

# Alpha-synuclein in cutaneous small nerve fibers

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**Abstract:** Despite progression in the development of pharmacological therapy, treatment of alpha synucleinopathies, such as Parkinson's disease (PD) and some atypical parkinsonism syndromes, is still challenging. To date, our knowledge of the mechanisms whereby the pathological form of alpha-synuclein causes structural and functional damage to the nervous system is limited and, consequently, there is a lack of specific diagnostic tools to evaluate pathology in these patients and differentiate PD from other neurodegenerative proteinopathies. Recent studies indicated that alpha-synuclein deposition in cutaneous small nerve fibers assessed by skin biopsies might be a valid disease marker of PD and facilitate early differentiation of PD from atypical parkinsonism syndromes. This observation is relevant since early diagnosis may enable timely treatment and improve quality of life. However, challenges include the necessity of standardizing immunohistochemical analysis techniques and the identification of potential distinct patterns of intraneural alpha-synuclein deposition among synucleinopathies. In this perspective, we explore the scientific and clinical opportunities arising from alpha-synuclein assessment using skin biopsies. These include elucidation of the peripheral nervous system pathology of PD and other synucleinopathies, identification of novel targets to study response to neuroprotective treatment, and improvement of clinical management. Furthermore, we discuss future challenges in exploring the diagnostic value of skin biopsy assessment for alpha-synuclein deposition and implementing the technique in clinical practice.

**Keywords:** Parkinson's disease, diagnosis, skin, immunohistochemistry, biopsy

## Introduction

Despite progression in the development of pharmacological therapy, treatment of the alpha synucleinopathy Parkinson's disease (PD) as well as treatment of atypical parkinsonism proteinopathies, including a range of synucleinopathies and nonsynucleinopathic neurodegenerative disorders, is still challenging. Early diagnosis and differentiation of PD from atypical parkinsonism syndromes are clinically important as it allows timely symptomatic treatment and improvement of quality of life.<sup>1</sup> To date, our knowledge of the mechanisms whereby the pathological form of alpha-synuclein causes structural and functional damage to the nervous system is limited. Consequently, there is a lack of specific diagnostic tools to allow early differentiation between idiopathic and atypical parkinsonism syndromes and optimization of individualized therapy. Therefore, there is an urgent need for novel disease markers. During the ongoing search for such markers, analysis of intraneural alpha-synuclein depositions in small autonomic nerve fibers in the skin has recently emerged as promising diagnostic target in patients with PD.<sup>2</sup> Skin samples are obtained in vivo via superficial punch biopsies and are immunohistochemically stained for alpha-synuclein and nerve fiber specific markers. The procedure was shown to be safe and quantitative results showed high sensitivity and specificity in the detection of PD.<sup>3,4</sup> However, challenges include the need for standardizing immunohistochemical analysis techniques

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and identifying distinct patterns of alpha-synuclein deposition among synucleinopathies in order to allow diagnostic discrimination.

In this perspective, we reflect on the scientific and clinical opportunities linked to alpha-synuclein assessment in skin biopsies. Potentialities include elucidation of the peripheral nervous system pathology in synucleinopathies, such as PD, identification of novel targets to study response to neuroprotective treatment, and improvement of clinical management in patients with neurodegenerative diseases. Moreover, we reflect on future challenges in exploring the diagnostic value of skin biopsy assessment for alpha-synuclein deposition and implementing the technique in clinical practice.

## Cutaneous small fiber alpha-synuclein in PD

The skin is innervated by afferent somatic nerves, unmyelinated C-fibers, and lightly myelinated A-delta fibers that originate in the dorsal nerve root ganglia (small fibers). In skin biopsy studies of PD, alpha-synuclein accumulation in cutaneous autonomic small fibers has been recently linked to autonomic nervous system disturbances even in the early stages of the disease, suggesting that quantification of this protein via immunohistochemical staining might be a valid biomarker of the disease and could help improve the accuracy of diagnosis and treatment.<sup>2</sup> This study also demonstrated that alpha-synuclein deposition normalized to intraepidermal nerve fiber density is increased in cutaneous sudomotor (sweat gland innervating) and pilomotor (pilomotor muscle innervating) nerve fibers in patients with PD and that more severe alpha-synuclein deposition relates to greater autonomic dysfunction and more advanced motor symptoms. Importantly, a clear increase in pathology was observed in the early stages of PD with substantially higher alpha synuclein depositions in patients at Hoehn and Yahr stage 2 compared to stage 1. This early increase of small fiber pathology indicates the possible utility of skin biopsy assessment in early diagnostic work-up and short-term clinical studies in patients with PD. Interestingly, another investigation found morphological similarities between alpha-synuclein deposition-related fiber damage in cutaneous small fibers and those present in cerebral neurites, which led the authors to postulate that the skin constitutes “a window into brain pathology” in patients with PD.<sup>3</sup> Additional research confirmed and further advanced these observations. A study in 28 patients with PD and 23 control subjects found >90% sensitivity and >90% specificity to distinguish PD from control participants with quantification of alpha-synuclein deposition in either pilomotor

or sudomotor nerve fibers.<sup>4</sup> Additional Class III evidence was provided in a study of 21 patients with PD, 30 control subjects, and 20 patients with parkinsonism syndromes assumed not to have alpha-synuclein deposits (ie, vascular parkinsonism, tauopathies, parkinsonism with evidence of parkin mutations). In this study, alpha-synuclein depositions were detected in all patients with PD but were not found in any skin sample in control subjects or patients with presumably nonsynucleinopathic parkinsonism syndromes.<sup>5</sup>

## Diagnostic discrimination of cutaneous alpha-synuclein deposition in synucleinopathies

Research indicated that alpha-synuclein deposition in cutaneous small fibers might facilitate early differentiation of PD from other synucleinopathies. In a disease comparison study, alpha-synuclein deposition in nerve fibers innervating pilosebaceous units (including pilomotor fibers) was found in 62% of PD patients but only in 7% of patients with atypical parkinsonism syndromes.<sup>6</sup> However, sample sizes were relatively small in this study (34 patients with PD, 33 patients with atypical parkinsonism syndromes, 20 control subjects) and the group of atypical parkinsonism syndromes included both synucleinopathic syndromes, such as dementia with Lewy bodies and multiple system atrophy (MSA), and tauopathies, such as progressive supranuclear palsy and Alzheimer’s disease, potentially limiting interpretability of the observed diagnostic discrimination.

Within the group of synucleinopathic neurodegenerative disorders, distinct distributions of alpha-synuclein deposition between the central and the peripheral nervous system have been assumed. Particularly, in contrast to PD, MSA has been traditionally believed to be a pure central disorder with deposition of the presumably pathological form of alpha-synuclein (phosphorylated alpha-synuclein) being limited to the brain and preganglionic nerve fibers.<sup>7</sup> Consistent with this assumption, a previous investigation in skin biopsies of patients with MSA found no accumulation of the presumably pathogenic form of alpha-synuclein (phosphorylated alpha-synuclein) in cutaneous autonomic adrenergic nerve fibers.<sup>8</sup> However, unexpectedly, another study also included assessment of phosphorylated alpha-synuclein in peripheral somatic nerve fibers in both MSA and PD patients and found pathological alpha-synuclein accumulation in sensory fibers in 67% of MSA patients, whereas a similar fraction of PD patients showed alpha-synuclein pathology in autonomic fibers.<sup>9</sup> This important observation does not only question the common conception of MSA being a pure or predominant

central nervous system disorder but also highlights the necessity of intraneural alpha-synuclein quantification beyond autonomic fibers in future research.

Similar to the PD phenotype, patients with pure autonomic failure (PAF) showed length-dependent somatic and autonomic small fiber loss, which increased with alpha-synuclein load.<sup>10</sup> Notably, in the comparison between PAF and MSA, differences in skin denervation patterns seem not to relate to patterns of autonomic function tests, including cardiovascular function assessed via head-up tilt test and sympathetic nerve activity assessed via microneurography.<sup>11</sup> By contrast, in PD patients, alpha-synuclein deposition in cutaneous small fibers strongly correlates with autonomic functions and severity of autonomic symptoms.<sup>2</sup> The discrepancy observed between peripheral alpha-synuclein pathology patterns in MSA and PD, as well as the differences between structural nerve fiber damage and autonomic functions in patients with MSA and PAF but not PD do not only highlight our limited understanding of the underlying mechanisms but also indicate the unique opportunity of the technique to identify disease-specific patterns. Defining these patterns might facilitate early diagnosis, disease monitoring, and investigation of response to neuroprotective treatment.

However, an encompassing characterization of alpha-synuclein deposition among autonomic and nonautonomic small nerve fibers in large populations of patients with synucleinopathies and longitudinal assessment of pathology related to disease progression are still lacking. Furthermore, it appears necessary to elucidate to what degree structural nerve fiber damage relates to loss of functional integrity of the affected nerve fiber types in order to improve our understanding of the functional relevance of alpha-synuclein deposition in cutaneous small fibers.

## Limitations and challenges

While quantification of alpha-synuclein deposition in pilomotor muscles might be a valid biomarker for PD, several current limitations would have to be overcome to enable broad clinical implication of the technique. First, and probably most important, consensus on the optimal technique of immunohistochemical staining methods and analysis appears to be necessary to generate comparable data and define clinical standards. Several techniques have been proposed including: 1) normalizing total alpha-synuclein burden to loss of intraepidermal nerve fibers to control for damage not related to alpha-synuclein; 2) using an antibody selective for the presumably neuropathy inducing phosphorylated form of alpha-synuclein; and 3) quantifying burden

of native alpha-synuclein.<sup>2,4,9</sup> While normalization to loss of intraepidermal nerve fibers and use of phosphorylated alpha-synuclein-specific antibodies seem superior over native alpha-synuclein quantification, the optimal analysis technique remains uncertain. Second, accumulations of alpha-synuclein were found in different types of autonomic cutaneous small fibers, including sudomotor (sweat gland innervating), vasomotor (blood vessel innervating), and pilomotor (pilomotor muscle innervating) fibers but it remains to be determined which fiber type (or which combination of types) is the optimal diagnostic target. However, two investigations assessing alpha-synuclein burden normalized to loss of intraepidermal nerve fibers found most severe pathology in pilomotor nerve fibers, potentially indicating high diagnostic value of this type of autonomic small fibers.<sup>2,4</sup> The uncertainty of the optimal neural target structure (or set of target structures) as well as the optimal skin site where the biopsy should be obtained from might, *inter alia*, explain why in previous studies positive staining for phosphorylated alpha-synuclein ranged between 0% and 70% in patients with PD.<sup>12-14</sup> Third, longitudinal studies in large populations of patients with PD and other synucleinopathies to relate cumulative nerve fiber damage over time to progression of clinical symptoms and confirm external validity of the technique are still lacking. Finally, immunohistochemical quantification of alpha-synuclein burden in small fibers captures structural pathology but may not reflect the functional integrity that results from structural damage.

Viewed in conjunction, these limitations and challenges underscore that consensus on standardized testing protocols as well as further assessment of sensitivity and specificity of cutaneous nerve fiber evaluation for both structural and functional damage related to alpha-synuclein deposition are needed.<sup>15-17</sup>

## Association of structural and functional nerve fiber damage related to alpha-synuclein deposition

While autonomic skin nerve fibers can be assessed for structural integrity using immunohistochemical quantification of nerve fiber densities in skin biopsies, several techniques have been designed to assess functional integrity of these fibers. In fact, functional assessment of autonomic skin nerves fibers has taken on increasing importance in the evaluation of autonomic neuropathy.<sup>18</sup> Cutaneous small fibers are responsive to a range of physical, chemical, and mechanical stimuli that can evoke an axon-reflex-mediated response. Controlled activation of cutaneous vasomotor (blood vessel innervating) fibers by acetylcholine administered via iontophoresis leads to orthodromic

conduction of an action potential to an axon branch point and, consecutively, antidromic conduction to a neighboring population of cutaneous blood vessels. On activation, both C-fibers and A-delta fibers release substance P and calcitonin gene-related peptide, leading to vasodilation and plasma extravasation in an indirect skin area, surrounding the area of acetylcholine application.<sup>19</sup> This neurally mediated response can be evoked by chemical (eg, acetylcholine, histamine, and capsaicin) and electrical stimulation and can be quantified via laser Doppler assessment as a measure of functional integrity of vasomotor fibers.<sup>20</sup> The most widely used application includes iontophoresis of acetylcholine using a predefined current and dosage. Similarly, axon-reflex responsiveness can be assessed in sudomotor nerve fibers via cholinergic and in pilomotor fibers via adrenergic stimulation.<sup>21,22</sup> Research has shown that sudomotor axon-reflex function is impaired in PD and impairment increases in severity with increasing disease stage.<sup>23</sup> However, to date, it is unknown whether axon-reflex responsiveness relates to alpha-synuclein load in pilomotor and vasomotor nerve fibers. Therefore, it remains to be defined to what degree structural nerve fiber damage related to alpha-synuclein deposition translates into dysfunction of the affected fiber. Axon-reflex-based assessment might be useful to elucidate this association.

## Conclusion

There is emerging evidence on an important pathogenic role of deposition of alpha-synuclein in cutaneous small nerve fibers, quantitative analysis of which might facilitate differentiation between synucleinopathies, early diagnosis, disease monitoring, and evaluation of response to neuroprotective treatment. Further research is warranted to standardize analysis techniques, elucidate progression of pathology over time, and define the association between structural and functional impairment of nerve fibers affected by alpha-synuclein deposition.

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