Standardized protocol to search phosphorylated α-synuclein in skin biopsy: recommendations from an expert panel

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Introduction

Skin biopsy is a promising diagnostic tool for the in vivo identification of α-synucleinopathies. However, despite increasing adoption, methodological variability exists among the different laboratories applying this technique. To move toward a formal standardized protocol, leading experts in the field convened for a meeting focused on discussing and harmonizing the main methodological differences. The outcome of this meeting was a consensus-based skin biopsy protocol, which is presented here

Methods

A formal expert opinion conference was held on November 15–16, 2024, at the IRCCS Institute of Neurological Sciences of Bologna (Italy). The consensus was reached by using a Question & Answer format following the PICO (Population, Intervention, Comparison, Outcome) methodology. A Delphi survey was developed based on the contrasting issues raised during the meeting.

Table. Delphy survey

Question 1	Question 2
Do you agree that the following two methods have similar diagnostic accuracy searching for m-α-syn: • Method 1: A biopsy of 3 skin sites (cervical, thigh and leg) with 1 sample for each skin site and analysis at 50 microns of 6 skin sections for each sample • Method 2: A biopsy of 2 skin sites (cervical and leg), with 2 samples for each site and analysis at 10 microns of 3 skin sections for each sample	What do you consider to be the optimal diameter of the punch, taking into account its advantages and disadvantages, for performing skin biopsies aimed at detecting abnormal deposits of α-synuclein?
Yes	3 µm
No	5 µm
I don't have the expertise to answer this question	I don't have the expertise to answer this question

Question 3	Question 4
In the case of a patient with Parkinson's Disease (PD) and asymmetric symptoms, which side of the body would you consider optimal for a biopsy to detect abnormal accumulations of m-α-syn?	Is the Distribution Coefficient (DC) or similar methods to describe the distribution pattern of p-α-syn helpful in differentiating PD from other α-synucleinopathies?
Symptomatic site	Yes
Asymptomatic site	No
I don't have the expertise to answer this question	I don't have the expertise to answer this question

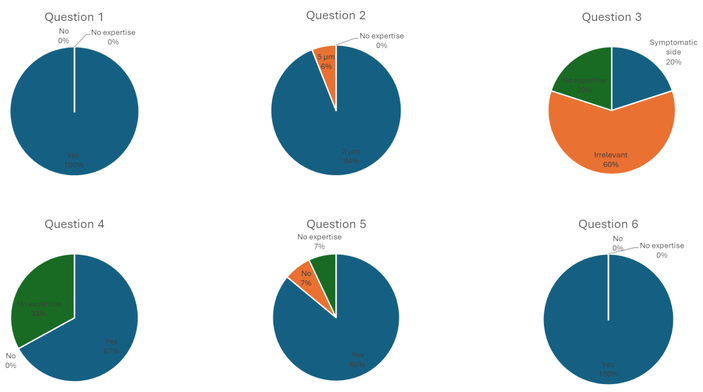

Figure 2 Survey results

The survey was developed based on the issues raised during the in-person meeting where unanimous agreement among the panel was not reached. It was built around the following questions: the methods of analyzing skin samples for p-α-syn detection, considering the number of samples to be used and the thickness of the skin sections to be analyzed; the diameter of the punch to be employed; the sampling sites in cases of asymmetric motor disturbance; the quantitative methods for assessing p-α-syn load; and p-α-syn as the optimal marker for misfolded α-synuclein in the skin. The consensus among participants was defined by considering a threshold of 75% of agreement of the responses provided. Consensus was not reached for questions 3 (Sampling sites in asymmetric motor involvement) and 4 (Distribution Coefficient as a quantitative method for p-α-syn load).

Conclusions

We have developed a consensus-based protocol for the detection of p-α-syn in skin samples from patients with suspected α-synucleinopathies, with the aim of promoting standardization, broader adoption of the method, and its integration into clinical practice.

Figure 1 Participants

The figure shows all participants of the formal expert opinion in-person meeting held on November 15–16, 2024, at the IRCCS Institute of Neurological Sciences of Bologna (Italy), with the aim of defining a consensus-based skin biopsy protocol for the identification of cutaneous p-α-syn deposits. From left to right, the panel was composed of Kazuto Tsukita (Kyoto, Japan), Cecilia Delprete (Bologna, Italy), Giogia Melli (Lugano, Switzerland), Alex Incensi (Standing in the back row, Bologna, Italy), Giovanni Rizzo (Bologna, Italy), Alessandro Furia (Crouching in the front row, Bologna, Italy), Roy Freeman (Boston, USA), Vincenzo Donadio (Bologna, Italy), Rocco Liguori (Bologna, Italy), Chris Gibbons (Boston, USA), Vincenzo Provitera (Benevento, Italy), Maria Nolano (Napoli and Benevento, Italy), Martin Ingelsson (Toronto, Canada and Uppsala, Sweden), Jing Yang (Zhengzhou, China) and Minglei Liu (Zhengzhou, China). Kathrin Doppler (Würzburg, Germany) attended the meeting remotely through an online connection.

Results

Unanimous consensus was reached on key elements, including the skin biopsy technique, the choice of fixative, the use of a cryostat, the staining method for phosphorylated α-synuclein (p-α-syn), the use of a high-power fluorescence microscope, co-localization of p-α-syn with neuronal or glial markers, and high-magnification analysis. Additional agreement, achieved through the Delphi method, concerned specific procedural parameters such as the number of samples and section thickness, punch diameter, preferred biopsy sites in cases of asymmetric motor involvement, quantitative methods for assessing p-α-syn load, and the use of p-α-syn as the optimal marker for identifying misfolded α-synuclein in the skin.

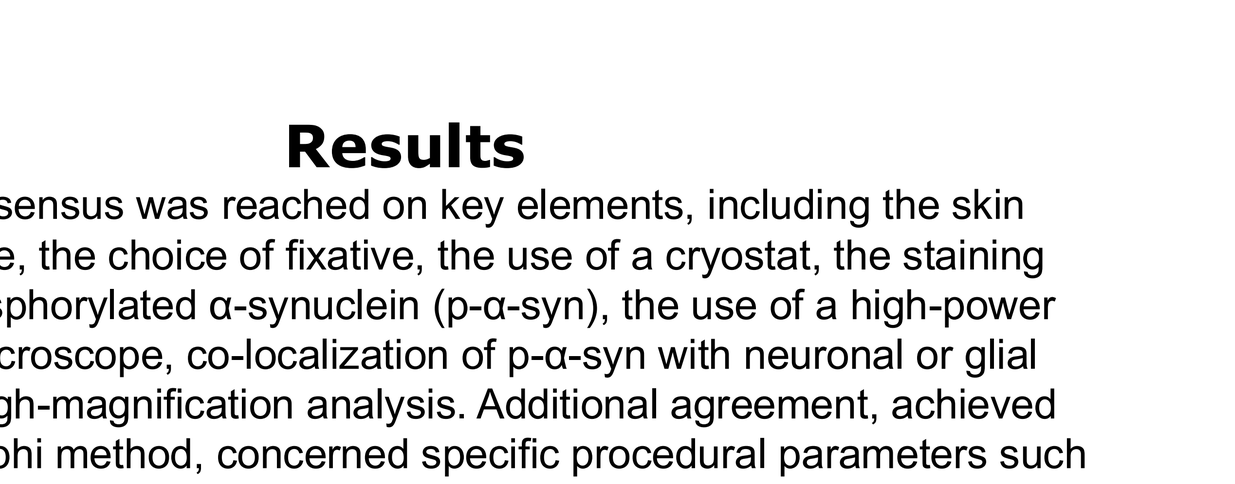

Figure 3 Skin biopsy protocol searching for p-α-syn

The figure outlines all the steps of the method, from skin sample collection to its analysis through indirect immunofluorescence, culminating in the visualization and quantification of abnormal p-α-syn deposits (see the Results section for further details).

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