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Non-inclusion α-synuclein co-pathology in neurodegenerative diseases

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Abstract

Neurodegenerative diseases (NDs) often present with co-pathologies from other NDs, affecting clinical presentation, disease progression, and therapeutic responses. Recent studies suggest that soluble α-synuclein oligomers, rather than Lewy body (LB) inclusions, are major contributors to neuronal dysfunction in Lewy body diseases. These oligomers are more abundant than LBs but are undetectable by standard immunohistochemical methods. This research aims to identify the level of α-synuclein non-inclusion co-pathology in ND cohorts previously examined for ND-related pathologies using immunohistochemistry (IHC). We utilize α-synuclein proximity ligation assay (PLA) with the MJFR-14-6-4-2 antibody to investigate post-mortem brain sections from individuals with various NDs and non-ND controls. Preliminary results indicate successful application of PLA on brain samples, revealing α-synuclein signals in non-ND controls, which necessitates further mapping to understand its distribution and emergence during aging. This study will enhance our understanding of α-synuclein PLA in healthy cases and its prevalence as co-pathology in NDs, crucial for patient stratification and optimizing treatments.

A. INTRODUCTION

It is well-documented that a significant fraction of patients diagnosed with a neurodegenerative disease (ND) present with co-pathology known from other, separate NDs at autopsy (Hamilton, 2000; McAleese *et al.*, 2017; Irwin and Hurtig, 2018). These co-pathologies have the potential to affect not only clinical presentation and symptomatology in the patients, but also disease progression and response to therapeutic interventions. There is, however, little knowledge of the exact roles and interactions of various pathologies in these mixed ND cases (Sengupta and Kayed, 2022; Shim *et al.*, 2022).

In recent years, soluble α-synuclein aggregates, so-called oligomers, not organized into neuronal Lewy body (LB)-like inclusions have been suspected as a major cause of neuronal dysfunction and toxicity in Lewy body diseases (Winner *et al.*, 2011; Ingelsson, 2016; Fusco *et al.*, 2017; Prots *et al.*, 2018; Alam *et al.*, 2019; Du, Xie and Liu, 2020; Kayed, Dettmer and Lesné, 2020). These oligomers are small aggregates, which are invisible to standard immunohistochemical methods that focuses on the inclusion pathology, but studies using α-synuclein aggregate proximity ligation assay techniques have shown that they are much more abundant than the LBs, our golden standard for α-synuclein pathology (Roberts, Wade-Martins and Alegre-Abarrategui, 2015; Sekiya *et al.*, 2019, 2022, Moeller Jensen *et al.*, 2024). As such, current estimates of α-

synuclein co-pathology (based on standard immunohistochemistry (IHC) for LBs) underestimates the degree of α-synuclein aggregate co-pathology in neurodegenerative diseases.

Correct assessments of the abundance of co-pathology in NDs is a crucial first step in determining the role of this co-pathology. This is of high importance to both understand the "natural history" of common ND and equally necessary for making informed choices regarding design of clinical trials.

The purpose of this research project is to identify the level of α-synuclein non-inclusion co-pathology in cohorts of neurodegenerative diseases, which have already been examined for ND-related pathologies by IHC (including α-synuclein (LBs), Aβ (plaques), tau (tangles), and TDP-43).

B. MATERIALS AND METHODS

In this study, we investigate post-mortem human brain sections from individuals with various neurodegenerative diseases as well as non-neurodegenerative controls. The study has been approved by the relevant ethical committee at Niigata University.

The study utilizes an α-synuclein proximity ligation assay (PLA) using the aggregation-specific antibody MJFR-14-6-4-2 (Abcam). PLA relies on antibody-based binding of proximity probes within approximately 40 nm, followed by a signal amplification to achieve high sensitivity, whereby structures with few epitopes (which are hard to detect using IHC) are labelled as efficiently as denser, epitope-rich structures (Fredriksson *et al.*, 2002). We are using antibody conjugation kits and PLA detection kits from Navinci, as recently developed and validated the PI (Moeller Jensen *et al.*, 2024, Moeller Jensen *et al.*, 2025, *in press*).

Regular immunostaining for neurodegenerative pathologies (such as α-synuclein (LBs), Aβ (plaques), tau (tangles), and TDP-43) is performed on parallel sections if these data are not already available.

All sections are digitalized on a slide scanner and subjected to quantitative image analysis on selected regions of interest to compare signal densities between cases and regions.

C. RESULTS/OUTCOMES

A pilot testing at Aarhus University using our proximity ligation assay (PLA) on post-mortem brain samples from BRI has proved successful compatibility with BRI brain bank samples. However, it also confirmed one of our observations from previous cohorts of post-mortem human brain samples, namely that some non-neurodegenerative controls contain α-synuclein PLA signal. At present, it has not been mapped in detail how this PLA signal is distributed in controls and when it appears during the life and aging process, which is important information for understanding the role of PLA in disease.

To increase our understanding of the PLA, we have therefore first collected brain sections from 5 younger non-neurodegenerative controls and 2 cases with Parkinson's disease for comparison. These sections have been shipped to Aarhus University and are currently undergoing PLA and subsequent image analysis.

D. DISCUSSION

α-Synuclein PLA has emerged as a technology capable of visualizing previously undetected α-synuclein pathology in Parkinson's disease and Multiple system atrophy. However, little is understood about the emergence of α-synuclein PLA during the course of life and its prevalence in non-neurodegenerative controls. Results from this project will increase our understanding of α-synuclein PLA in healthy cases, as it is the first study evaluating younger non-neurodegenerative controls.

Additionally, it is well-known that α-synuclein aggregates frequently occur as a co-pathology in other neurodegenerative diseases, but this has not yet been explored using PLA. Results from this research will pro-

vide important information about the level of co-pathology in various neurodegenerative diseases, which could be crucial for the stratification of cases by their co-pathologies in order to understand the patients' symptomatology and ultimately optimize treatments.

E. CONCLUSION

We have successfully applied α-synuclein PLA with the MJFR-14-6-4-2 antibody to post-mortem brain samples from BRI. Analyses are on-going and will substantially increase our understanding of both the natural occurrence of α-synuclein PLA in human brain and its prevalence as a co-pathology in other neurodegenerative diseases.

F. PUBLICATIONS

1. PAPERS

Moeller Jensen, N. *et al*. 'MJF-14 proximity ligation assay detects early non-inclusion alpha-synuclein pathology with enhanced specificity and sensitivity', *npj Parkinsons Dis*, 10, 277 (2024).

Moeller Jensen, N. *et al*. 'Abundant non-inclusion α-synuclein pathology in Lewy body-negative LRRK2-mutant cases', *Acta Neuropathologica* (2025, *in press*).

2. PRESENTATIONS

The LRRK2-Lewy body conundrum: Demonstration of widespread alpha-synuclein aggregate pathology in Lewy body-negative LRRK2-mutant cases, *AD/PD 2025*, April 2025, Vienna, Austria.

G. APPLICATION AND REGISTRATION STATUS ON INTELLECTUAL PROPERTY RIGHTS

1. PATENT

Not applicable

2. UTILITY MODEL REGISTRATION

Not applicable

3. OTHERS

Not applicable