and disease duration.

The outcome measures to assess the diagnostic validity of misfolded α-syn deposits in somatic and autonomic innervations in distinguishing MSA-P from PD+OH were sensitivity, specificity, and likelihood ratios. The post hoc diagnostic accuracy of the combination of the results in the 2 types of nerves was also explored.

Results

The gender distribution between MSA-P and PD+OH patients was not significantly different. Mean age was higher in the patients with PD+OH (74 vs. 66 years; Table 1), whereas the difference in mean disease duration, although slightly higher in the patients with PD+OH compared with the patients with MSA-P (6.8 vs. 4.9 years), did not reach statistical significance ($P = 0.061$; Table 1). L-dopa was higher in the patients with PD+OH, and dopamine agonists were mainly taken by patients with PD+OH. Autonomic and motor involvement were similar in the 2 groups because no differences were found in the COMPASS-31, Movement Disorder Society–Unified Parkinson's Disease Rating Scale, Hoehn and Yahr scale, or UMSARS-II scale. A significant difference was found in the Non-Motor Symptoms Scale and UMSARS-I scores, reflecting the more widespread and greater disability attributed to nonmotor symptoms and autonomic dysfunction in patients with MSA-P compared with patients with PD+OH (Table 1).

Intraneural p-syn Deposits

MSA-P

Abnormal α-syn deposits were found in 18 patients (72%) with a distal-proximal gradient (Suppl. Fig. 1). Considering both skin samples, the leg site was positive in all patients, whereas the thigh and C7 sites were positive in 78% and 67% of patients, respectively. Overall,

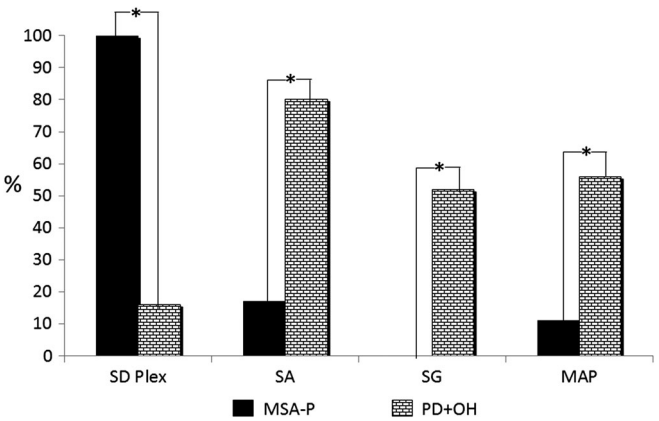

FIG. 1. Phosphorylated α-synuclein distribution among skin annexes. Analysis of phosphorylated α-synuclein distribution among skin annexes revealed a significant difference between multiple system atrophy parkinsonism type (MSA-P) and Parkinson's disease with orthostatic hypotension (PD+OH). In fact, MSA-P mainly showed phosphorylated α-synuclein deposits in the somatic fibers of subepidermal plexi of the superficial dermis (SD Plex) as opposed to PD+OH that mainly showed phosphorylated α-synuclein deposits in autonomic annexes such as skin arterioles (SA), sweat glands (SG), and muscle arrector pilori (MAP). * = χ^2 test: $P < 0.001$.

63% of analyzed skin samples showed p-syn positivity: the first sample analyzed showed abnormal deposits in 40% of patients, whereas by adding the second sample, the positivity increased to 72%. The data supported a rather patchy p-syn distribution in these patients. The 3 patients excluded from the innervation analysis were positive (1 patient) and negative (2) for p-syn. However, abnormal p-syn deposits were characteristically found in the somatic nerves of the subepidermal plexi in the superficial dermis in all patients (χ^2 test: $P < 0.001$ MSA-P vs. PD+OH; Figs. 1 and 2A). In addition, scant p-syn positivity was found in the autonomic adrenergic fibers of skin arterioles (3 patients; χ^2 test: $P < 0.001$ MSA-P vs. PD+OH) and muscle arrector pilori (2 patients; χ^2 test: $P < 0.001$ MSA-P vs. PD+OH), whereas no abnormal deposits were found in the cholinergic fibers of the sweat gland (χ^2 test: $P < 0.001$ MSA-P vs. PD+OH). Interestingly, cardiac MIBG in 1 of these patients showed cardiac autonomic adrenergic denervation.

No differences in age, gender distribution, disease duration, L-dopa dosage, clinical scales, or the degree of OH were found between patients who were positive or negative for p-syn. The amount of p-syn deposits in skin samples was not correlated with age, disease duration, L-dopa dose, or clinical scales.

PD+OH

All patients displayed p-syn deposits without a proximal–distal gradient (Suppl. Fig. 1). The first skin sample was positive in 92% of patients, and when the second sample was added, the positivity increased to 100%. The amount of p-syn deposits was higher than in the patients with MSA-P (Mann-Whitney U test, $P < 0.001$;

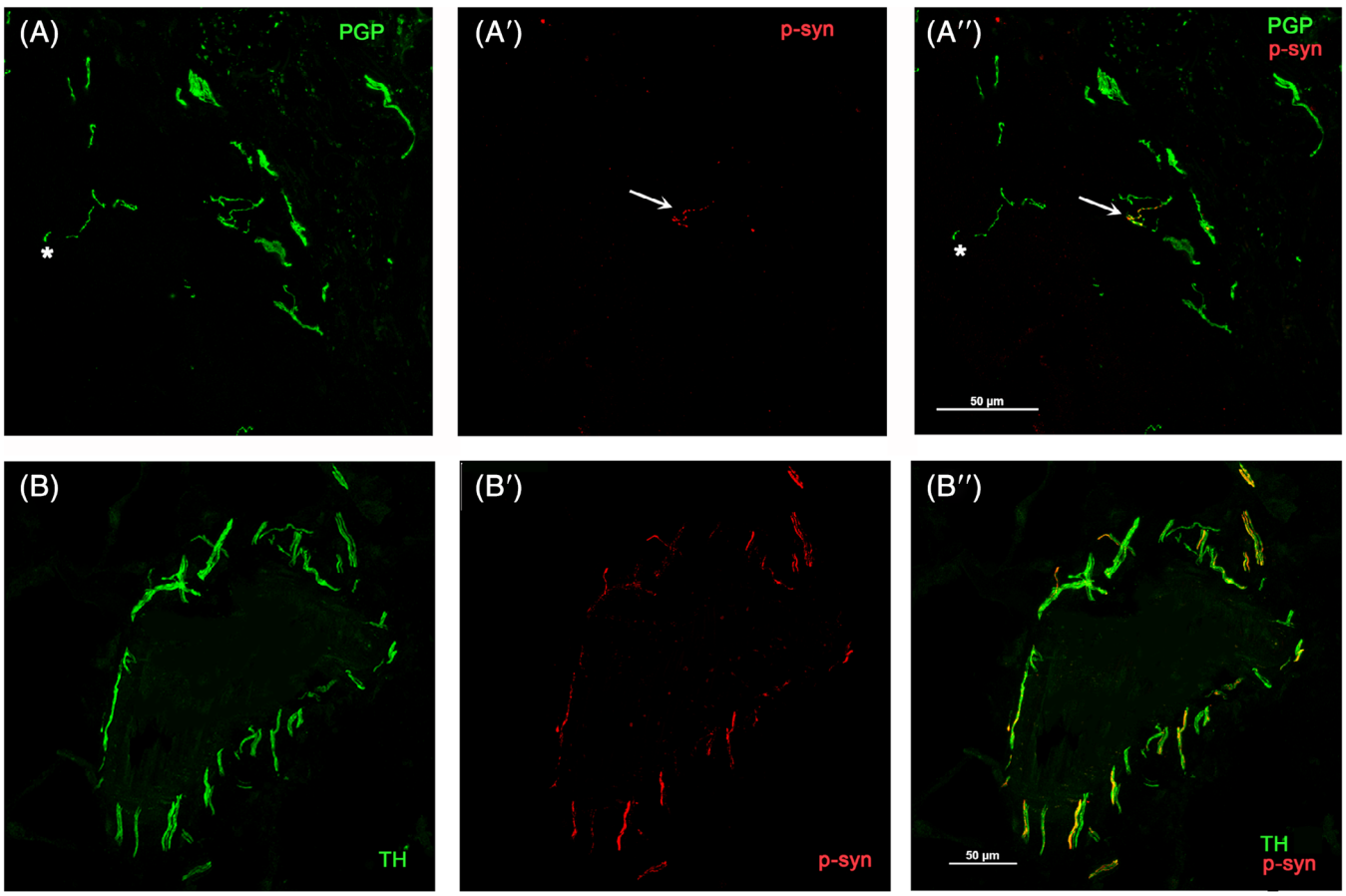

FIG. 2. Phosphorylated α-synuclein (p-syn) deposition in multiple system atrophy parkinsonism type and Parkinson's disease with orthostatic hypotension. Confocal microscope (×400) study of p-syn deposits in cutaneous nerves of a patient with multiple system atrophy parkinsonism type and a patient with Parkinson's disease with orthostatic hypotension. **A–A^{II}**, In multiple system atrophy parkinsonism type, abnormal p-syn deposits were characteristically found in the somatic nerves of subepidermal plexi in the superficial dermis. These plexi are identified by pan-neuronal marker protein gene product (PGP) 9.5 close to the epidermal fibers (indicated by the asterisk). p-syn was depicted (arrow) by staining the phosphorylation at Ser 129 (**A^{I}**). Abnormal p-syn deposits were neuritic inclusions, as shown by the merged image (arrow), in the subepidermal plexus typically running parallel to the epidermis just below the basal membrane (**A^{II}**). **B–B^{II}**, By contrast, abnormal α-synuclein aggregates were found in the autonomic fibers of the deep dermis in Parkinson's disease with orthostatic hypotension. Adrenergic nerve fibers identified by tyrosine-hydroxylase (TH) were found around an arteriole in the deep dermis (**B**). Most of the TH immunoreactive fibers showed positive p-syn (**B^{I}**) as neuritic inclusions demonstrated by the merged image (**B^{II}**). [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 2. Diagnostic validity of misfolded p-syn deposits in somatic and autonomic cutaneous innervation in the diagnosis of MSA versus PD-OH

Test	Sensitivity (95% CI)	Specificity (95% CI)	LR+	LR–
Positive p-syn deposits in somatic nerves	72% (70–74)	84% (82–86)	4.5	0.33
Negative p-syn deposits in autonomic nerves	88% (86–90)	100% (98–100)	1	0.12

p-syn, phosphorylated α-synuclein; MSA-P, multiple system atrophy parkinsonism type; PD+OH, Parkinson's disease with orthostatic hypotension; CI, confidence interval; LR+, likelihood ratio of positive test; LR–, likelihood ratio of negative test.

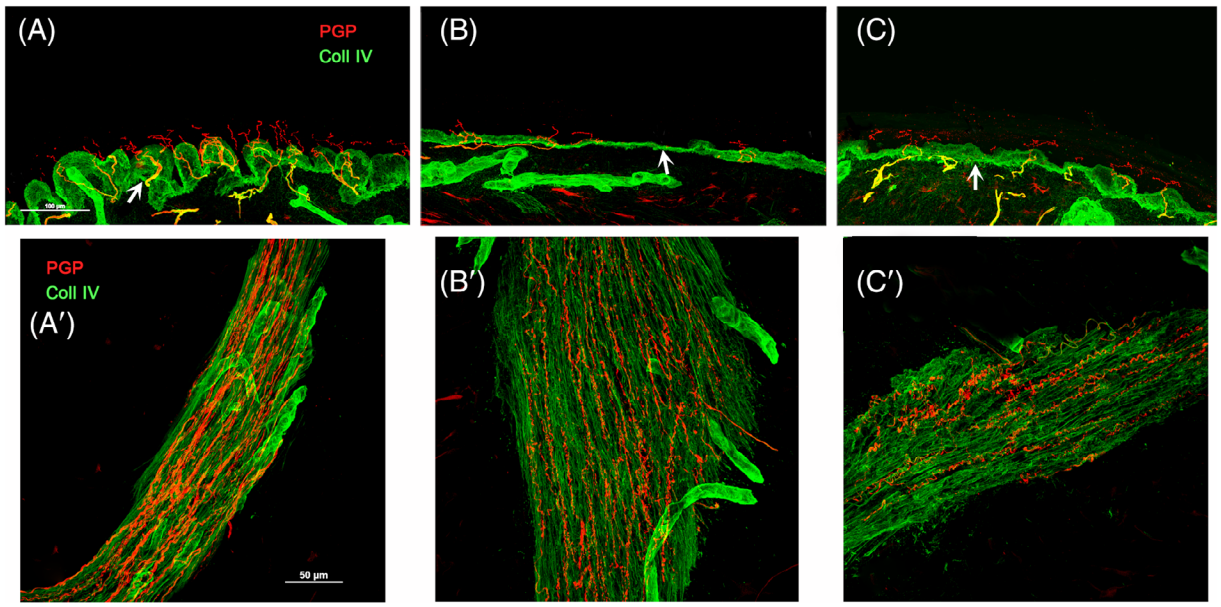

FIG. 3. Somatic and autonomic patterns of innervation in multiple system atrophy parkinsonism type (MSA-P) and Parkinson's disease with orthostatic hypotension (PD+OH) patients and a healthy control subject. Epidermal and muscle arrector pilori autonomic innervations disclosed by confocal microscope (×400) in an age-matched healthy control subject (**A,A^{I}**), MSA-P (**B,B^{I}**), and PD+OH (**C,C^{I}**) patients. Nerve fibers are marked in red by a pan-neuronal marker protein gene product (PGP) 9.5, whereas the collagen (Coll IV) staining is shown in green. **A–C**, Free-ending PGP immunoreactive nociceptive fibers are evident in the epidermis of the control (**A**). The basement membrane separating epidermis from dermis is marked by Coll IV staining and indicated by an arrow. Nociceptive fibers were markedly decreased in the patient with MSA-P (**B**) and to a lesser extent in the patient with PD+OH (**C**). **A^{I}–C^{I}**, Muscle arrector pilori showed a rich density of fibers running in a longitudinal and wavy pattern in the healthy subject (**A^{I}**), but these fibers were poor and had a deranged pattern of innervation in the patient with PD+OH (**C^{I}**). The patient with MSA-P presented a pattern of innervation with a slight decrease in fibers (**B^{I}**). [Color figure can be viewed at wileyonlinelibrary.com]

χ^2 test, $P < 0.05$; Suppl. Fig. 1), even after adjusting for age, gender, and disease duration (multiple linear regression). These data underline the presence of diffuse and uniform skin nerve p-syn deposits in these patients, even in the patient excluded from the innervation analysis. However, there was a clear difference in p-syn deposits when compared with the patients with MSA-P, since they were found in autonomic fibers both adrenergic, mainly of skin arterioles (Fig. 2B), and cholinergic innervation of sweat glands and plexi close to the autonomic structures in the lower dermis (Fig. 1). Four patients had scant abnormal deposits, even in the plexi of the superficial dermis, in addition to the involvement of autonomic fibers. p-syn deposits in skin nerves were not correlated with age, disease duration, L-dopa dose, or clinical scales.

Diagnostic Accuracy of p-syn Deposits in Somatic and Autonomic Skin Innervation

The distribution of patients according to the results of misfolded α-syn deposits in somatic and autonomic innervations is shown in Supplementary Figure 2. The diagnostic validity measures (sensitivity and specificity) used to test p-syn deposits in the 2 types of nerves (somatic and autonomic) are reported in Table 2. By combining the results of the test (Suppl. Tables 2 and 3), maximizing sensitivity, the best post hoc accuracy was found when p-syn positivity was detected in somatic nerves or when somatic and autonomic nerves were both negative (sensitivity 100%, 95% confidence interval [CI], 98–100; specificity 84%, 95% CI, 82–86). Maximizing specificity, the best accuracy was

found when the autonomic nerves were negative with any result for somatic nerve (sensitivity 88%, 95% CI, 86–90; specificity 100%, 95% CI, 98–100). This result supports a different distribution of p-syn in the skin nerves in MSA-P (mainly affecting somatic nerves) compared with PD+OH (autonomic nerves) or a clear diagnostic indication in favor of MSA-P when both nerves were negative.

Peripheral Skin Innervation

MSA-P

The patients with MSA-P showed a pronounced decrease of ENFs that was higher than the patients with PD+OH in all skin sites, although the difference was not significant (Suppl. Table 1). However, autonomic innervation was relatively preserved compared with the patients with PD+OH, particularly for the adrenergic fibers of muscle arrector pilori in the leg, although distal autonomic innervation of the sweat glands was slightly decreased when compared with the healthy controls (Fig. 3, Suppl. Table 1). Somatic and autonomic innervation did not differ between patients who were positive versus negative for p-syn and were not correlated with age, gender distribution, disease duration, L-dopa, clinical scales, or the degree of OH. However, an inverse linear correlation was found between p-syn deposits and cervical ENFs (Pearson $r = -0.6$; $P < 0.01$) or the autonomic innervation of sweat glands in the leg ($r = -0.8$; $P < 0.01$).

PD+OH

This group displayed somatic and autonomic small-fiber neuropathy mainly affecting the legs (Fig. 3, Suppl. Table 1). Innervation scores were not correlated with age, gender distribution, disease duration, p-syn deposits, or clinical scales.

Discussion

The main results of our study indicate the following: (1) p-syn in the skin nerves allows differentiation of MSA-P from PD+OH; (2) MSA-P shows mainly somatic fiber involvement with relatively preserved autonomic innervation; (3) by contrast, PD+OH displays mainly abnormal p-syn deposits and denervation in the autonomic postganglionic nerves. These data extend the current knowledge about the involvement of autonomic peripheral innervation in patients with parkinsonism and autonomic dysfunction.

The differential diagnosis between patients with MSA-P and PD+OH is particularly challenging because of the wide overlap of clinical signs and symptoms.[1,3] However, the prognosis and disabilities of these 2 disorders differ, with patients with MSA-P having greater disability, poor prognosis, and a lack of L-dopa response.[1,3,31] Accordingly, our data emphasize that motor and autonomic involvement did not differ in the 2 groups of patients because the clinical scales (ie, COMPASS-31, Movement Disorder Society–Unified Parkinson's Disease Rating Scale Part III, Hoehn and Yahr, and UMSARS-II) and the degree of neurogenic OH were similar. By contrast, the findings showed higher values on the Non-Motor Symptoms Scale and UMSARS-I scale, consistent with the greater overall disability in patients with MSA-P.

Cardiac sympathetic innervation can be included in the diagnostic tests used in the differential diagnosis of these 2 conditions to distinguish patients with MSA from patients with PD+OH because of the preserved cardiac sympathetic innervation in patients with MSA.[8,9] However, a minority of patients with MSA had cardiac sympathetic denervation,[11] similar to patients with PD +OH, with the neuroimaging and postmortem neuropathologic analysis. These findings have been correlated with the deposition of Lewy bodies or neurites in sympathetic ganglia or nerves associated with glial cytoplasmic inclusions of α-syn.[11] Conversely, cardiac MIBG could be relatively preserved in the early stages or in patients with a short duration of PD.[31] This is the likely reason for the absence of a significant difference in MIBG accumulation between patients with PD and patients with MSA-P.[32] In addition, the presence of predisposing causes for peripheral neuropathy makes the study of cardiac innervation unreliable as the result of a possible underlying autonomic neuropathy.[33,34] An olfactory test showed good specificity for discriminating patients with MSA-P from patients with PD, but the sensitivity was rather low.[32] Several fluid proteins have been tested in both cerebrospinal fluid and serum to differentiate patients with MSA from patients with PD, but no single reliable biomarker with accurate sensitivity and specificity has been identified.[35] Patterns of decreased glucose metabolism disclosed by FDG-PET have been shown to distinguish MSA-P from PD with a high positive predictive value,[36] but more data are needed to confirm these findings.

Skin biopsy is a low-cost and an easy-to-perform technique that provides promising data for the in vivo diagnosis of synucleinopathies, although the analysis of the skin sample is a technically challenging process and cannot be done in a routine laboratory setting.[12-17,21,37] A recent meta-analysis demonstrated that this technique presented the best diagnostic accuracy in revealing in vivo abnormal α-syn deposits in patients with PD.[38]

Our study provides additional important data on the possibility of differentiating the clinical variants of synucleinopathies that present with parkinsonism and OH, such as MSA-P and PD+OH, through the use of skin biopsy. These disorders last for a long period of time, with a similar clinical picture, and they are difficult to identify.[1,3] Accordingly, identifying biomarkers that

can help to differentiate these 2 conditions may facilitate their clinical management, allowing for more focused treatment and preparing patients and caregivers to deal with the more severe disability levels of MSA. Thus, the presence of a useful biomarker may prevent pitfalls in selecting patients for clinical trials and represent an important instrument for testing a disease-modifying treatment when it becomes available.

Our data demonstrate that skin biopsy, by searching for p-syn in cutaneous nerves, can be used to make a clear-cut differentiation between patients with MSA-P and PD+OH. In fact, the patients with MSA-P had p-syn deposits in the somatic nerves of the subepidermal plexi of the superficial dermis of all patients positive for p-syn; on the other hand, all patients with PD+OH had abnormal p-syn deposits in the autonomic nerves. These findings have been reported in smaller studies,[17,18,21] but in this study we demonstrated the possibility of differentiating patients with MSA-P from patients with PD+OH by searching for p-syn in the cutaneous nerves in a large cohort of patients with similar clinical involvement. The reason behind p-syn deposits in different skin fibers is unknown and requires further investigation; however, it could be related to different strains affecting these disorders.[39] p-syn deposits were not found in 28% of MSA-P patients, which is similar to the findings of a previous article.[18] Based on our data, it is difficult to understand the reason for the p-syn negativity in these patients who showed no differences in disease duration, clinical scales, degree of autonomic failure, or cutaneous denervation compared with patients with p-syn positivity. It should be underlined that the correlation between p-syn deposits and disease duration needs further studies that analyze patients with longer disease duration than this study, which involved a circumscribed disease duration in both patient cohorts. However, the presence of epidermal denervation found in the p-syn-negative patients suggests the possibility that abnormal deposits are present in these patients but were not found because of their patchy distribution, as we found when considering the second skin sample and also as suggested previously.[18] A focused study analyzing more biopsy samples or slides, which are crucial to increase the p-syn positivity,[37] is needed to clarify this point.

However, the p-syn positivity in all patients with PD +OH supports the possibility of reliably differentiating this disorder from p-syn-negative patients with MSA-P. The highly prevalent damage of somatic fibers in patients with MSA-P implies the need to evaluate nociceptor function in these patients to look for neuropathic pain not previously reported in MSA.

Our findings, particularly the patient with MSA with cardiac denervation, may support the finding of abnormal p-syn deposits in autonomic neurons in a subgroup of patients with MSA, which may explain abnormal cardiac innervation shown by MIBG, as demonstrated in an autopsy-based study.[11] In fact, p-syn deposits were typically found in this patient in somatic nerve fibers but to a lesser extent also in adrenergic autonomic fibers, justifying the underlying autonomic neuropathy and MIBG abnormalities. If confirmed in a large cohort of patients, this could be an important demonstration of the higher sensitivity and specificity of skin biopsy compared with cardiac MIBG as an MSA biomarker.

The analysis of skin innervation showed interesting findings. Skin denervation found in the 2 groups of disorders mirrored p-syn deposits, since MSA showed a marked decrease in epidermal (ie, somatic) nerve fibers, where p-syn was mainly found. This conclusion was also supported by the inverse linear correlation between the p-syn deposits and EFNs we found in patients with MSA-P. By contrast, in the patients with PD+OH, we found involvement of autonomic fibers damaged by p-syn deposits associated with the epidermal denervation that could be attributed to secondary tissue change because of abnormal autonomic innervation producing abnormal blood flow, shunting with hypoperfusion in nutritive vessels, hypoxia, and acidosis, or alternatively it may be the result of more proximal p-syn deposits (ie, ganglia and dorsal root or spinal cord horn).[40]

In addition, preserved autonomic innervation in the majority of patients with MSA supported preganglionic autonomic involvement in this disorder,[6-8] although a minority of patients may have an extension of misfolded α-syn deposition in autonomic neurons, possibly explaining the inverse linear correlation between p-syn deposits and autonomic innervation. By contrast, our data supported postganglionic damage underlying autonomic dysfunction in PD, as previously reported.[8] However, the characteristic pattern of innervation with preserved autonomic innervation in MSA may also help to differentiate MSA from PD.

The main limitation of our study is the lack of autopsy-confirmed diagnosis of patients with MSA-P and PD+OH, which are disorders with a similar clinical picture. Nevertheless, we selected only patients who fulfilled the validated clinical criteria, and the clinical diagnosis was supported by specific diagnostic tests. However, the clear-cut differences we found in p-syn deposits between the 2 disorders demonstrate that the selection was made correctly.

In addition, the question as to whether the findings we show in this work in well-characterized patients could potentially be helpful for differentiating the conditions earlier in the course of the disease, when clinical differences between MSA and PD can be quite challenging, is still open and requires additional future studies.

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