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AUTONOMIC INNERVATION IN MULTIPLE SYSTEM ATROPHY AND PURE AUTONOMIC FAILURE

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Abstract

Background. Pure autonomic failure (PAF) and multiple system atrophy (MSA) are both characterized by chronic dysautonomia although presenting different disability and prognosis. Skin autonomic function evaluation by indirect tests has disclosed conflicting results in these disorders. Here we report the first direct analysis of skin sympathetic fibers including structure and function in PAF and MSA to ascertain different underlying autonomic lesion sites which may help differentiate the two conditions.

Methods. We studied 8 patients with probable MSA (mean age 60±5 years) and 9 patients fulfilling diagnostic criteria for PAF (64±8 years). They underwent head-up tilt test (HUTT), extensive microneurographic search for muscle and skin sympathetic nerve activities from peroneal nerve and punch skin biopsies from finger, thigh and leg to evaluate cholinergic and adrenergic autonomic dermal annexes innervation graded by a semiquantitative score presenting a high level of reliability.

Results. MSA and PAF patients presented a comparable neurogenic orthostatic hypotension during HUTT and high failure rate of microneurographic trials to record sympathetic nerve activity, suggesting a similar extent of chronic dysautonomia. In contrast, they presented different skin autonomic innervation in the immunofluorescence analysis. MSA patients showed a generally preserved skin autonomic innervation with a significantly higher score than PAF patients showing a marked postganglionic sympathetic denervation. In MSA patients with long disease duration morphological abnormalities and/or a slightly decreased autonomic score could be found in the leg reflecting a mild postganglionic involvement.

Conclusion. Autonomic innervation study of skin annexes is a reliable method which may help differentiate MSA from PAF.
Introduction

Pure Autonomic Failure (PAF) and multiple system atrophy (MSA) are characterized by severe orthostatic hypotension with a different clinical course. The mean survival time after diagnosis of MSA is 9 years, whereas the autonomic dysfunction in PAF shows little progression over time and the disease may last for decades. Due to an overlap of several autonomic failure symptoms the differential diagnosis of these two diseases is not straightforward.

Skin autonomic function evaluation by indirect tests has disclosed conflicting results. Sudomotor tests failed to differentiate the autonomic site dysfunction in MSA and PAF while skin vasomotor reflex (SVR) was recently reported to be normal in MSA but not in PAF.

Tests directly disclosing sympathetic fiber morphology (skin biopsy) and function (microneurography) are lacking in these two conditions. Recently we reported that skin biopsy in association with microneurography proved reliable diagnostic tools in detecting the sudomotor lesion site in patients with anhidrosis.

Here we extend this approach analyzing the autonomic innervation in PAF and MSA patients to ascertain different underlying autonomic lesion sites which may help differentiate the two conditions.

Methods

Patients

Seventeen patients with chronic autonomic failure were examined, including 8 MSA (6 men and 2 women; mean age 60±5 years) and 9 PAF (7 men and 2 women; 64±8 years). Probable MSA was diagnosed according to the consensus statement of MSA diagnostic categories whereas PAF patients showed orthostatic hypotension and other autonomic dysfunctions without more widespread neurological involvement for more than 5 years fulfilling diagnostic criteria established by a consensus statement. The clinical profile of patients is reported in the Table.
Five MSA and eight PAF patients were taking medications for orthostatic hypotension (fludrocortisone, midrodrine or dihydroergotamine) and two MSA patients levodopa. Serologic screening for microbiologic, autoimmune (including antibodies against autonomic ganglia nicotinic acetylcholine receptor) and paraneoplastic disorders was negative. Motor and sensory nerve conduction studies (median, ulnar, sural and tibial nerves) were normal. Brain MRI was normal except in two MSA patients showing cerebellar atrophy (patient with predominant cerebellar signs: MSA-C) and brainstem atrophy with the cross sign (predominant Parkinsonian features patient: MSA-P).

Twenty age-matched subjects (16 males and 4 females; 61±10 years) without clinical signs of neurological dysfunction were prospectively enrolled and used as controls.

The experimental procedures were approved by the Human Ethics Committee of Bologna and Göteborg University, and all subjects gave written informed consent to the study.

**Head-up tilt test (HUTT)**

Patients were studied in a temperature-controlled clinical investigation room (23 ± 1°C). Patients had to fast the night before the test except for small amount of water if they were thirsty. They had to abstain from drinking alcohol or coffee the day before the study.

Systolic and diastolic blood pressure (SBP, DBP; Portapres model 2, TNO-TPD Biomedical Instrumentation, Delft, the Netherlands), heart rate (HR; Grass 7P511; Astro-Med West Warwick, RI, USA), oronasal and abdominal breathing (Grass DC preamplifier 7P1) were monitored continuously.

After 30 min of supine rest, the HUTT (10 min at 65°) was performed using previously described procedures. At each minute of HUTT the changes in SBP, DBP and HR were calculated with respect to basal values. Pre-HUTT supine values (baseline) for SBP, DBP and HR were set at 0, and changes were expressed as Δ (raw data) from baseline. Orthostatic hypotension is defined by
consensus as a fall in blood pressure (BP) of at least 20 mmHg systolic and 10 mmHg diastolic within 3 min in the upright position.\textsuperscript{9}

\textit{Microneurographic recording}

Patients lay semi-reclining in an ambient temperature of 20-25°C and relative humidity of 20-30\%. A microneurographic search for multiunit efferent postganglionic sympathetic nerve activity was performed in the left peroneal nerve, posterior to the fibular head.\textsuperscript{11} Muscle sympathetic nerve activity (MSNA) was considered acceptable when it revealed spontaneous, pulse-synchronous bursts of neural activity that fulfilled the criteria previously described.\textsuperscript{11}

A burst of skin sympathetic nerve activity (SSNA) was considered if it: 1) showed irregular occurrence varying in strength and duration, unrelated to heart beats; 2) at rest was followed by changes in SVR (recorded by an infrared photoelectric transducer, model PPS, Grass Instruments: filter setting 0.2 – 100 Hz) and/or sympathetic skin response (SSR recorded by an Ag-AgCl surface electrodes: filter setting 0.2 – 100 Hz); 3) was evoked by various arousal stimuli, including surface electrical stimulation. A search for SSNA and MSNA bursts was done in the same recording session by exploring several nerve fascicles during a maximum of 70 minutes.

In case of absent spontaneous sympathetic bursts several manoeuvres were used to elicit sympathetic activity. Electrical stimulation of the right median nerve at wrist (maximum stimulus 99 mA and 1 s duration) or arithmetic mental stress (consisting in complex subtractions) were used to evoke SSNA activity and inspiratory/expiratory apneas associated with a clear BP decrease were used to evoke MSNA bursts. The absence of sympathetic nerve activity was established after exploring at least 5 different nerve fascicles. SSR and SVR were considered abnormal when no response was obtained with the strongest electrical stimulus used (99 mA and 1 s).

\textit{Skin biopsy}
To visualize somatic and autonomic skin nerve fibers, three mm punch biopsies were taken from glabrous skin, i.e. fingertip, and hairy skin, i.e. distal leg (10 cm above the lateral malleolus) and thigh (15 cm above the patella). According to previously published procedures\textsuperscript{12} skin samples were immediately fixed in cold Zamboni’s fixative and held at 4°C overnight. Sixty μm-thick sections were obtained using a freezing sliding microtome (2000R, Leica, Deerfield, IL, USA). Free floating sections were incubated overnight with a panel of primary antibodies, including the pan-neuronal marker protein gene product 9.5 (PGP 9.5, 1:800; Biogenesis, Poole, UK), collagen IV (mColIV, 1:800, Chemicon, Temecula, CA, USA), and autonomic markers like dopamine-beta-hydroxylase (DβH; 1:100, Chemicon, Temecula, CA, USA), to identify the noradrenergic fibers\textsuperscript{13} and vasoactive intestinal peptide (VIP, 1:1000; Incstar, Stillwater, MN, USA), co-localized in the sudomotor cholinergic fibers.\textsuperscript{14} Sections selected for VIP and DβH were pre-incubated in citrate buffer at 60°C to increase the specific staining. After an overnight incubation, sections were washed and secondary antibodies, labeled with cyanine dye fluorophores 2 and 3.18 (Jackson ImmunoResearch, West Grove, PA, USA), were added. A biotinylated endothelium binding lectin, ULEX europæus, (Vector laboratories Burlingame, CA, USA) was added along with primary antibodies to show the endothelium, sweat gland tubules and hair follicles. This staining was visualized by cyanine dye fluorophore 5.18 coupled with streptavidin (Jackson ImmunoResearch, West Grove, PA, USA). From double or triple stained sections, digital images were acquired and studied using a laser-scanning confocal microscope (Leika DMIRE 2, TCS SL, Leika Microsystems, Heidelberg, GmbH). Each image was collected in successive frames of 1-2 μm increments on a Z-stack plan at the appropriate wavelengths for cyanine 2, 3 and 5 fluorophores with a x40 plan apochromat objective and successively projected to obtain a 3D confocal image by a computerized system (LCS lite, Leica Microsystems, Heidelberg, GmbH). Epidermal nerve fiber density (ENFs: number of unmyelinated fibers per linear millimeter of epidermis) was calculated by considering single epidermal nerve fiber crossings of the dermal–epidermal junction.\textsuperscript{12}
As previously described, autonomic innervation of skin annexes were semiquantitatively graded by considering the whole recognizable target structure on a Z-stack plan, i.e. sweat gland (SG) for the cholinergic innervation in both glabrous and hairy skin; arteriovenous anastomoses (AVAs) for adrenergic innervation in glabrous skin; and muscle errector pilorum (MEP) for the adrenergic score in hairy skin. It included: 0= absent autonomic innervation; 1= severe fibers loss showing morphological abnormalities and/or destroyed pattern of innervation; 2= discrete loss of autonomic fibers showing no or sparse morphological changes with a recognizable but abnormal pattern of innervation; 3= slightly reduced autonomic fiber density without morphological abnormalities and preserved pattern of innervation; 4= a full nerve fiber density with preserved pattern of innervation (Figure 1A and 1B). An intermediate score (i.e. 2.5) was used when the innervation finding did not completely fit one established point. In each skin site the autonomic score represented the mean of three different target structures. The lowest score obtained in controls was considered the cut-off value between normal and abnormal findings. At each skin site and for both cholinergic and adrenergic innervation this value was 3 which was generally considered the cut-off value. The score analysis was made blinded to the clinical diagnosis of the patients.

As a measure of internal consistency and reliability, we evaluated intraobserver (V.Don.) and interobserver (V.Don. and E.B.) autonomic innervation variability by blinded comparison.

**Statistics**

All values are expressed as mean ± SD. Two-tailed Student’s t-test for unpaired data was used to compare a) mean BP and HR changes during HUTT in the two group of patients; b) the autonomic innervation score between group of subjects and between different skin sites and c) ENFs between groups.

Microneurographic failure difference between groups was checked by a Fisher exact test.

The correlation between autonomic score, disease duration and/or BP and HR changes during HUTT was assessed with Pearson linear regression analysis. Intraclass correlation coefficient (ICC)
performed with the SPSS statistical package (SPSS Interactive Graphics, Version 10.00, SPSS Inc, Chicago, IL) was used to assess intraobserver and interobserver variability with values >0.8 being considered as excellent reproducibility; p<0.05 was considered significant.

**Results**

The mean disease duration was significantly shorter in MSA (4±2 years) than PAF (8±4 years; p<0.05).

**HUTT**

Supine SBP, DBP and HR did not differ between MSA (140±8, 80±5 mmHg and 73±6 beats/min respectively) and PAF (130±13, 78±13 mmHg and 65±6 beats/min; p>0.1) patients.

During HUTT a neurogenic orthostatic hypotension with pronounced BP fall and absent or small HR increase was found in all patients. After 10 min of HUTT the mean SBP, DBP and HR changes did not differ between MSA (-74±14, -40±14 mmHg and 5±2 beats/min) and PAF (-65±10, -40±7 mmHg and 3±2 beats/min; p>0.2).

**Microneurographic recording**

*Controls*. Sympathetic activity (MSNA and/or SSNA) was recorded in all cases except one subject (5%). MSNA showed a normal cardiac rhythmicity with a mean activity of 58±15 bursts/100 HB and 37±10 bursts/min. The mean activity of SSNA was 11±5 bursts/min.

*Patients*. Sympathetic activity was often absent during microneurography despite an extensive search procedure. Microneurography failed to record sympathetic bursts in 6 MSA (75%) and 8 PAF (89%) patients. SSNA bursts with normal characteristics and within the normal range of incidence were obtained in 2 patients (one MSA-C and one PAF), and 1 MSA-C showed MSNA with normal cardiac rhythmicity and incidence. The disease duration was relatively short in these patients (Table).
The microneurographic failure to record sympathetic activity was comparable between MSA and PAF and significantly higher in both groups of patients compared to controls (p<0.01).

**Skin biopsy**

The ICC for interobserver and intraobserver reproducibility of autonomic score analysis was 0.86 and 0.96 respectively (p<0.0001), indicating a high level of reliability.

**Controls.** PGP immunoreactive (PGP-ir) fibers were abundant around dermal annexes in both glabrous and hairy skin (Figs. 2A<sup>l</sup>, 3A<sup>l</sup> and 4A<sup>l</sup>) and the majority of these fibers were DβH-ir or VIP-ir. The adrenergic DβH-ir fibers were prevalent around AVAs (Fig. 2A) and in the MEP (Fig. 3A), whereas the cholinergic VIP-ir fibers were mainly localized around SG (Fig. 4A). A proximal-distal gradient with higher score in the thigh compared to the leg was found in the lower limb cholinergic (p< 0.05) and adrenergic innervation (p=0.06) (table).

**Patients.** Autonomic skin innervation clearly differed in the two group of patients with chronic dysautonomia.

MSA showed a preserved PGP-ir innervation of MEP, AVAs and SG (Figs. 2B<sup>l</sup>, 3B<sup>l</sup> and 4B<sup>l</sup>). Adrenergic DβH-ir innervation was expressed around AVAs (Figs. 2B and 2B<sup>l</sup>) and in the MEP (Figs. 3B and 3B<sup>l</sup>). The mean adrenergic score did not differ from controls in any skin site (p> 0.1) although it was below the cut-off value in the leg in one patient with fairly long duration (patient 16 of table). Similarly, cholinergic VIP-ir fibers were represented around sweat glands (Figs. 4B and 4B<sup>l</sup>). Cholinergic innervation did not show a significant difference from controls in finger and thigh (p> 0.1) although the leg score was at the significance level (p= 0.05) (Table). Further, the individual analysis disclosed a slightly reduced leg score in 3 patients (table). Additionally, patients with longer disease duration often showed in the distal site (i.e. leg) morphological abnormalities of both adrenergic (arrows and arrowheads in Figs. 3B and 3B<sup>l</sup>) and cholinergic (arrows and arrowheads in Figs. 4B and 4B<sup>l</sup>) fibers. Epidermal innervation was significantly reduced compared to controls in any skin site (5 ± 2, 11 ± 2, 10 ± 2 ENFs/mm for finger, thigh and leg respectively; p<
without appreciable differences between patients with normal (9 ± 3 ENFs/mm) and abnormal (11 ± 2 ENFs/mm) leg autonomic scores. No correlation was found between MSA disease duration and either autonomic score or ENFs.

By contrast, PAF patients presented a poor and deranged PGP-ir innervation of dermal annexes (Figs. 2C1, 3C1 and 4C1). A marked loss of adrenergic fibers was observed around AVAs (Figs. 2C and 2C1) and in MEP (Figs. 3C and 3C1). The mean adrenergic score was significantly reduced compared to MSA patients and controls in any skin sites (p< 0.001). Cholinergic VIP-ir nerve fibers around sweat glands were also significantly reduced compared to MSA and controls (p< 0.001) (Table and Figs. 4C and 4C1). Decreased cholinergic and adrenergic innervation in the leg were both significantly correlated to the disease duration (r= 0.8; p<0.05) but not to BP and HR changes during HUTT. To exclude the effect of disease duration, we compared patients with similar disease duration (PAF: patients 1, 3, 4, 5, 8, 9 of table; MSA: patients 11, 12, 13, 15, 16, 17). A significant difference between MSA and PAF was still disclosed for both adrenergic (p< 0.05) and cholinergic (p< 0.05) innervation score. Epidermal innervation was similar to the MSA group (p>0.2) but lower than controls in any skin site (4 ± 1, 10 ± 4, 7 ± 2 ENFs/mm for finger, thigh and leg respectively; p< 0.001) with no correlation with the disease duration.

**Discussion**

The main finding of our study is that MSA and PAF, both presenting a similar degree of chronic dysautonomia as suggested by HUTT and microneurography, show different skin autonomic innervation findings at immunofluorescence analysis which may help differentiate these two disorders characterized by different disability and prognosis. We report the first direct analysis of sympathetic fibers including structure and function suggesting a preganglionic dysfunction underlying dysautonomia in MSA and a postganglionic denervation in PAF patients.

Our data confirm that chronic dysautonomia characterizes MSA and PAF to a similar extent. During HUTT pronounced BP loss with small HR changes was disclosed in all patients. The degree
of these changes did not differ between MSA and PAF patients. In addition, extensive microneurographic search procedures usually failed to identify sympathetic bursts with established characteristics. The failure was similar in both disorders but significantly greater in patients than in controls, indicating that sympathetic activity was weak or absent in most PAF and MSA patients.\textsuperscript{16,17} Recordable sympathetic activity in patients with shorter disease duration suggested a progressive loss of peripheral sympathetic function positively correlated with the disease duration. However, the new finding of our study is the morphological analysis of skin innervation by immunofluorescence. Epidermal innervation was decreased in both MSA and PAF patients. Nevertheless a small fiber neuropathy (SFN) seems unlikely because ENFs was reduced even in MSA patient showing a preserved postganglionic sympathetic innervation and because patients did not complain of burning paresthesia, a key symptom of SFN. This finding could be due to secondary damage of epidermal nerve fibers induced by a tissue change caused by dysautonomia, i.e. abnormal blood flow shunting with hypoperfusion in nutritive vessels, hypoxia and acidosis.\textsuperscript{18} In agreement with cardiovascular\textsuperscript{19,20} and pharmacologic tests\textsuperscript{21,22}, the immunofluorescence analysis disclosed a different skin autonomic innervation in MSA and PAF. MSA patients had a preserved cholinergic and adrenergic autonomic innervation of dermal annexes significantly greater than PAF patients although microneurography failed to disclose any sympathetic bursts in most of these patients. These data suggested a preganglionic dysfunction underlying the chronic dysautonomia in MSA and a functional inactivity of postganglionic autonomic fibers. The MSNA or SSNA bursts with normal characteristics in two patients may reflect a residual preganglionic sympathetic activity. It should be noted that MSA patients with long disease duration showed a slight decrease of the autonomic score (mainly cholinergic) in the leg and/or morphological abnormalities of autonomic fibers that could be considered predegenerative aspects similar to those seen in epidermal nerve fibers.\textsuperscript{23} This may reflect the early postganglionic involvement in MSA, positively correlated with the duration of the disease, reported in a study of cardiac sympathetic innervation.
and attributed to a transsynaptic mechanism or to a concurrent deposition of α-synuclein inclusions in the sympathetic ganglia.24

By contrast, PAF showed a marked loss of cholinergic and adrenergic autonomic skin innervation prevalently in the distal site (i.e. leg) outlining a length-dependent sympathetic postganglionic involvement responsible for the SSNA absence during microneurographic search. SSNA was recordable in one PAF patient although he showed an abnormal sympathetic innervation score of dermal annexes (patient 5 in the table). This apparently contrasting finding could be explained by the chance to record a sympathetic nerve discharge during microneurographic search from the few preserved peripheral sympathetic fibers still functionally active. The autonomic score of PAF patients was correlated with the disease duration and they presented a significantly longer disease duration than MSA. This may suggest that a shorter disease duration was responsible for preserved skin autonomic innervation in MSA. A direct comparison of patients with similar disease duration disclosed a still significant higher autonomic score in MSA than PAF making this hypothesis unlikely.

Our data differ slightly from previous studies of skin autonomic function in PAF and MSA and this may depend on the different methods used. Skin autonomic activity was previously analyzed by autonomic function tests (mainly pharmacological) based on the activation of skin sympathetic effectors (mainly sweat glands).2,4 However, the diagnostic utility of the pharmacologic sweat tests in disclosing a preganglionic dysfunction during the course of the illness may be time dependent and confined to the onset of symptoms25 suggesting that decentralized preganglionic neurons such as in MSA may lose early fiber excitability although their structure may still be preserved. Immunofluorescence analysis adds further information on the peripheral autonomic innervation providing a direct detailed visualization of sympathetic postganglionic skin neurons structure, thereby overcoming the limitation of functional tests and helping to clarify uncertain data,2,4 although a direct comparison between functional and structural tests of postganglionic nerve fibers is needed to confirm this conclusion. Of specific interest will be a comparison between Quantitative
Sudomotor Axon Reflex Test (QSART), a reliable and objective test of postganglionic cholinergic functional activity\textsuperscript{26} and morphological skin innervation analysis by immunofluorescence. The main finding of our study is supported by morphological data showing preserved unmyelinated fibers in the sural nerve of MSA patients\textsuperscript{16,27} and a clear reduction in PAF\textsuperscript{16,28} and by a recent report of preserved dermal innervation in MSA.\textsuperscript{29}

Accordingly, a degeneration of sympathetic neurons of ventrolateral medulla\textsuperscript{30} and preganglionic sympathetic neurons in the intermediolateral cell column of the spinal cord\textsuperscript{31} have been recognized as the main substrate of sympathetic failure in MSA, whereas the main autonomic lesions in PAF are considered the sympathetic and parasympathetic postganglionic neurons with Lewy bodies and α-synuclein inclusions primarily affecting the autonomic ganglia.\textsuperscript{31,32}

The limitation of this study concerns the immunofluorescence analysis we used which did not express a quantitative measure of sympathetic innervation although it showed a high reproducibility rate suggesting a reliable method. A quantitative method valuable for clinical purposes to study skin sympathetic innervation by immunofluorescence is needed and future efforts should focus on this aim.

**Acknowledgements**

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References


Legend for Table and Figures

**Table Clinical and autonomic findings in patients**

Values expressed as mean ± SD. OH= orthostatic hypotension; UI= urinary incontinence; GD= genital dysfunction; ID= intestinal dysfunction; SD= sweat dysfunction; a= absent; ND= not done.

**Fig. 1 Cholinergic and adrenergic skin autonomic score.**

Confocal images of cholinergic (A) and adrenergic (B) innervation selectively marked by VIP and DβH respectively. Autonomic fibers were graded by a semiquantitative score assigned by observers considering both the amount and type of the innervation pattern (see text for details). Examples of the five different degrees of innervation considered are shown in the figure with the corresponding scores reported above the SG (A) and the MEP (B) (in green VIP and DβH-ir fibers; in blue Collagen staining). The analysis was highly reliable in terms of intra and inter-subject variability. A similar analysis including five different scores was made for the DβH adrenergic innervation of AVAs in glabrous skin.

**Fig. 2 Adrenergic innervation around arteriovenous anastomoses**

Confocal images of finger AVAs innervation in an age-matched subject (A, A₁), in patient 17 of table with MSA of long duration (B, B₁) and patient 8 with PAF (C, C₁) and a similar disease duration. AVAs in control subject are heavily innervated by PGP-ir fibers (A₁) and the majority of these fibers are adrenergic DβH-ir (A). The adrenergic fibers showed a typical encircling pattern of innervation constituting a very dense tangle around the AVAs canal (A, A₁). Adrenergic DβH-ir innervation was well expressed in the MSA patient (B, B₁) showing a typical pattern of innervation. By contrast, the PAF patient showed a marked loss of PGP-ir and DβH-ir fibers (C, C₁).
**Fig. 3 Adrenergic innervation in the errector pilorum muscle**

Leg adrenergic innervation of MEP disclosed by confocal microscopy in an age-matched subject (A, A¹), in patient 16 with MSA of long duration (B, B¹) and patient 3 PAF with the same disease duration (C, C¹). The control subject showed PGP-ir and DβH-ir fibers running with a longitudinal wavy pattern in the muscle (A, A¹).

MEP adrenergic innervation was preserved in the MSA patient (B, B¹) showing a recognizable pattern of innervation although adrenergic fibers often presented morphological abnormalities (i.e. linear aspect losing the typical wavy pattern, excessive fragmentation and swelling as indicated by arrowheads or tangled and interconnected fibers indicated by arrows) which could be considered predegenerative aspects. PGP-ir fibers and adrenergic innervation (C, C¹) were clearly decreased in the PAF patient with a poor and deranged innervation with fibers showing fragmentation and/or swelling.

**Fig. 4 Cholinergic innervation around sweat glands**

SG innervation from the leg visualized by confocal microscopy in an age-matched subject (A, A¹), in patient 17 with MSA (B, B¹) and patient 2 with PAF (C, C¹) both presenting long disease duration. In control subject SG showed a dense PGP-ir (A¹) innervation with fibers mainly encircling the sweat tubules and most were VIP-ir (A). Cholinergic fibers were represented around sweat glands showing a typical innervation in the MSA patient (B, B¹) although morphological abnormalities of nerve fibers were evident, i.e. swelling indicated by arrows and/or fragmentation indicated by arrowheads, and probably expressing a predegenerative state. No PGP-ir and VIP-ir fibers were detected around sweat tubules in the PAF patient (C, C¹).
Table: Clinical and autonomic findings in patients

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