

# Skin alpha-synuclein deposit patterns: A predictor of Parkinson's disease subtypes



Yihang Han,<sup>1</sup> Di Wu,<sup>1</sup> Yanjuan Wang, Jian Xie, and Zhijun Zhang \*

Department of Neurology, Affiliated Zhongda Hospital, Research Institution of Neuropsychiatry, School of Medicine, Southeast University, 87 Dingjia Qiao, Nanjing, Jiangsu 210009, China

## Summary

Parkinson's disease (PD) is a neurodegenerative disease characterized pathologically by the formation of Lewy bodies comprised mainly of  $\alpha$ -synuclein. Assessment of skin synuclein has the potential as an excellent diagnostic method with high sensitivity, specificity, and reproducibility that is also convenient and acceptable to patients. In this review, we summarize findings regarding the characteristics of cutaneous nerve p- $\alpha$ -syn or  $\alpha$ -syn deposits and their correlations with clinical phenotypes in PD patients with and without orthostatic hypotension and *LRRK2*, *GBA*, and *SNCA* gene mutations. This review can serve as a reference for the diagnosis and classification of PD based on  $\alpha$ -syn deposit patterns and to deeply explore its pathogenesis.

eBioMedicine 2022;80:  
104076  
Published online xxx  
<https://doi.org/10.1016/j.ebiom.2022.104076>

**Funding statement** The work was partly supported by the National Natural Science Key Foundation of China (No. 81830040 and No 82130042) and the Program of Excellent Talents in Medical Science of Jiangsu Province (No. JCRCA2016006) .

**Copyright** © 2022 The Author(s). Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>)

**Keywords:** Parkinson's disease; Alpha-synuclein; Autonomic dysfunction; LRRK2; GBA; SNCA

## Introduction

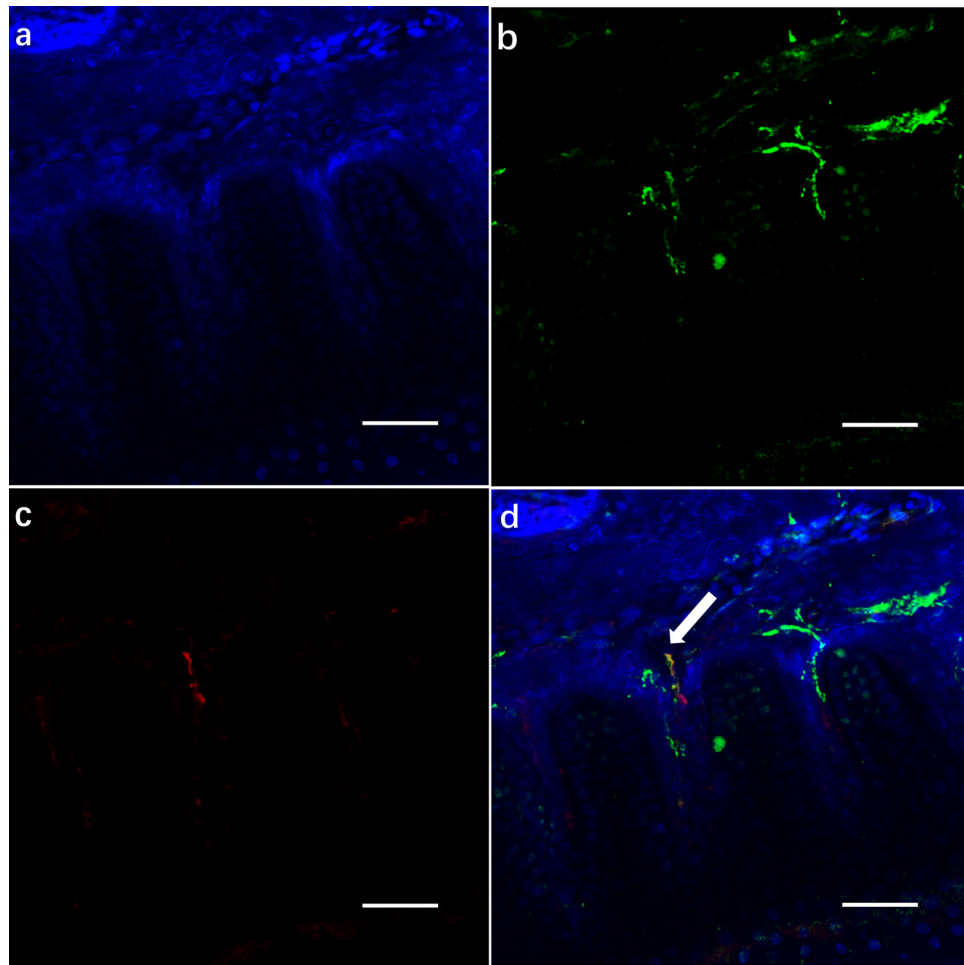
Parkinson's disease (PD) is a common neurodegenerative disorder associated with  $\alpha$ -synuclein ( $\alpha$ -syn) misfolding and aggregation, mitochondrial dysfunction and mitophagy, neuroinflammation, oxidative stress, and neuromelanin pigmentation.<sup>1–5</sup> The main neuropathological characteristics of PD are Lewy bodies (LBs) and progressive degeneration of dopaminergic neurons in the substantia nigra.<sup>3</sup> The principal component of LBs is  $\alpha$ -syn, 90% of which is phosphorylated at Ser129, i.e., phosphorylated alpha-synuclein (p- $\alpha$ -syn).<sup>6</sup> The misfolding and aggregation of  $\alpha$ -syn are the major pathogenic events in PD. The aggregation of  $\alpha$ -syn is a complicated process involving the formation of oligomers, protofibrils, and fibrils.<sup>1</sup> Toxic oligomers may be the primary cause of neurotoxicity but are not responsible for propagation, as shown by *in vitro* studies.<sup>7</sup> Fibrils are the elements that propagate in a prion-like manner.<sup>7</sup> Recently, the potential for two-way propagation between the enteric nervous system (ENS) and central nervous system (CNS) was demonstrated.<sup>8</sup> Pathological biopsies or autopsies of brain tissue from PD patients are not

technically difficult, but are obviously not possible in living patients. Accordingly, recent studies have focused on peripheral tissues. Some studies have found deposition of  $\alpha$ -syn and p- $\alpha$ -syn in the cerebrospinal fluid (CSF). However, a meta-analysis concluded there was no significant difference in total  $\alpha$ -syn between patients with PD and other synucleinopathies or atypical parkinsonism. The sensitivity and specificity for distinguishing total  $\alpha$ -synuclein in PD and controls were 0.72 and 0.65, respectively, and 0.71 and 0.64 for oligomeric  $\alpha$ -syn.<sup>9</sup> The detection of different pathology-related  $\alpha$ -syn species may be informative for diagnosis. There are reports of  $\alpha$ -syn and p- $\alpha$ -syn deposits in the skin of PD patients, and p- $\alpha$ -syn deposits were found in the hand skin nerves of PD patients in our study (unpublished, [Figure 1](#)). Furthermore, we compared known pathologies of the skin and CNS in PD ([Table 1](#)). The  $\alpha$ -syn staining patterns, p- $\alpha$ -syn deposition, and loss of neurons/nerve fibers in the skin are similar to the CNS pathology, and skin pathology may reflect CNS pathology to some degree. Notably, there are p- $\alpha$ -syn deposits in 82% of PD patients using freshly cut serial skin sections, and among patients positive for p- $\alpha$ -syn, 94% were also detected for truncated  $\alpha$ -syn (syn105), 90% and 69% positive for aggregated  $\alpha$ -syn as detected by 5G4 and syn211 after PK digestion, respectively, and 89% for oligomeric  $\alpha$ -syn (ASyO5).

\*Corresponding author.

E-mail address: [janemengzhang@vip.163.com](mailto:janemengzhang@vip.163.com) (Z. Zhang).

<sup>1</sup> These authors contributed equally to this work.



**Figure 1.**  $\alpha$ -syn deposits in the skin nerves of PD.

Confocal microscope ( $\times 400$ ) study of  $\alpha$ -syn deposits in the hand superficial dermal nerves which are stained with mouse protein gene product 9-5 (PGP, Bio-Rad Cat# MCA4750, RRID: AB\_2210503). a. Nucleus marked with DAPI (Abcam Cat# ab104139, RRID: no) (blue), it is superficial derma (\*); b. Skin nerves marked with PGP 9-5 (green); c.  $\alpha$ -syn deposits marked with rabbit monoclonal  $\alpha$ -syn (Abcam Cat# ab51253, RRID: AB\_869973) (red); d. Merged images, there are  $\alpha$ -syn deposits in the superficial dermal nerves (arrow). Scale bars: 50  $\mu$ m. These figures are taken by Di Wu and Yanjuan Wang, and all authors confirm originality of it and retain copyright to it.

Also, it is positive for the same antibodies in the substantia nigra of one PD patient, whereas they are all negative in normal control.<sup>10</sup>

It is usually difficult to distinguish PD from other synucleinopathies including MSA, dementia with Lewy bodies (DLB), and pure autonomic failure (PAF). The nerve subtype, close skin annexes, and distribution patterns of p- $\alpha$ -syn deposits may contribute to distinguishing these conditions. It has been reported that MSA shows p- $\alpha$ -syn mainly in subepidermal somatosensory (i.e., non synaptic) fibers.<sup>19,20</sup> This finding differs from IPD, DLB, and PAF, which showed p- $\alpha$ -syn deposits mainly in autonomic fibers. Autonomic fibers are differently affected in IPD, showing p- $\alpha$ -syn deposits mainly around skin vessels (SV) and PAF presenting with

widespread extension of deposits that also involve sweat glands (SG) and muscle arrector pilorum (MAP). Abnormal aggregates in autonomic annexes showed an intermediate degree of extension in DLB.<sup>20</sup> The different patterns of p- $\alpha$ -syn distribution may help distinguish these conditions as follows: in PAF, there is homogenous distribution of p- $\alpha$ -syn in proximal and distal sites, whereas there is higher positivity of p- $\alpha$ -syn in proximal sites, mainly C7, in IPD and DLB.<sup>20</sup>

This review article summarizes and further analyzes the characteristics of  $\alpha$ -syn and p- $\alpha$ -syn deposition in the skin nerves of different PD subtypes and their correlation with clinical symptoms and characteristics, thereby providing a reference for diagnosing PD by cutaneous synucleinopathy.

|                                | Skin pathology   | CNS pathology  |
|--------------------------------|--|--|
| $\alpha$ -syn staining pattern | Immunoreactive for p- $\alpha$ -syn, 5G4, syn211 after PK digestion, syn105, and AsyO5 antibodies <sup>10</sup>  | Immunoreactive for p- $\alpha$ -syn, 5G4, syn211 after PK digestion, syn105, and AsyO5 antibodies <sup>10</sup>  |
| p- $\alpha$ -syn deposition    | Restricted to unmyelinated fibers <sup>11</sup><br>VIP-, TH-, SP- and CGRP- positive fibers <sup>11</sup><br>Autonomic >> somatosensory small fibers <sup>14</sup>                                     | Restricted to unmyelinated fibers <sup>12</sup><br>SP- and TH- immunoreactive neurons/fibers <sup>11,13</sup><br>Autonomic structures at early stages, sensory association areas at later stages <sup>15</sup>                           |
| Loss of neurons/nerve fibers   | IENFD, MAL, IETNFL/Area, and IETNFL/Length are significantly reduced <sup>16</sup><br>length-dependent loss of intraepidermal fibers <sup>17</sup><br>Marked loss of SP- positive fibers <sup>11</sup> | loss of neurons in the substantia nigra, vagal/glossopharyngeal<br>nucleus, reticular formation, coeruleus complex <sup>15</sup><br>impaired axonal transport <sup>18</sup><br>Loss of SP- levels and SP- positive neurons <sup>11</sup> |

**Table 1: Pathologies in the skin and CNS of PD. IENFD: Intraepidermal Nerve Fiber Density, MAL: Mean Axonal Length, IETNFL: Intraepidermal Total Nerve Fiber Length.**

### PD with and without autonomic dysfunction

The non-motor features of PD include sensory dysfunction, sleep disorders, dysautonomic features, and neuropsychiatric symptoms. Autonomic dysfunction is characterized by abnormal function of the cardiovascular, digestive, urogenital systems, and thermoregulation, all of which seriously affect PD patients' quality of life. Orthostatic hypotension (OH) is the most common cardiovascular autonomic dysfunction in PD patients with a prevalence of 9.6%–64.9%.<sup>21–23</sup> OH is not just a complication of late-stage PD, but can also occur during the premotor stages.<sup>24</sup> When patients with OH change positions, they may experience orthostatic intolerance such as amaurosis, dizziness, blurry vision, fatigue, loss of consciousness, and syncope, resulting in falls and fractures. As a result, some patients are afraid of moving and potential falls. This can have an additive effect on motor symptoms, leading to muscle atrophy and movement disorders. Furthermore, cerebral and cardiac hypoperfusion can cause cerebral infarction, cognitive impairment, coronary heart disease, heart failure, and arrhythmia, which affect PD patients' quality of life, aggravate the medical burden, and substantially increase morbidity and mortality.<sup>25</sup> PD patients with asymptomatic OH are more likely than those without OH to experience greater deterioration in daily activities and quality of life.<sup>26</sup> Therefore, it is vital to screen for OH in all PD patients.

As a scientific and feasible diagnostic method, skin biopsy can provide guidance for early diagnosis and intervention of OH for PD patients. Donadio et al. studied 28 PD patients, including 14 patients with OH (PD + OH) and 14 matched patients without OH (PD – OH), and performed skin biopsy at proximal and distal hairy sites. The proximal site was the cervical C7 paravertebral area and the distal sites were the thigh and distal leg. They found that the distribution of p- $\alpha$ -syn deposits was more homogenous for PD + OH patients

compared to PD – OH patients in all three areas. Specifically, PD + OH patients showed a similar distribution of p- $\alpha$ -syn deposits between proximal and distal skin sites, whereas there was a pronounced proximal-distal gradient in PD – OH patients. Furthermore, 90% of all PD + OH skin samples showed p- $\alpha$ -syn deposits, while only 38% of all PD – OH skin samples showed p- $\alpha$ -syn deposits. The low percentage in PD – OH cases, namely 38%, may be the result of less p- $\alpha$ -syn-positive skin samples in the leg compared with the cervical C7 paravertebral area and the thigh. These results indicate differences in both the distribution and percentage of skin samples showing p- $\alpha$ -syn deposits between PD + OH and PD – OH patients. Notably, there was a similar mean amount of p- $\alpha$ -syn deposits around skin vessels in PD + OH and PD – OH patients. In contrast, the deposits in the sweat glands, muscle arrector pilorum, and skin plexuses of PD + OH patients were much higher than those in PD – OH patients. These findings support that PD + OH patients manifest widespread extension of p- $\alpha$ -syn in cholinergic and adrenergic autonomic nerves, which is different from PD – OH patients, indicating mainly restricted involvement of vessel adrenergic nerves.<sup>27</sup> Donadio et al. further studied 25 MSA-P patients and 25 PD + OH patients undergoing skin biopsy from the cervical area, thigh, and leg. These results suggested a different distribution of p- $\alpha$ -syn in the skin nerves of MSA-P + OH patients (mainly affecting somatic nerves) compared with PD + OH patients (mainly affecting autonomic nerves). Interestingly, abnormal p- $\alpha$ -syn deposits were also found in 18 MSA-P + OH patients (72%) with a distal-proximal gradient (with the highest positivity in leg), which is opposite to previous findings in PD – OH patients. Although these findings should be confirmed in a larger cohort, they suggest that skin biopsy could be informative for discriminating synucleinopathies in OH or healthy controls.<sup>28</sup> Infante et al. evaluated a PD + OH

patient 10 months after symptom onset undergoing annual skin biopsy of the cervical C7 paravertebral area, thigh, and distal leg. The first skin biopsy of the distal leg revealed p- $\alpha$ -syn deposits in autonomic nerve bundles of the deep dermis (40%) and autonomic nerve terminals of muscle arrector pilorum (25%). The second skin biopsy found p- $\alpha$ -syn deposits spread from autonomic nerve bundles (5%) to adrenergic autonomic nerves terminals of skin vessels (31%) in the thigh and distal leg. The third skin biopsy demonstrated that p- $\alpha$ -syn was widely involved in cholinergic and adrenergic autonomic nerves terminals of sweat glands (40%) and muscle arrector pilorum (29%), as well as nerve bundles of the deep dermis (9%) in the distal leg, thigh, and cervical area.<sup>29</sup> These findings suggest that, with progression of the disease, there is decreasing p- $\alpha$ -syn deposited in the dermis autonomic ganglia but increasing p- $\alpha$ -syn deposited in autonomic nerve terminals. In other words, in PD + OH patients, p- $\alpha$ -syn may display widely centrifugal diffusion from autonomic ganglia to nerve terminals. In this specific patient, p- $\alpha$ -syn deposits firstly involved the lumbar ganglia, which differs from the reported involvement of the cervical ganglia.<sup>11,30</sup> This could relate to the fact that this patient underwent skin biopsy at a very early stage and performed the colocalization analysis of p- $\alpha$ -syn with multiple autonomic markers.

Overall, these prior studies suggest that p- $\alpha$ -syn distribution is homogeneous in the skin of PD + OH patients, but shows a proximal-distal gradient in PD – OH patients. Furthermore, the distribution and percentage of skin samples displaying p- $\alpha$ -syn in PD + OH patients were higher than those in PD – OH patients. PD + OH patients manifest wider extension of p- $\alpha$ -syn in more autonomic nerves compared to PD – OH patients. Some patients in the prodromic stage of PD suffer from autonomic non-motor symptoms including orthostatic hypotension and sweating dysfunction. And these symptoms suggest early pathology involvement of skin nerves. Moreover, a significant increase in  $\alpha$ -syn oligomeric species within the synaptic terminals of autonomic nerve fibers has been reported in the skin of PD patients compared with controls using the proximity ligation assay technique.<sup>31</sup> In addition,  $\alpha$ -syn oligomerization has been described as an early event in the pathology,<sup>32,33</sup> although toxic oligomers are not responsible for propagation.<sup>34</sup> Therefore, there may exist an intracellular event *in situ* skin that is not associated with the propagation of  $\alpha$ -syn from other organs. This indicates that the pathogenesis may differ between PD + OH and PD – OH, and that skin biopsy can help differentiate them. The skin p- $\alpha$ -syn distribution pattern and mechanism may also differ between PD patients with and without autonomic dysfunction, such as dysfunction of the digestive and urogenital systems and thermoregulation. Given the limited number of relevant studies in this area, further research is warranted.

### PD with different genetic mutations

An increasing number of studies have examined the role and contribution of genetic factors in familial and sporadic PD (sPD), concluding that a significant source of PD risk is genetic.<sup>35</sup> Nalls et al. identified 90 independent genome-wide significant risk signals by meta-analysis, including the common risk variants leucine-rich repeat kinase 2 (*LRRK2*), glucocerebrosidase (*GBA*), and *SNCA*.<sup>36</sup>

### *LRRK2* gene mutations

*LRRK2* mutation is the most common cause of familial PD and a risk factor for sPD. *LRRK2* variants have been proven to cause autosomal dominant PD.<sup>37</sup> In the Asian population, the most frequent PD risk factor is Gly2385Arg (G2385R, p.2385G>R) variant. In the Chinese population it may increase PD risk by nearly two-fold.<sup>38</sup> Both cellular and animal model studies have found that *LRRK2* plays a role in kinase activity and may cause  $\alpha$ -syn to be phosphorylated.<sup>39</sup> Kinase activity is elevated in individuals with Gly2019Ser or Ile2020Thr mutations, which cause PD via “gain-of-function” or hyperactivity of *LRRK2*.<sup>40</sup> Compared with idiopathic PD (iPD) patients, patients with the *LRRK2* G2385R variant require a larger levodopa equivalent dose and manifest a specific clinical profile including gait dysfunction and postural instability, motor fluctuations, fatigue, and rapid eye movement sleep behavior disorders.<sup>38</sup>

Preliminary work has explored the effect of *LRRK2* mutation on the features of  $\alpha$ -syn deposits in the skin. Isonaka et al. examined 7 PD patients with *LRRK2* mutations and 16 control participants without PD that underwent skin biopsy of the C2 cervical area. Skin total  $\alpha$ -syn deposition was quantified using the  $\alpha$ -syn tyrosine hydroxylase colocalization index, which is based on immunoreactive total  $\alpha$ -syn and tyrosine hydroxylase (a marker of adrenergic neurons) signal intensities. The results showed significantly more intraneuronal total  $\alpha$ -syn depositions in skin sympathetic noradrenergic nerve fibers of PD patients with *LRRK2* mutations compared to controls.<sup>35</sup> Total  $\alpha$ -syn comprises the different elements from p- $\alpha$ -syn in the pathology, and numerous Lewy body and neurite like accumulations of  $\alpha$ -syn along with a diffuse neuropil signal were found when staining for total  $\alpha$ -syn (syn211) in PD brains.<sup>10</sup> We assume that staining for total  $\alpha$ -syn and p- $\alpha$ -syn are both meaningful. However, it is unclear which method is more scientific, more feasible, or better reflects the nature of the disease. Yang et al. performed skin biopsy at the distal leg and cervical C7 paravertebral area in 12 *LRRK2* G2385R carrier PD patients and 47 *LRRK2* G2385R noncarrier PD patients. The results showed that in G2385R noncarriers the positivity rate of p- $\alpha$ -syn deposition at C7 was higher than that at the distal leg

[33/47 (70.2%) vs. 17/47(36.2%),  $P=0.001$ ], whereas in G2385R carriers the rates were similar between C7 and the distal leg [8/12 (66.7%) vs. 7/12 (58.3%),  $P=1.000$ ]. Notably, the nonmotor symptoms scale (NMSS) score, percentage of patients with rapid eye movement sleep behavior disorders (RBD), and levodopa equivalent dose (LED) were all higher in the G2385R carrier group than the noncarrier group.<sup>38</sup> However, there are no prior studies about the correlation between p- $\alpha$ -syn deposition and clinical outcomes. These results may suggest that the distribution pattern of skin p- $\alpha$ -syn in PD patients with the *LRRK2* G2385R variant is homogeneous, whereas PD patients without this variant show a p- $\alpha$ -syn distribution with a proximal-distal gradient. These differences suggest that PD clinical phenotype is related to skin p- $\alpha$ -syn distribution patterns and could thus hint at the presence or absence of *LRRK2* mutation in PD patients. Moreover, pathogenesis between PD patients with and without *LRRK2* mutations may differ.

### GBA gene mutations

Heterozygous *GBA* mutations are thought to be the most common and significant genetic risk factor for PD.<sup>41,42</sup> Such mutations occur in 4–10% of all PD patients<sup>41,43,44</sup> and increase the risk of PD up to 20-fold.<sup>44</sup> The gene *GBA* encodes the lysosomal enzyme glucocerebrosidase (GCase) and its mutations may result in the autosomal recessively inherited disorder Gaucher disease. GCase is synthesized and glycosylated in the endoplasmic reticulum, but is only active when transferred to the acidic lumen of lysosomes.<sup>45</sup> *GBA* variants affect the development of PD through multiple factors. For example, they may cause glucocerebrosidase (GCase) functional loss, thereby damaging lysosomal  $\alpha$ -syn degradation. As aggregation of  $\alpha$ -syn results in neurotoxicity, this inhibits lysosomal activity of normal GCase.<sup>46</sup> Changes in the composition of cellular or lysosomal membranes and autophagy may contribute to reduced *GBA* activity.<sup>47</sup> *GBA* dysfunction has also been shown to cause microglial and immune activation, and the activation of microglia cells may play a role in reduced striatal dopamine release and  $\alpha$ -syn aggregation.<sup>48</sup> There is a higher proportion of males with *GBA* mutations than females, and *GBA* mutation carriers have an earlier age of PD onset, more rapid progression, more common non-motor symptoms, and reduced survival.<sup>41,42</sup> Isonaka et al. reported skin  $\alpha$ -syn deposition in the C2 cervical area of 6 PD patients with *GBA* heterozygous mutations and 16 control participants. They found that 83% of patients with *GBA* variants had higher total  $\alpha$ -syn deposition in the skin noradrenergic nerves compared to controls.<sup>35</sup> Doppler et al. performed skin biopsy at the distal, proximal leg, paravertebral back Th10, and neck C7 of 10 PD patients with different *GBA* mutations (6 N370S, 3 E326K, 1 L444P) and 10 healthy controls. They found that p- $\alpha$ -syn deposition in

PD patients was mainly distributed in autonomic fibers, with vasomotor fibers showing predominant autonomic involvement, and that p- $\alpha$ -syn deposition was not restricted to one *GBA* mutation. No healthy control showed p- $\alpha$ -syn deposition, whereas p- $\alpha$ -syn was found in 60% of PD patients (3 N370S, 3 E326K), which is comparable with rates in previous iPD reports using similar protocols.<sup>49</sup> These findings demonstrate that a similar distribution and frequency of skin p- $\alpha$ -syn is irrespective of *GBA* mutations.

### SNCA gene mutations

The first single genetic mutation identified to cause autosomal-dominant PD was for *SNCA*. Mutations in the *SNCA* gene, which encodes the protein  $\alpha$ -syn and is located on human chromosome 4, may result in misfolding and aggregation of  $\alpha$ -syn protein, damaging neurons and intracellular ultrastructures including mitochondria and the degradative system. Compared with iPD, *SNCA*-mutated PD usually has an earlier age of onset (<50 years), faster deterioration of motor symptoms, and conspicuous levodopa-responsiveness in the early stage but less sustained alleviation of symptoms. It may occur along with dementia, respiratory hypoventilation, myoclonus, mental disorders, and seizures.<sup>40,50</sup> The E46K-*SNCA* mutation is strongly linked to fibrogenesis and pathogenicity,<sup>51,52</sup> with particular clinical manifestations of early-onset parkinsonism and obvious non-motor features, such as dementia, autonomic dysfunction, and sleep disorders.<sup>53–55</sup> Isonaka et al. performed C2 cervical skin biopsies of 2 PD patients with *SNCA* mutations and 16 control participants, and found that total  $\alpha$ -syn deposition in noradrenergic nerves was higher in PD patients compared to controls.<sup>35</sup>

Carmona-Abellan et al. studied 7 E46K-*SNCA* carriers, 2 *PARK2* carriers, and 2 healthy controls. The E46K-*SNCA* carriers included 3 cases with dementia with LBs, 2 cases with pure autonomic failure, 1 PD case, and 1 asymptomatic case. All participants underwent skin biopsy of the cervical C7 region. The results showed a distinctly inverse correlation between nerve fiber density and the degree of p- $\alpha$ -syn aggregation ( $r=-0.889$ ,  $p=0.002$ ). Moreover, in epidermal and dermal structures, all E46K-*SNCA* carriers showed moderate to severe small fiber neurodegeneration and p- $\alpha$ -syn deposition, which was particularly distinct in patients with pure autonomic failure.<sup>56</sup> Taken together, these findings imply that autonomic nerves are affected by abnormal synuclein deposition in E46K-*SNCA* carriers, ultimately leading to autonomic failure.

### Discussion

As summarized above, there is abundant evidence of variation in p- $\alpha$ -syn deposits in the skin of PD patients with autonomic dysfunction, *LRRK2*, *GBA*, and *SNCA*



mutations. These differences may suggest different pathogenesis in different subtypes of PD. In this review, we examined possible pathological mechanisms, clinical manifestations, and cutaneous synuclein deposition in order to correlate cutaneous synuclein pathology with clinical symptoms and features and deeply explore the pathogenesis of PD. The relatively small sample sizes of the studies is a notable limitation, and the findings should be validated in larger samples. In the future, the plantar skin of mouse model hind paws and full-thickness three-dimensional (3D) skin models could potentially help address this issue. Another limitation is the lack of data of PD patients with dementia and DLB, which is often difficult to differentiate. It should be noted that some described studies of PD with genetic mutations include healthy controls and lack the inclusion of idiopathic PD patients. In addition, some of the existing studies lack autopsy pathology to validate the diagnosis.

According to the common pathogenicity, symptom severity, significance of early diagnosis and other reasons in different subtypes of PD, the above four types of PD were selected. At present, there are few studies about skin synuclein deposits in any other PD subtypes. In future studies, the distribution and mechanism of synuclein in PD patients with non-motor symptoms or other genetic mutations, such as *PARK2* or *DJ-1*, should be considered.

Detection of p- $\alpha$ -syn by skin biopsy has numerous advantages for the diagnosis of PD. Tsukita et al. used a univariate random effect model to analyze 41 case-control biopsy studies. They assessed the biopsy sensitivity and specificity of the skin, gastrointestinal tract, minor salivary glands, and submandibular glands for diagnosing PD utilizing native  $\alpha$ -syn or p- $\alpha$ -syn antibody. The highest specificity [1.00, 95% confidence interval (CI) 0.98-1.00] and relatively satisfied sensitivity (0.76, 95% CI 0.69-0.82) were obtained for the skin.<sup>57</sup> It is possible that these results are related to the biopsy site and sample number. Compared with gastrointestinal tissue biopsy under endoscopic surgery, submandibular tissue biopsy, positron emission computed tomography, single-photon emission computed tomography, or <sup>123</sup>I-MIBG myocardial scintigraphy, skin biopsy is easier to perform, more acceptable to patients, and less expensive. Donadio et al. performed an experiment in the Würzburg and Bologna laboratories and demonstrated that detecting p- $\alpha$ -syn by skin biopsy is highly reproducible and technically reliable. Their sample included 21 PD patients, 1 patient with dementia with LBs, 11 patients with rapid eye movement sleep behavior disorder, 4 patients with multiple system atrophy, and 6 patients with small fiber neuropathy. Skin biopsy was performed at the C7 paravertebral spine region and distal skin areas (thigh or leg). The researchers then randomly selected 25 skin sections from C7 and 25 from the distal areas for analysis at two laboratories in

different locations. The results indicated superb reproducibility of the intra-laboratory analysis both in Würzburg (concordance of classification 100% of sections;  $K=1$ ;  $P<0.001$ ) and Bologna (96% of sections;  $K=0.92$ ;  $P<0.001$ ) laboratories. Moreover, the reproducibility of the inter-laboratory analysis (90%;  $K=0.8$ ;  $P<0.001$ ) was excellent.<sup>58</sup> In conclusion, detection of p- $\alpha$ -syn by skin biopsy may be a potential PD diagnostic method with higher sensitivity, specificity, and reproducibility. It is also convenient to perform, highly acceptable to patients, and facilitated to follow up.

### Outstanding questions

Synuclein has been detected in skin biopsy from PD patients for more than a decade. However, biopsy of skin nerves is only carried out at a few large hospitals. Thus, there are difficulties that must be quickly resolved to promote the clinical application of skin biopsy and realize its value for PD diagnosis. Unified selection of an antibody, biopsy site, fixative method, section thickness, and other characteristics is still needed. Furthermore, neuropathologists to analyze biopsy results and experimental technicians to perform biopsy procedures are needed. In the near future, it is possible that skin biopsy will become a widely recognized and high-quality tool for the diagnosis of PD and other neurodegenerative diseases.

### Search strategy and selection criteria

Data for this review were identified by searching PubMed using the search terms “Parkinson's disease”, “skin biopsy”, “synuclein”, “orthostatic hypotension”, “gene”, “*LRRK2*”, “*GBA*”, and “*SNCA*”. Only articles published in English between 2003 and 2021 were included.

### Contributors

Yihang Han - Writing - original draft & editing; Di Wu - Writing - review & editing; Yanjuan Wang - review & editing; Jian Xie - review & editing; Zhijun Zhang - Conceptualization, Supervision, Writing - review & editing. All authors read and approved the final version of the manuscript.

### Declaration of interests

The authors have declared that no competing interest exists.

### Acknowledgments

The work was partly supported by the National Natural Science Key Foundation of China (No. 81830040 and No

82130042) and the Program of Excellent Talents in Medical Science of Jiangsu Province (No. JCRCA2016006).

### Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.ebiom.2022.104076.

### References

- Mehra S, Sahay S, Maji SK.  $\alpha$ -Synuclein misfolding and aggregation: implications in Parkinson's disease pathogenesis. *Biochim Biophys Acta (BBA) Proteins Proteom.* 2019;1867(10):890–908.
- Malpartida AB, Williamson M, Narendra DP, Wade-Martins R, Ryan BJ. Mitochondrial dysfunction and mitophagy in parkinson's disease: from mechanism to therapy. *Trends Biochem Sci.* 2021;46(4):329–343.
- Ho MS. Microglia in Parkinson's disease. *Adv Exp Med Biol.* 2019(1175):335–53.
- Trist BG, Hare DJ, Double KL. Oxidative stress in the aging substantia nigra and the etiology of Parkinson's disease. *Aging Cell.* 2019;18(6).
- Lotankar S, Prabhavalkar KS, Bhatt LK. Biomarkers for Parkinson's disease: recent advancement. *Neurosci Bull.* 2017;33(5):585–597.
- Xu Y, Deng Y, Qing H. The phosphorylation of  $\alpha$ -synuclein: development and implication for the mechanism and therapy of the Parkinson's disease. *J Neurochem.* 2015;135(1):4–18.
- Tofaris GK. Initiation and progression of  $\alpha$ -synuclein pathology in Parkinson's disease. *Cell Mol Life Sci.* 2022;79(4).
- Horsager J, Andersen KB, Knudsen K, et al. Brain-first versus body-first Parkinson's disease: a multimodal imaging case-control study. *Brain.* 2020;143(10):3077–3088. 2020-10-01.
- Eusebi P, Giannandrea D, Biscetti L, et al. Diagnostic utility of cerebrospinal fluid  $\alpha$ -synuclein in Parkinson's disease: a systematic review and meta-analysis. *Mov Disord.* 2017;32(10):1389–1400.
- Kuzkina A, Schulmeyer L, Monoranu CM, Volkman J, Sommer C, Doppler K. The aggregation state of  $\alpha$ -synuclein deposits in dermal nerve fibers of patients with Parkinson's disease resembles that in the brain. *Parkinsonism Relat Disord.* 2019;64:66–72.
- Doppler K, Ebert S, Üçeyler N, et al. Cutaneous neuropathy in Parkinson's disease: a window into brain pathology. *Acta Neuropathol.* 2014;128(1):99–109.
- Braak H, Rüb BU, Gai WP, Del Tredici K. Idiopathic Parkinson's disease: possible routes by which vulnerable neuronal types may be subject to neuroinvasion by an unknown pathogen. *J Neural Transm.* 2003;110(5):517–536. 2003-05-01.
- Chu Y, Morfini GA, Langhamer LB, He Y, Brady ST, Kordower JH. Alterations in axonal transport motor proteins in sporadic and experimental Parkinson's disease. *Brain.* 2012;135(7):2058–2073. 2012-07-01.
- Brumberg J, Kuzkina A, Lapa C, et al. Dermal and cardiac autonomic fiber involvement in Parkinson's disease and multiple system atrophy. *Neurobiol Dis.* 2021;153:105332.
- Braak H, Tredici KD, Rüb U, de Vos RAJ, Jansen Steur ENH, Braak E. Staging of brain pathology related to sporadic Parkinson's disease. *Neurobiol Aging.* 2003;24(2):197–211. 2003-01-01.
- Jeziorska M, Atkinson A, Kass-Iliyya L, Javed S, Kobylecki C, Gosal D, et al. Increased intraepidermal nerve fiber degeneration and impaired regeneration relate to symptoms and deficits in Parkinson's disease. *Front Neurol.* 2019;10:1–8. 2019-02-14.
- Donadio V, Incensi A, Piccinini C, et al. Skin nerve misfolded  $\alpha$ -synuclein in pure autonomic failure and Parkinson disease. *Ann Neurol.* 2016;79(2):306–316.
- Prots I, Veber V, Brey S, et al.  $\alpha$ -Synuclein oligomers impair neuronal Microtubule-Kinesin interplay. *J Biol Chem.* 2013;288(30):21742–21754.
- Donadio V. Skin nerve  $\alpha$ -synuclein deposits in Parkinson's disease and other synucleinopathies: a review. *Clin Auton Res.* 2019;29(6):577–585.
- Donadio V, Incensi A, El-Agnaf O, et al. Skin  $\alpha$ -synuclein deposits differ in clinical variants of synucleinopathy: an *in vivo* study. *Sci Rep UK.* 2018;8(1).
- Euesden J. PRSice: polygenic risk score software. *Bioinformatics.* 2015;31(9):1466–1468.
- International Parkinson Disease Genomics Consortium. Imputation of sequence variants for identification of genetic risks for Parkinson's disease: a meta-analysis of genome-wide association studies. *Lancet.* 2011;377(9766):641–649.
- International Parkinson S Disease Genomics Consortium IPDGC WTCC, WTCCC. A two-stage meta-analysis identifies several new loci for Parkinson's disease. *PLoS Genet.* 2011;7(6):e1002142. [Journal Article; Meta-Analysis; Research Support, N.I.H., Extramural; Research Support, N.I.H., Intramural; Research Support, Non-U.S. Gov't; Research Support, U.S. Gov't, Non-P.H.S.]2011-06-01.
- Cutsforth-Gregory JK, Low PA. Neurogenic orthostatic hypotension in Parkinson disease: a primer. *Neurol Ther.* 2019;8(2):307–324.
- Palma J, Kaufmann H. Orthostatic hypotension in Parkinson disease. *Clin Geriatr Med.* 2020;36(1):53–67.
- Merola A, Romagnolo A, Rosso M, et al. Autonomic dysfunction in Parkinson's disease: a prospective cohort study. *Mov Disord.* 2018;33(3):391–397.
- Donadio V, Incensi A, Del Sorbo F, et al. Skin nerve phosphorylated  $\alpha$ -synuclein deposits in Parkinson disease with orthostatic hypotension. *J Neuropathol Exp Neurol.* 2018;77(10):942–949. 2018-10-01.
- Donadio V, Incensi A, Rizzo G, et al. Skin biopsy may help to distinguish multiple system Atrophy–Parkinsonism from Parkinson's disease with orthostatic hypotension. *Mov Disord.* 2020;35(9):1649–1657.
- Infante R, Scaglione C, Incensi A, Rizzo G, Liguori R, Donadio V. A longitudinal skin biopsy study of phosphorylated  $\alpha$ -synuclein in a patient with parkinson disease and orthostatic hypotension. *J Neuropathol Exp Neurol.* 2020;79(7):813–816. 2020-07-01.
- Donadio V, Incensi A, Rizzo G, et al. Spine topographical distribution of skin  $\alpha$ -synuclein deposits in idiopathic Parkinson disease. *J Neuropathol Exp Neurol.* 2017;76(5):384–389. 2017-05-01.
- Mazzetti S, Basellini MJ, Ferri V, et al.  $\alpha$ -Synuclein oligomers in skin biopsy of idiopathic and monozygotic twin patients with Parkinson's disease. *Brain.* 2020;143(3):920–931. 2020-03-01.
- Roberts RF, Wade-Martins R, Alegre-Abarrategui J. Direct visualization of alpha-synuclein oligomers reveals previously undetected pathology in Parkinson's disease brain. *Brain.* 2015;138(6):1642–1657.
- Bengoa-Vergniory N, Roberts RF, Wade-Martins R, Alegre-Abarrategui J. Alpha-synuclein oligomers: a new hope. *Acta Neuropathol.* 2017;134(6):819–838.
- Nunilo Cremades S. Direct observation of the interconversion of normal and toxic forms of  $\alpha$ -synuclein. *Cell.* 2012;149(1):1048–1059.
- Isonaka R, Goldstein DS, Zhu W, et al.  $\alpha$ -Synuclein deposition in sympathetic nerve fibers in genetic forms of Parkinson's disease. *Mov Disord.* 2021;36(10):2346–2357.
- Vallegra CL, Tan M, Kia DA, et al. Identification of novel risk loci, causal insights, and heritable risk for Parkinson's disease: a meta-analysis of genome-wide association studies. *Lancet Neurol.* 2019;18(12):1091–1102. 2019-01-01.
- Kluss JH, Mamais A, Cookson MR. LRRK2 links genetic and sporadic Parkinson's disease. *Biochem Soc Trans.* 2019;47(2):651–661. 2019-04-30.
- Yang J, Wang H, Yuan Y, et al. Peripheral synucleinopathy in Parkinson disease with LRRK2 G2385R variants. *Ann Clin Transl Neurol.* 2021;8(3):592–602. [Journal Article; Research Support, Non-U.S. Gov't]2021-03-01.
- Melli G, Vacchi E, Biemmi V, et al. Cervical skin denervation associates with alpha-synuclein aggregates in Parkinson disease. *Ann Clin Transl Neurol.* 2018;5(11):1394–1407. [Journal Article]2018-11-01.
- Cherian A, Divya KP. Genetics of Parkinson's disease. *Acta Neurol Belg.* 2020;120(6):1297–1305.
- Berge-Seidl V, Pihlstrøm L, Maple-Grødem J. The GBA variant E326K is associated with Parkinson's disease and explains a genome-wide association signal. *Neurosci Lett.* 2017;658(29):48–52.
- Riboldi GM, Di Fonzo AB. GBA, gaucher disease, and Parkinson's disease: from genetic to clinic to new therapeutic approaches. *Cells.* 2019;8(4):364. Basel2019-04-19.
- Sidransky E, Nalls MA, Aasly JO, et al. Multicenter analysis of glucocerebrosidase mutations in Parkinson's disease. *N Engl J Med.* 2009;361(17):1651–1661. 2009-01-01.
- Schapira AHV. Glucocerebrosidase and Parkinson disease: recent advances. *Mol Cell Neurosci.* 2015;66:37–42.
- Do J, McKinney C, Sharma P, Sidransky E. Glucocerebrosidase and its relevance to Parkinson disease. *Mol Neurodegener.* 2019;14(1).

- 46 Brockmann K, Srulijes K, Pflederer S, et al. GBA-associated Parkinson's disease: reduced survival and more rapid progression in a prospective longitudinal study. *Mov Disord.* 2014;30(3):407-411.
- 47 Gegg ME, Sweet L, Wang BH, Shihabuddin LS, Sardi SP, Schapira AHV. No evidence for substrate accumulation in Parkinson brains with GBA mutations. *Mov Disord.* 2015;30(8):1085-1089.
- 48 Adler CH, Beach TG, Shill HA, et al. GBA mutations in Parkinson disease: earlier death but similar neuropathological features. *Eur J Neurol.* 2017;24(11):1363-1368. [Journal Article]2017-11-01.
- 49 Doppler K, Brockmann K, Sedghi A, et al. Dermal phospho-alpha-synuclein deposition in patients with Parkinson's disease and mutation of the glucocerebrosidase gene. *Front Neurol.* 2018;9. 2018-12-17.
- 50 Anne Weissbach C, Kleina C. Atypical' Parkinson's disease - genetic. *Int Rev Neurobiol.* 2019;149(149):207-235.
- 51 Fredenburg RA, Rospigliosi C, Meray RK, et al. The impact of the E46K mutation on the properties of  $\alpha$ -synuclein in its monomeric and oligomeric states. *Biochemistry.* 2007;46(24):7107-7118. US2007-06-01.
- 52 Íñigo-Marco I, Valencia M, Larrea L, et al. E46K  $\alpha$ -synuclein pathological mutation causes cell-autonomous toxicity without altering protein turnover or aggregation. *Proc Natl Acad Sci.* 2017;114(39):E8274-E8283. 2017-09-26.
- 53 Tijero B, Gómez-Esteban JC, Lezcano E, et al. Cardiac sympathetic denervation in symptomatic and asymptomatic carriers of the E46K mutation in the  $\alpha$ -synuclein gene. *Parkinsonism Relat Disord.* 2013;19(1):95-100.
- 54 Somme JH, Gomez-Esteban JC, Molano A, Tijero B, Lezcano E, Zarranz JJ. Initial neuropsychological impairments in patients with the E46K mutation of the  $\alpha$ -synuclein gene (PARK 1). *J Neurol Sci.* 2011;310(1-2):86-89.
- 55 Zarranz JJ, Fernández-Bedoya A, Lambarri I, et al. Abnormal sleep architecture is an early feature in the E46K familial synucleinopathy. *Mov Disord.* 2005;20(10):1310-1315.
- 56 Carmona-Abellan M, Gabilondo I, Murueta-Goyena A, et al. Small fiber neuropathy and phosphorylated alpha-synuclein in the skin of E46K-SNCA mutation carriers. *Parkinsonism Relat Disord.* 2019;65:139-145.
- 57 Tsukita K, Sakamaki Tsukita H, Tanaka K, Suenaga T, Takahashi R. Value of *in vivo*  $\alpha$ -synuclein deposits in Parkinson's disease: a systematic review and meta-analysis. *Mov Disord.* 2019;34(10):1452-1463.
- 58 Donadio V. Abnormal  $\alpha$ -synuclein deposits in skin nerves: intra- and inter-laboratory reproducibility. *Eur J Neurol.* 2019;26(26):1245-1251.