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Phosphorylated α-synuclein deposits in sural nerve deriving from Schwann cells: A biomarker for Parkinson's disease

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ABSTRACT

Introduction: Paresthesia is common in Parkinson's disease (PD) patients. We assumed that peripheral nerve might be implicated. This study aimed to investigate whether phosphorylated α-synuclein (pSNCA) pathology occurred in sural nerve fibers and to explore the underlying pathogenesis of paresthesia of lower limbs associated with PD.

Methods: Clinical assessments and sural nerve biopsy were performed to evaluate clinical characteristics and the deposition of total α-synuclein (tSNCA) and pSNCA in biopsy pieces using immunochemistry methods on 16 PD patients and 15 controls. In addition, immunofluorescence staining was performed using certain antibodies to characterize the component of sural nerve and to localize the expression of pSNCA.

Results: Deposition of pSNCA was found in 16/16 PD patients with a high positive percentage of 100% but in 0/15 controls, however, all biopsy pieces showed positive response to tSNCA immunohistological staining in nerve fibers. pSNCA was expressed mainly in Schwann cells but scarcely in axons, demonstrating a novel pattern of pSNCA expression in peripheral nervous system.

Conclusion: Our findings suggest that peripheral somatic sensory nerve is also involved in SNCA pathology in PD. The search for pSNCA in sural nerve might serve as a novel biomarker for early diagnosis of PD and pSNCA in sural nerve may derive from Schwann cells rather than propagate retrograde along the primary sensory neurons from the central nervous system.

1. Introduction

The hallmark of brain pathology in PD is intraneuronal Lewy bodies (LBs) and Lewy neurites (LNs) consisting of aggregated α-synuclein (SNCA) in midbrain nigral neurons and striatum. Nowadays there are still certain difficulties diagnosing PD, especially for those in the early or prodromal stage, because of hard work of brain biopsy [1]. Although some recent studies indicated that some kinds of biopsies such as colon [2], skin [3], submandibular gland [4] etc. could be used as useful biomarkers for diagnosis in living PD patients, their results were very conflicting [5–7] arising from the diversity of research methods including different biopsy specimens, different locations, protocols details and the difference of evaluation criteria [8].

30–85% of PD patients suffer from sensory disturbances including olfactory loss, visual changes, burning sensation, pain and other peripheral paresthesia [9]. Peripheral neuropathy has been found to occur with high frequency in PD patients [10]. Limb paresthesia may precede the onset of motor symptoms by up to 10 years [11], especially in the lower limbs. Previous preliminary investigations strongly suggested that sensory disorders are specific non-motor symptom in PD patients which might result from pathological changes within somatosensory nerves [12]. However, to date, the pathological feature of primary sensory neurons has not been widely investigated and little is known about whether peripheral nerve biopsy could serve as a novel diagnostic biomarker for PD.

Sural nerve biopsy is a routine procedure assisting in the diagnosis of many peripheral neuropathies. Histologically, sural nerve is composed of the axons of primary sensory neurons and Schwann cells sheathing around them. Aggregated pSNCA, the major ingredient of Schwann cell cytoplasm inclusion (SCCI), was found in post-mortem

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multiple system atrophy (MSA) patients indicating that Schwann cells were also implicated in such α-synucleinopathies [13]. For these reasons, the aims of the current study were to investigate the clinical value of sural nerve biopsy in the early detection and differential diagnosis of PD and clarify the precise location of pathological SNCA deposition in sural nerve.

2. Material and methods

2.1. Patients

Sixteen patients with well-characterized PD were recruited from the Department of Neurology of the First Affiliated Hospital of Nanjing Medical University, China from July 2015 to October 2017. Each patient was treated with L-dopa alone or in combination with dopamine agonists and had a good response to symptom control for more than one year. All patients were diagnosed by two experienced neurologists according to the UK Parkinson's Disease Society Brain Bank criteria. Exclusion criteria included all kinds of definite secondary parkinsonism, atypical parkinsonism suggestive of multiple system atrophy, corticobasal degeneration, progressive supranuclear palsy and essential tremor. Those patients who lacked good response to levodopa treatment or had good response to levodopa but with significant cognitive dysfunction (mini-mental state exam (MMSE) score < 24) were also excluded; each patient with a history of diabetes, alcoholism, chronic inflammatory demyelinating polyradiculoneuropathy, hereditary peripheral neuropathy, polyneuritis, HIV, syphilis, cancer, poliomyelitis or with abnormal laboratory tests that are predisposing causes for peripheral neuropathy (e.g., fasting plasma glucose, glycosylated haemoglobin, fT3, fT4, TSH, serum vitamin B12 and folate, autoimmune antibodies) was also excluded. The initial clinical diagnosis was confirmed by a clinical follow-up in all cases (mean follow-up period: 12 ± 6.3 months).

2.2. Control subjects

Fifteen controls who had previously been identified as no related neurodegenerative disorders and volunteered to participate in scientific studies were selected. Detailed clinical information of control subjects was listed in Table 2. This study was approved by the ethics committee of the First Affiliated Hospital of Nanjing Medical University, and all participants gave their informed written consent before taking part in the experiment.

Table 2
The demographic and clinical characteristics of controls.

Case	M/F	Age, y	Disease duration	Diagnosis(additional remarks)
CON1	F	50	2 year	Somatization disorder
CON 2	M	61	2 years	Nerve root compression
CON 3	M	47	3 months	Peripheral neuropathy (ethylene oxide poisoning)
CON 4	M	54	1 year	Acute myelitis sequelae
CON 5	M	33	4 years	Kennedy disease (abnormal amplification of CAG gene: 59times)
CON 6	M	50	5 years	HMSN (repeating mutations of PMP 22 gene)
CON 7	M	58	12 years	HMSN (heterozygous missense mutations of MPZ gene)
CON 8	M	43	3 years	HSMN (repeating mutations of PMP 22 gene)
CON 9	M	62	21 days	AIDP
CON 10	F	49	1 year	PMA
CON 11	M	61	1year	ALS
CON 12	M	52	4 months	CIDP
CON 13	F	49	2 years	CIDP
CON 14	M	63	NA	Healthy control
CON 15	M	56	NA	Healthy control
Mean ± SD	NA	52.5 ± 8.1	NA	NA

Abbreviations: HMSN: Hereditary motor and sensory neuropathy; AIDP: Acute inflammatory demyelinating polyneuropathies; PMA: Progressive muscular atrophy; ALS: Amyotrophic lateral sclerosis; CIDP: Chronic inflammatory demyelinating polyradiculoneuropathy; CON: control; M = male; F = female; NA: not applicable.

2.3. Clinical evaluation

Disease severity of PD was evaluated using the Unified Parkinson's Disease Rating Scale (UPDRS) and the Hoehn and Yahr (H-Y) staging. The mini-mental state examination (MMSE) was applied to screen patients for global cognitive function. Levodopa equivalent daily dose (LEDD) was calculated according to established methods for patients with anti-parkinsonian treatments.

All PD patients underwent nerve conduction velocity (NCV) and sensory nerve action potentials (SNAP) amplitudes of bilateral sural nerve in our laboratory by conventional surface electrodes using six channel electromyogram (Keypoint, Dantec corporation, Danmark) before the sural nerve biopsy. According to the collected age-adjusted standardized reference ranges at our laboratory, the results of NCV and SNAP were classified as normal or abnormal.

Table 1
The demographic and clinical characteristics of PD patients.

Case	M/F	Age, y	Disease duration, y	UPDRS Total score	Hoehn and Yahr stage	Sensory symptoms	LEDD mg/d
PD1	F	54	9	50	3	+	1000
PD2	F	70	10	57	3	+	800
PD3	M	59	3	60	1.5	+	400
PD4	F	67	4	80	2.5	–	800
PD5	F	71	1.5	37	1	–	400
PD6	F	32	3	41	3	–	1025
PD7	F	60	10	48	2.5	+	375
PD8	M	65	1	41	1.5	–	500
PD9	M	72	1.5	48	1.5	–	300
PD10	F	60	3	49	2	+	600
PD11	M	73	3	25	2	–	600
PD12	F	59	7	83	3	+	650
PD13	F	62	6	42	2.5	–	450
PD14	F	68	6	80	2.5	–	875
PD15	M	71	5	34	2	–	475
PD16	M	50	2	43	2.5	–	225
Mean ± SD	NA	62.0 ± 10.5	4.7 ± 3.0	51.1 ± 17.0	2.3 ± 0.6	NA	592.2 ± 235.9

Abbreviations: PD = Parkinson disease; NA = not applicable; UPDRS = Unified Parkinson's Disease Rating Scale; LEDD = Levodopa equivalent daily dose; M = male; F = female; NA: not applicable.

2.4. Tissue biopsy and morphological observations

Sural nerve biopsy was operated meeting standardized procedures under local anaesthesia. Nerve specimens were processed for standard methodology as follows: fixed in 10% neutral formalin for 2 weeks, dehydrated and embedded in paraffin, 3 μm in thickness of serial sections cut on a freezing sliding microtome (Leica, Germany). To observe if there were morphological changes in sural nerve of PD patients, we performed toluidine blue staining on all samples. 3 μm sections were stained with 1% toluidine blue and then photographed with Leica APERIO CS2.

2.5. Immunohistochemistry

Sections were immunostained with a panel of primary antibodies (three serial sections per antibody), including mouse monoclonal antibody to neurofilament 200kD (NF) (MAB5256, Millipore, USA, 1:100) as the marker of axons, mouse monoclonal antibody to α-synuclein (tSNCA) (Syn204, Cell signaling, USA, 1:50), and mouse monoclonal antibody to pSNCA (phosphorylated at Ser 129, pSyn#64, WAKO, JAPAN, 1:1000). Phosphate buffered saline (PBS) instead of primary antibody was used as negative controls. All slides were processed by the same technician under the same laboratory conditions. The serial sections after staining were observed by light microscopy in the same settings (Olympus microscope, Japan). The analysis was made in blinded manner by two doctors with expertise in neuropathology (Pan Ji and Zhen Wang) who had no knowledge of the patients. The positive or negative judgment was reached in condition of full agreement by the two analyzers without any discordance or uncertain classification.

Referring to a previous study [14], in each section, tSNCA- and pSNCA-positive inclusions were semi-quantitatively counted by the same pathologists (Pan Ji and Zhen Wang) using a four-point scale A (0 = absent, 1 = light yellow, 2 = light brown, 3 = dark brown) to determine the staining intensity, a five-tier scale B (0 = none, 1 = 1–25%, 2 = 26–50%, 3 = 51–75%, 4 = 76–100%) as the positive range. The total score of each slice equals the product of scale A and B and take the average score of the two graders as the final result. Then, we divided PD patients into two groups according to whether they had sensory symptoms or not and compared the expression of tSNCA and pSNCA between the two groups.

2.6. Immunofluorescence

To detect if there is autonomic nerve fibers in sural nerve, sections were first incubated with the two following primary antibodies respectively at 4 °C overnight: recombinant rabbit polyclonal antibody to vasoactive intestinal polypeptide(VIP) (ab22736, ABCAM, UK, 1:1000) for cholinergic fibers and recombinant rabbit polyclonal antibody to dopamine beta hydroxylase(DβH) (ab96615, ABCAM, UK, 1:1000) for noradrenergic fibers. After washing with PBS, the sections were incubated with Alexa Fluor 488 conjugated secondary antibody (ab150077, Abcam, UK, 1:400) for 2 h at room temperature avoiding light. Then the sections were washed again in PBS and covered with 4′,6-diamidino-2-phenylindole(DAPI).

To characterize the location of pSNCA, double immunofluorescence was performed with the following combined primary antibodies: mouse monoclonal antibody to pSNCA(pSyn#64, WAKO, JAPAN, 1:4000) with recombinant rabbit monoclonal antibody to NF heavy polypeptide (ab207176, Abcam, UK, 1:1000), pSNCA with recombinant rabbit monoclonal antibody to glial fibrillary acidic protein(GFAP) (ab33922, Abcam, UK, 1:400). GFAP was used as the marker of Schwann cells. The sections were then incubated with Alexa Fluor 488 (ab150077, Abcam, UK, 1:400) and 594(ab150108, Abcam, UK, 1:1000) conjugated secondary antibodies. Slices were viewed under a fluorescent microscope (Zeiss, Germany) for co-localization images.

2.7. Statistical analysis

Statistical analyses were performed with SPSS 20.0 statistical analysis software (SPSS Inc. Chicago, IL, USA). All data were shown as the mean ± SD. Comparisons of clinical data between the groups of PD patients and control subjects were performed using Mann-Whitney *U* test. Spearman correlation analysis was used to assess the univariate correlations between semi-quantitative results and clinical parameters in PD patients. Fisher's Exact Test was used to compare the difference of the positive rate between two groups.

3. Results

3.1. The demographic and clinical characteristics

The demographic and clinical characteristics of PD patients and controls were shown in Tables 1 and 2 respectively. In the PD group, the range of age was from 32 to 73 years and the mean age was 62.0 ± 10.5 years; H-Y staging: 12 cases were early stage (1.5–2.5stage) and 4 cases advanced stage (3stage) of PD patients; disease duration: 7 patients ≥ 5 years and the others < 5 years. The mean scores of H-Y staging, UPDRS and disease duration of PD patients were 2.3 ± 0.6, 51.1 ± 17.0 and 4.7 ± 3.0 years, respectively. 13 patients showed a late-onset disorder (older than 50 years); 3 patients were an early-onset disorder (younger than 50 years), and one of them was an autosomal recessive disorder with a pathogenetic parkin mutation (involving repeat mutations of PARK2 gene exons10). There were 6 patients with PD complaining about all kinds of abnormal sensations such as numbness, tingling, pain, often affecting extremities or trunk. Among them, numbness was the most popular symptom and often appeared in lower limbs. One patient with PD showed a serious continuous hyperalgesia in double lower limbs and was accompanied with lower extremities weakness. In the control group, the range of age was from 33 to 63 years and the mean age was 52.5 ± 8.1 years. There was significant difference in the age ($p = 0.004$) and sex distinction ($p = 0.045$) between two groups.

Seven PD patients (four with paresthesia and three without) showed abnormal electrophysiological results with a mean decrease of 53.6% in NCV and 48.3% in SNAP amplitudes. PD patients with paresthesia showed lower mean numerical value of NCV and SNAP amplitudes than PD patients without paresthesia but the difference was not statistically significant($p = 0.469$ for NCV and $p = 0.404$ for SNAP amplitudes).

3.2. Sural nerve biopsy findings

Firstly we checked the results of toluidine blue staining. In this study, controls were composed of 10 different kinds of neuropathies at different degrees which did not meet the standard of comparative analysis, so we picked the CIDP samples which represented the worst neuropathy and the healthy control (HC) sample where there was no neuropathy. In HC, the nerve fibers were tightly arranged and the shape of Schwann cells and axons were clearly visible. In contrast, the Schwann cells and axons in CIDP were totally disorganized and fragmented and there was onion bulb formation which is commonly observed in CIDP nerve biopsy. It was hard to capture a complete nerve fiber which made counting the amount of nerve fibers inaccurate. We found that the status of sural nerve fibers in PD was interposed between CIDP and HC. Nerve fibers were distributed loosely accompanied with swollen and fragmentized Schwann cells and only partial visible axons (Fig. 1).

Initial microscopic examination of immunohistochemistry showed identifiable abundant nerve tissue and a little connective tissue in each biopsy from all subjects. Nerve-related protein NF200kD and tSNCA were equally expressed in sural nerve tracts, showing puncta or ring (cross section) or linear (longitudinal section) staining in sample images from all PD patients and control subjects. Surprisingly, pSNCA was

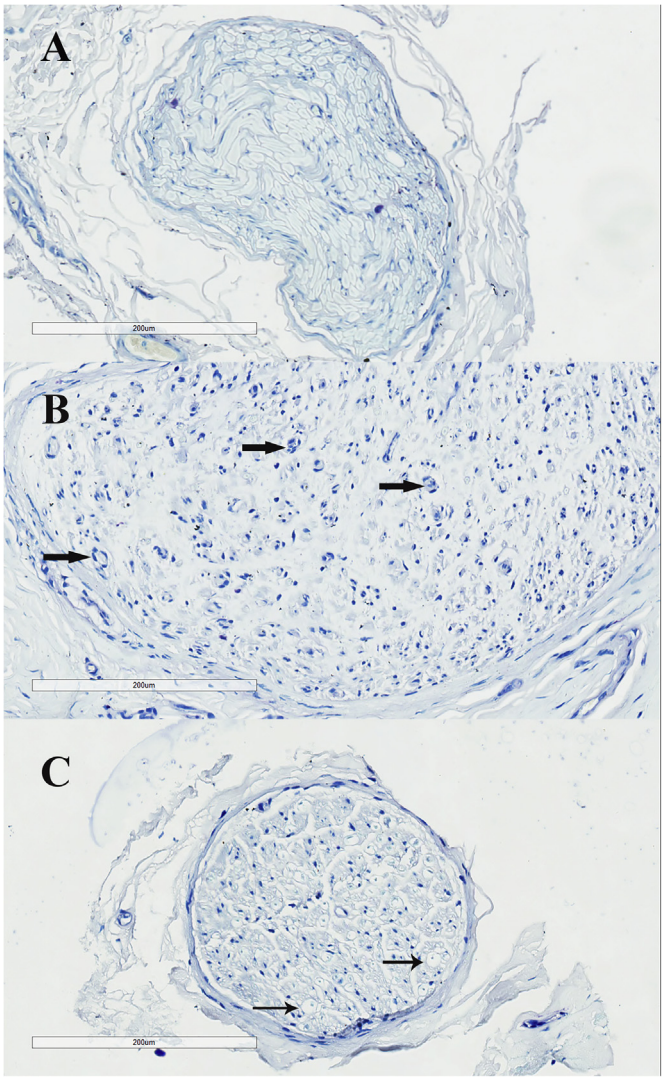

Fig. 1. Photomicrographs of toluidine blue stained sural nerve biopsy from HC (A), CIDP (B) and PD (C).

A. Nerve fibers in HC were tightly arranged and the shape of Schwann cells and axons were clearly visible. B. Schwann cells and axons were totally disorganized and fragmented. There was onion bulb formation (*thick arrows*) indicated by clustered Schwann cell nucleus, which is commonly found in CIDP nerve biopsy samples. C. The status of sural nerve fibers in PD was interposed between CIDP and HC. Nerve fibers were distributed loosely accompanied with swollen and fragmentized Schwann cells and only partial visible axons (*thin arrows*). Bar indicates 200 μm.

Abbreviations: HC: healthy control; CIDP: Chronic inflammatory demyelinating polyneuropathy; PD: Parkinson's disease. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

expressed in all PD patients but not in any control subject ($p = 0.000$). Positive staining to pSNCA presented not only in advanced stage PD patients (Fig. 2. PD12), but also in early stage ones (Fig. 2.PD5). Among PD patients, one was 32 years old and the age of onset was 29, with an autosomal recessive Parkin gene mutation, also showing positive pSNCA expression in sural nerve (Fig. 2.PD6).

There was no significant difference in the semi-quantitative results of tSNCA($p = 0.225$) and pSNCA($p = 0.869$) between PD patients with and without sensory symptoms. Besides, no correlations were obtained between the scores and clinical parameters such as UPDRS scores ($p = 0.686$), disease duration($p = 0.504$) or LEDD($p = 0.755$).

With immunofluorescent staining, no VIP or DβH staining were seen in all sections (Fig. 3), indicating that there were no autonomic fibers including cholinergic or noradrenergic fibers in sural nerve. To ascertain the pattern of pSNCA expression, co-localization of pSNCA/NF and pSNCA/GFAP were assessed from sections of one PD patient (PD7 in Table 1). In pSNCA/NF sections, pSNCA was mostly expressed as ring or half ring shape and NF was all granular or liner surrounded by pSNCA (Fig. 4a–c); and in pSNCA/GFAP sections, pSNCA was co-localized precisely with GFAP, both of which were ring or half ring shape (Fig. 4d–e). The results demonstrated that pSNCA was mainly expressed in Schwann cells but scarcely in axons.

4. Discussion

The main results of our study are that (1) the deposition of pSNCA in sural nerve was found in all PD patients but none in controls, rendering the examination for pSNCA in sural nerve a useful biomarker for PD diagnosis, especially for those patients in early stage; (2) the component of sural nerve is simply somatic sensory fibers without any autonomic fibers, so pSNCA in these fibers may contribute to some kinds of somatosensory symptoms; (3) pSNCA was expressed mainly in Schwann cell and very little was observed in the axons; the expression pattern of pSNCA suggested that the pathophysiological mechanism in PNS may be different from that in CNS.

In the present study, pSNCA immunohistochemistry staining in sural nerve was found in PD patients with a high percentage of 100.0%, but not in any control subjects, demonstrating extremely high sensitivity and specificity. Interestingly, abundant pSNCA expression was shown in those PD patients who didn't suffer from frequent lower limbs paresthesia, suggesting that pSNCA deposits in sural nerve might occur in the preclinical stage. More importantly, all of 12 early-stage PD patients in this study showed significant pSNCA expression, so it can be inferred that the detection of pSNCA in sural nerve may be a useful biomarker for PD diagnosis even in the early stage. Those PD patients with pathogenetic parkin mutation were usually deemed to have no α-synuclein deposits because the underlying pathogenesis is thought to be related to mitochondrial function and oxidative damage [15]. However, in our study, one PD patient with an autosomal recessive parkin mutation (involving repeat mutations of PARK2 gene exons10) also appeared pSNCA expression in sural nerve. The underlying pathogenesis is still poorly understood.

It is widely acknowledged that SNCA was expressed in multiple organs in human body [16] and vast studies have been searching for a suitable peripheral biomarker for PD, among which skin biopsy was currently deemed as the optimal one [17]. Both tSNCA and pSNCA were found in cutaneous nerve including autonomic and sensory fibers in PD patients, accompanying different levels of nerve fibers impairment [18,19]. Besides, skin biopsy could differentiate PD with other parkinsonisms [20] and pSNCA was detected in skin biopsy from rapid-eye-movement sleep behavior disorder (RBD) patients implying the value of skin biopsy in the diagnosis of prodromal PD [21]. However, because of the nonuniformity of tissue innervation, the different biopsy site often varies the positive rate. One multiple sites skin biopsy study found pSNCA in cutaneous autonomic fibers but the positive rate for pSNCA was associated with the site, ranging from 24% to 100% [20]. By contrast, sural nerve biopsy eliminates the influence due to a stable nerve tissue acquisition. Therefore, sural nerve biopsy to detect pSNCA expression can be served as a relatively optimal diagnostic method for PD diagnosis.

Paresthesia is one of the common NMS in patients with PD. In current study, six patients complained about all kinds of abnormal sensory symptoms which were often seen in the lower limbs, indicating that peripheral somatic sensory nerve were also involved in PD patients. However, we found only four PD patients with paresthesia displaying decreased SNAP amplitudes and NCV. Since Sumikura et al. found pSNCA in the spinal dorsal horn and the dorsal root ganglia (DRG) in patients with PD at autopsy [12]. We thought complaints of

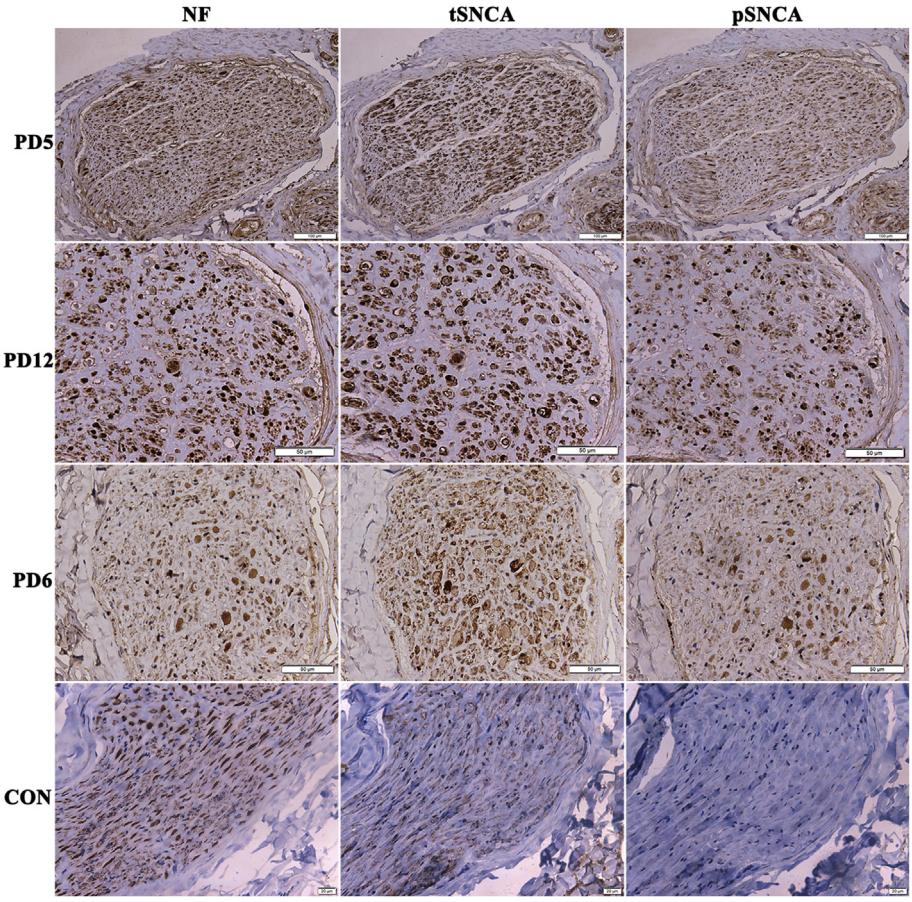

Fig. 2. Representative photomicrographs of paraffin-embedded immunohistochemistry sections of sural nerve from PD patients and controls.
NF and tSNCA were expressed in sural nerve from PD patients and control subject. pSNCA was only expressed in PD patients but not in control subjects. Bar was shown inside the pictures.
Abbreviations: PD: Parkinson disease; CON: control; tSNCA: total α-synuclein; pSNCA: phosphorylated α-synuclein; NF: neurofilament.

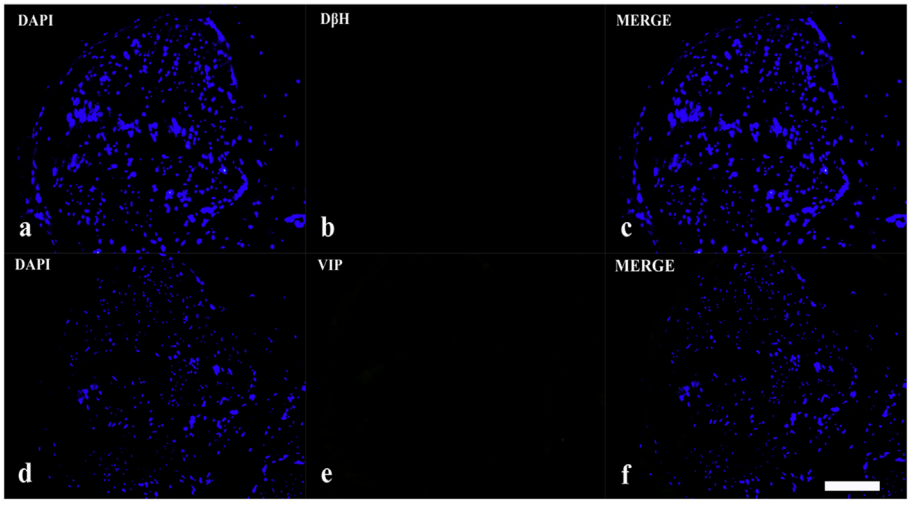

Fig. 3. VIP and DβH immunofluorescent staining of sural nerve in PD.
No positive staining of VIP or DβH was seen (b, e). DAPI labels the nuclei of Schwann cells (a, d). Bar indicates 50 μm.
Abbreviations: VIP: vasoactive intestinal polypeptide; DβH: dopamine beta hydroxylase; PD: Parkinson disease.

paresthesia from the other two patients with normal electrophysiological results may originated from pSNCA deposition in dorsal horn and DRG, which could not be detected by electrophysiological examination. What's more, another three PD patients without paresthesia showed abnormal electrophysiological results. In general, the subjective sensory abnormality in lower limbs of PD patients was not completely consistent with sural nerve electrophysiological examination. The pSNCA positive rate of sural nerve biopsy was significantly higher than that of electrophysiological examination. Previous investigations verified changes of unmyelinated nerve fibers and peripheral cutaneous denervation of sensory nerve in PD patients [11]. We found no autonomic fibers in sural nerve in this study and certificated that sural nerve belongs to pure primary sensory neurons. In addition, the results of morphological observation have demonstrated intuitive damage in sural nerve of PD patients. Hence, the abundant pSNCA deposited in sural nerve fibers might contribute to paresthesia in the lower limbs in PD patients because of their direct pathological harmful mechanism.

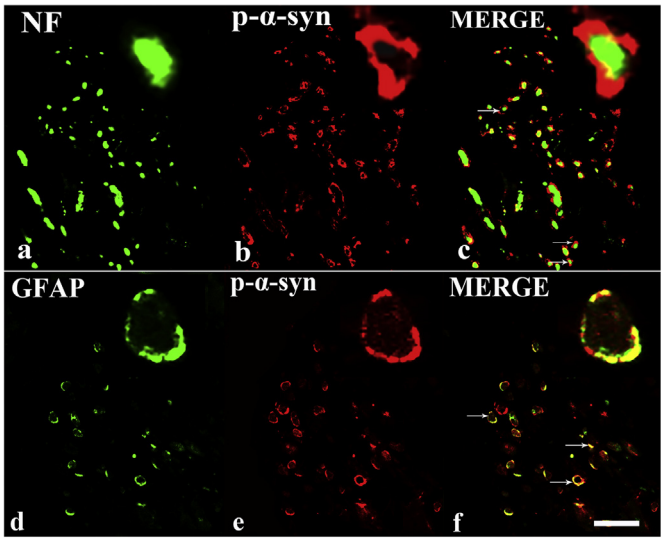

Fig. 4. pSNCA/NF and pSNCA/GFAP double immunofluorescent staining of sural nerve in PD. pSNCA (b, e) was expressed as ring or half ring shape. GFAP (d) was also ring or half ring shape and precisely co-localized with pSNCA (f). NF was granular or ribbon-like (a) surrounded by pSNCA (c). White arrows indicated the patterns of NF/pSNCA and GFAP/pSNCA expression which were magnified in the insets. Bar indicates 20 μm.
Abbreviations: pSNCA: phosphorylated α-synuclein; NF: neurofilament; GFAP: glial fibrillary acidic protein; PD: Parkinson disease.

The present study firstly verified the pathological pSNCA expression in somatic primary sensory nerve in PD patients by means of sural nerve biopsy. Because sural nerve fibers were composed of Schwann cells and axons, we performed immunofluorescence experiment to co-localize GFAP, NF and pSNCA in sural nerve and found pSNCA mainly in Schwann cells but scarcely in axons. In multiple system atrophy (MSA), another α-synucleinopathy, accumulation of pSNCA was detected in the cytoplasm of Schwann cells, mostly in the anterior nerve of the sacral cord, indicating that Schwann cells were also involved in the disease pathophysiological process [13].

There are some limitations in the present study. Firstly, a relatively small sample size was collected in this study; thus, it is necessary to enroll a larger sample to further testify the findings. Secondly, because of experimental conditions, we couldn't precisely evaluate neuropathologic damage of nerve tissue and it is better to use confocal microscopy to display immunofluorescence pictures. Thirdly, sural nerve biopsy is an invasive test producing permanent lesion to subjects, so this test could not be proposed as routine diagnostic tool for PD. Last but not least, due to the lack of other kinds of neurodegenerative disease as a control, the specificity of the findings for PD diagnosis is yet to be fully examined. We hope these limitations of the current study will be overcome in the future studies.

In summary, we present firstly in vivo pSNCA deposition in sural nerve fibres in PD patients, and pSNCA aggregated mostly in Schwann cells but scarcely in axons. Analysis of peripheral pSNCA pathology in sural nerve as a novel biomarker for PD diagnosis shows a high sensitivity and specificity. pSNCA deposits in sural nerve fibers may contribute to all kinds of somatosensory symptoms in the lower limbs in patients with PD.

Authors' contributions

Ke-Zhong Zhang: study concept or design, acquisition of clinical data, writing of the first draft, revising the manuscript for content, study supervision, obtaining fundings. Hui Zhang: acquisition of clinical data and sural nerve, revising the manuscript for content. Lin Zhu: conception of the study, acquisition of clinical data and sural nerve, revising the manuscript for content. Yan Zhi: acquisition of clinical data, revising the manuscript for content, statistical analysis. Jian Ding: acquisition of clinical data, revising the manuscript for content. Yong-Sheng Yuan: acquisition of clinical data and sural nerve. Fei-Fei Shen: acquisition of clinical data and sural nerve. Xiao Li: acquisition of neuropathology data. Pan Ji: acquisition of neuropathologic data. Zhen Wang: acquisition of neuropathologic data. Qi Niu: acquisition of clinical data, revising the manuscript for content. Li Sun: acquisition of clinical data, revising the manuscript for content. All authors approved the final version to be submitted.

Declarations of interest

None.

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