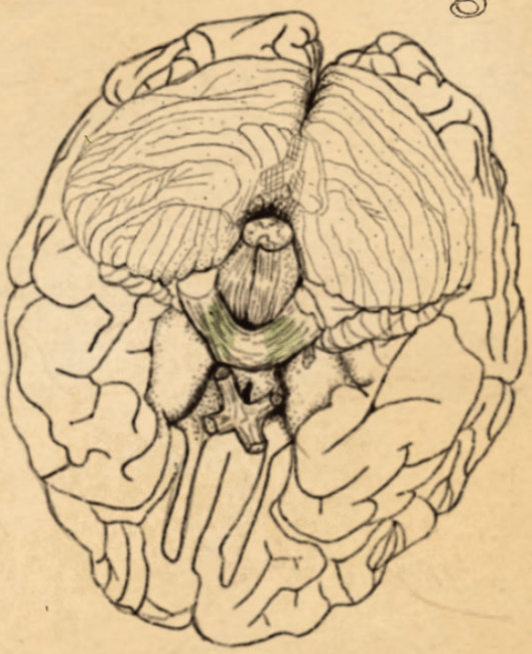
Fig 1.



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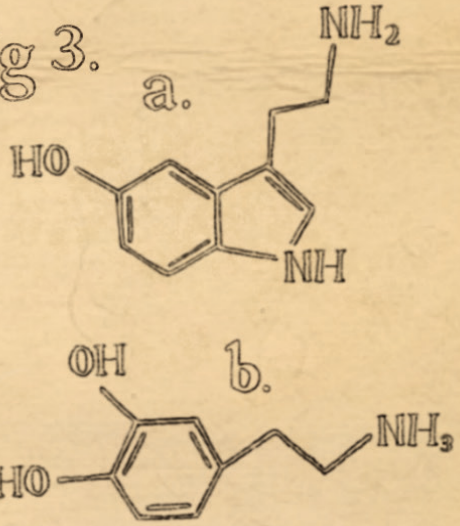

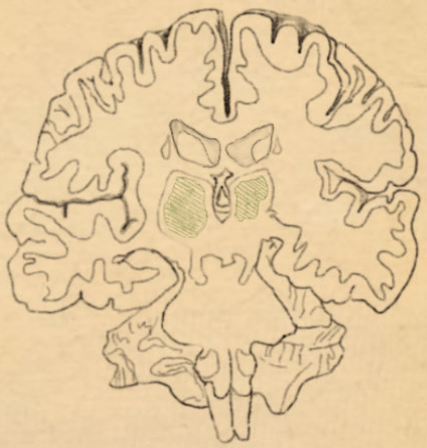

Fig 4.

An evaluation of a novel method for the detection of Parkinson's disease

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ABSTRACT

Parkinson's Disease (PD) is a neurodegenerative disorder affecting millions of individuals worldwide. However, current diagnostic tools are limited to the clinical assessment of overt symptoms, after PD has already progressed into the clinical stage. A novel PD testing method, α-synuclein seed amplification assay (αsyn-SAA), may revolutionize the testing by allowing clinicians to detect PD before symptoms arise. The α-synuclein protein abundant in pre-synapse is typically involved in the release of dopamine. However, in the development of PD, αsyn proteins become misfolded and infect their pathogenic conformations to other αsyn proteins through the prion-like process of seeding. αsyn-SAA testing identified PD progression by amplifying and measuring the accumulation of misfolded α-synuclein proteins in an individual's cerebrospinal fluid. This critical review aims to appraise the mechanisms of αsyn-SAA testing, exploring its benefits and drawbacks. Notably, αsyn-SAA has been found to have over 90% sensitivity to PD and other synucleinopathies, while being able to distinguish between PD patients and healthy subjects with a high degree of accuracy. However, there are notable limitations and future longitudinal studies are necessary to optimize the specificity of αsyn-SAA testing.

INTRODUCTION

PD is a progressive neurodegenerative disease that causes uncontrollable movements, typically characterized by tremors, stiffness, and progressively deteriorating motor function impairment.[1] PD affects 1 million Americans, and causes immeasurable suffering for both patients and their communities.[2] Scientists believe that PD is associated with the death or malfunction of nerve cells in the basal ganglia, responsible for body movement through the secretion of dopamine.[1] Although much remains unknown, research suggests that most cases of PD involve both genetic and environmental components.[2]

The onset of PD begins prior to the emergence of symptoms, in a phase known as the preclinical stage. However, most diagnoses of PD occur when individuals are symptomatic when the disease has progressed to the clinical stage.[2,5] Much research is currently focused on improving early detection of PD in the preclinical stage to prevent its progression. Additionally, since many symptoms of PD are similar to other disorders, many cases of PD are initially misdiagnosed, which results in delayed treatment.[1,8] Considering these factors and the ageing population of the U.S., much effort has gone into developing better tools for diagnosing subclinical PD, with the αsyn-SAA being analysed as the aim of this study.[1]

MECHANISMS OF ACTION FOR THE aSYN-SAA TEST

In healthy brains, the α-synuclein (αsyn) protein can be found in nerve cells and is responsible for the storage and release of neurotransmitters like dopamine and serotonin.[2] However, abnormalities involving this protein, like occurrence of clumps of αsyn known as Lewy bodies, play a key role in the development of PD.[1,2] For example, αsyn proteins aggregate and propagate in misfolded fibrous shapes within the nervous system.[3,4] PD progression may be attributed to toxic inclusions of the αsyn protein, in which pathological, misfolded αsyn proteins proliferate through the brain, eliciting neurodegeneration and synaptotoxicity.

αsyn-SAA testing is a prospective diagnostic technique for subclinical PD detecting aggregating αsyn proteins as a biomarker to distinguish PD patients from healthy individuals.[3] αsyn-SAA tests exploit the self-proliferating, prion-like nature of the aggregated proteins, which are able to 'infect' unaggregated proteins via seeding. To test for misfolded αsyn seeds in samples of cerebrospinal fluid (CSF) via αsyn-SAA, scientists add fluorescently tagged, non-aggregated α-syn proteins to the medium. If misfolded α-syn is present in a patient's sample, the tagged αsyn proteins are converted to aggregated αsyn through the αsyn-seeding process.[6] Interactions between monomeric (non-misfolded) and aggregated αsyn fibrils induce the unfolding of monomeric αsyn and their aggregation-prone surfaces being exposed, generating new αsyn amyloid fibrils.[10] Thus, concentrations of aggregated αsyn in the sample are expected to be amplified exponentially. Thioflavin T (ThT) binds to αsyn fibrils, intensifying fluorescence, which is then measured. Positive αsyn-SAA test results are indicated by a threshold fluorescence of greater than 100 relative fluorescent units.[6]

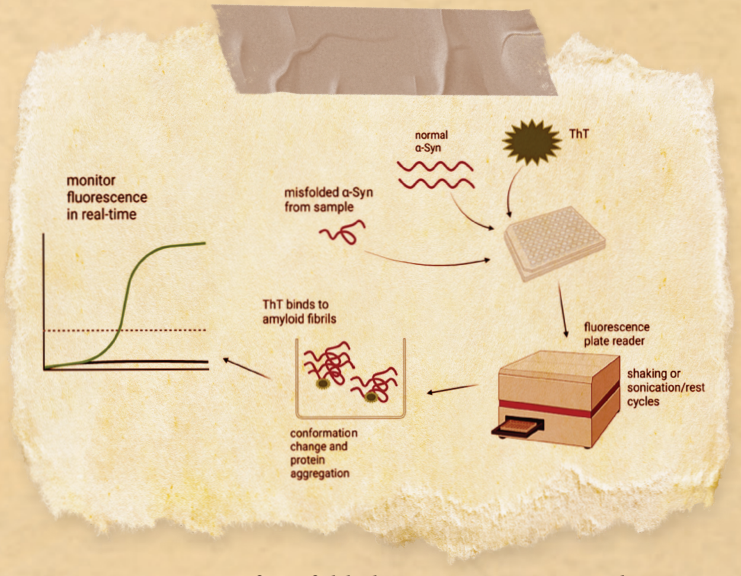


Figure 1: Detection of misfolded αsyn proteins using the αsyn-SAA test. The fluorescently tagged, normal αsyn proteins are integrated among the misfolded αsyn proteins and placed in a fluorescence plate reader; if misfolded αsyn are in the sample, fluorescence will increase, as depicted by the graph on the left.[9]

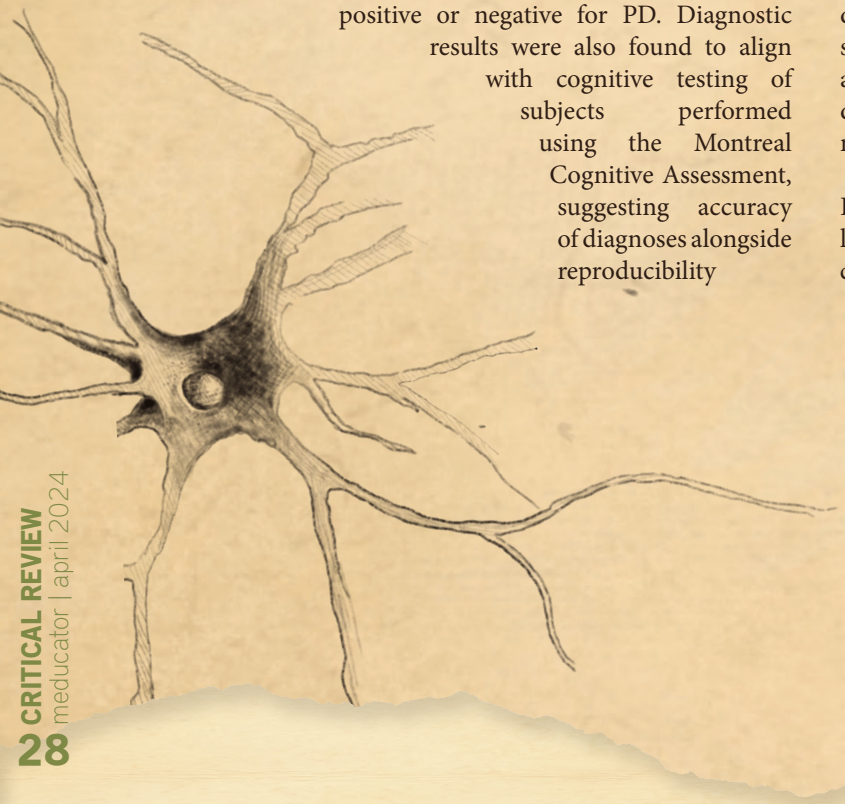
STRENGTHS AND IMPLICATIONS

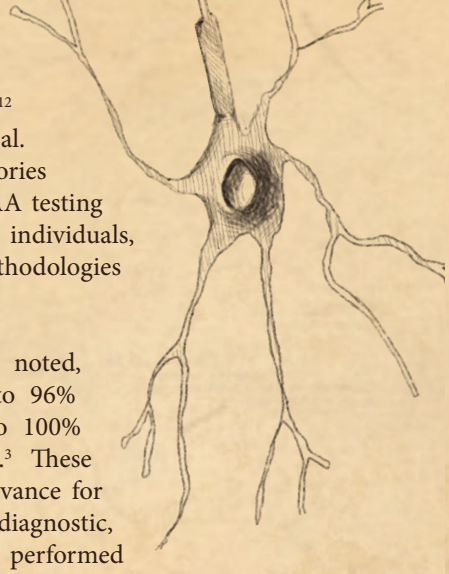
αsyn-SAA testing is of particular interest because of its proven accuracy in diagnosing individuals with PD. αsyn-SAA helps identify various synucleinopathies, a group of neurodegenerative diseases characterized by α-syn aggregates, including PD or multiple system atrophy.[7] αsyn-SAA testing displays robust sensitivity

and specificity, identifying PD patients with a positive test result while identifying healthy individuals as negative, respectively.[11] Upon sampling 55 PD patients, Gomes et al. found that all were correctly given positive results (100% sensitivity). αsyn-SAA also differentiated these patients from 24 healthy controls with a specificity of 70.8%. Additionally, αsyn-SAA testing discerns synucleinopathies from other movement disorders, a previously difficult task due to their similar symptoms. This includes tauopathies such as supranuclear palsy, a neurological disease associated with the buildup of pathological tau proteins, that αsyn-SAA can discriminate with 75% specificity.[6]

αsyn aggregation is found to occur prior to the onset of PD symptoms, such as dopamine deficiency, which hints at its pivotal ability to serve as a biomarker for PD years before symptoms arise. In a cross-sectional study performed by Siderowf et al., prodromal carriers of gene mutations associated with PD were found to have misfolded αsyn accumulation prior to biomarker modifications or overt clinical symptoms.[11] This has significant potential to become one of the initial diagnostic tools for PD, allowing for preemptive refined treatments for patients. Furthermore, early diagnosis of PD through αsyn-SAA has implications for research in this field as researchers can explore various mechanisms that underlie the development of PD, and search for biomarkers in presymptomatic patients which may assist in comprehending disease progression and severity. Finally, αsyn-SAA is able to recognize small concentrations of αsyn, which can have useful applications for preclinical stages of PD, of which individuals typically display minute αsyn levels.[13]

In efforts to standardize the procedures of αsyn-SAA testing, a concern of αsyn-SAA testing research is the reproducibility and accuracy of results across different laboratories. However, various laboratories confirm the validity of αsyn-SAA as a diagnostic tool.[3] For instance, Bräuer et al. tested this in two labs using the same procedure to test 55 samples of PD patients or healthy controls for αsyn aggregation. In 54 of 55 samples, the two labs obtained matching results when distinguishing subjects as either positive or negative for PD. Diagnostic results were also found to align with cognitive testing of subjects performed using the Montreal Cognitive Assessment, suggesting accuracy of diagnoses alongside reproducibility between laboratories.[12] Furthermore, Russo et al. compared three laboratories which undertook αsyn-SAA testing of 30 healthy and 30 PD individuals, using their own methodologies without coordination.





Consistent results were noted, with sensitivities of 86 to 96% and specificities of 93 to 100% found in all three labs.[3] These findings illustrate the relevance for αsyn-SAA as a future PD diagnostic, which can be universally performed in laboratories regardless of location.

LIMITATIONS

While αsyn-SAA is highly effective at distinguishing between PD and healthy patients, Siderowf et al. identified varying results between various forms of PD. Particularly, αsyn-SAA testing had lower sensitivity in patients with a LRRK2 gene variant than individuals with typical or sporadic forms of PD. This indicates that despite having identical clinical symptoms, αsyn-SAA may not be able to properly distinguish between different subtypes of PD. LRRK2-related PD may have a different method of pathology compared to other αsyn-positive types, such as PD associated with the GBA gene mutation, which is found to have high rates of positive αsyn-SAA results. Diverse ages, sex, and motor abilities amongst individuals with LRRK2 variants were also associated with varying αsyn-SAA results. Further research is necessary to understand how heterogeneity in various subgroups affects accuracy of PD diagnoses, and to compare αsyn-SAA results in individuals with variants across different PD-associated genes, such as PINK1 and PRKN.[11]

Though the αsyn-SAA test can detect subtle quantities of aggregated αsyn in samples, it still does not allow researchers to reliably discriminate between different synucleinopathies.[13] Furthermore, since the detection of PD is based on a αsyn threshold that only allows for positive or negative tests, it is difficult to quantify differential amounts of αsyn aggregates, so this test cannot monitor the extent of disease progression in its current state.

In all, current research in αsyn proteins in biological samples is limited by several factors.[13] First, there is a lack of standardized collection guidelines for sample collection and analysis, as well as patient selection criteria for trials. Therefore, results may be inconsistent and confounding variables are bound to be introduced across different studies. Additionally, current assays only focus on detecting one specific αsyn protein, which does not account for the nuance of modified forms of different αsyn markers. Research also found that most studies on αsyn proteins have small sample sizes, which is likely a consequence of the invasive nature of CSF collection, low participation in research studies, and requirement of multiple strenuous sample donations. Consequently, research in αsyn-SAA and PD detection account for variability and consistency poorly, which casts doubts on the prospective benefits of αsyn-SAA testing.

NEXT STEPS

Considering the invasive collection methods to obtain CSF samples for the αsyn-SAA test, this PD testing method is difficult to implement in current clinical settings. Further research is required to develop a method that can procure samples less invasively, perhaps from saliva or blood, and requires larger sample sizes to ensure its reliability. Moreover, standardized protocols for sample collection and analysis, more rigorous participant cohort selection, as well as larger sample sizes across more trials, would improve the reliability of the results and the aforementioned limitations of αsyn-SAA tests. Further longitudinal studies may also assist in elucidating the relationship between αsyn concentration and PD severity and progression, as well as the accuracy of positive test results in individuals with pre-symptomatic or prodromal conditions.[11,13]

CONCLUSION

αsyn-SAA is an innovative early-detection method for the diagnosis of PD, introducing a novel and sensitive way to screen individuals. While further studies would allow for optimization of αsyn-SAA for specific synucleinopathies and types of PD, current research is promising. If implemented in the future, αsyn-SAA is expected to complement traditional clinical diagnoses, as well as allow for earlier detection and implementation of treatment options to assist in slowing the progression of PD. The implications of αsyn-SAA for PD research and clinical trials also cannot be disregarded, as early detection measures of PD and other neurological disorders may aid in the search for new related biomarkers and therapeutics for patients.

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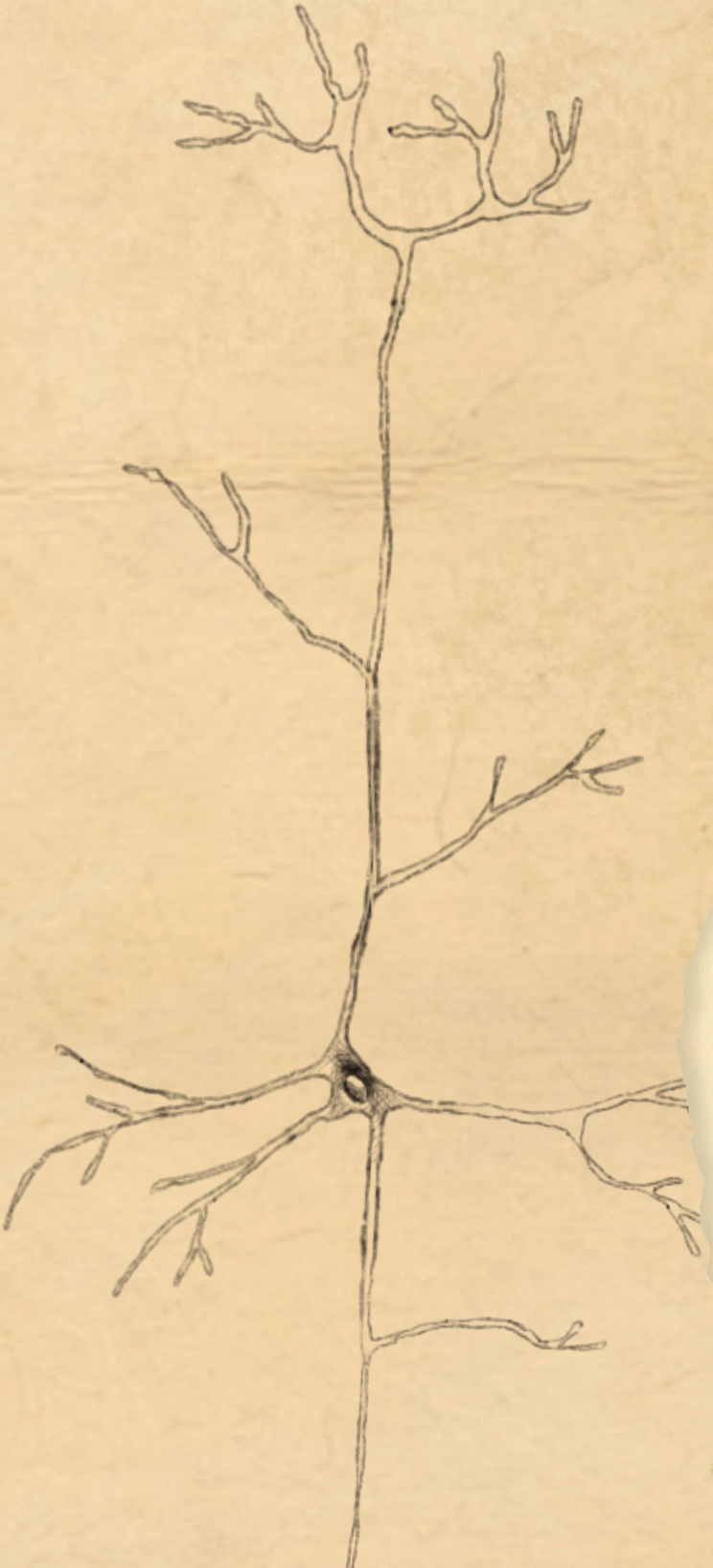
EDITED BY: ANGELA HONG, OLIVIA KIM, ZAHRA

1. NIH National Institute on Aging (NIA). Parkinson's Disease: Causes, Symptoms, and Treatments [Internet]. National Institute on Aging. 2022. Available from: https://www.nia.nih.gov/health/parkinsons-disease/parkinsons-disease-causes-symptoms-and-tre atments
2. Parkinson's Disease: Challenges, Progress, and Promise | National Institute of Neurological Disorders and Stroke [Internet]. www.ninds.nih.gov. 2023 [cited 2024 Jan 7]. Available from: https://www.ninds.nih.gov/current-research/focus-disorders/parkinsons-disease-research/parkins ons-disease-challenges-progress-and-promise
3. Russo MJ, Orru CD, Concha-Marambio L, Giaisi S, Groveman BR, Farris CM, et al. High diagnostic performance of independent alpha-synuclein seed amplification assays for detection of early Parkinson's disease. *Acta Neuropathol Commun*. 2021 Nov 6;9(1). Available from: doi:10.1186/s40478-021-01282-8.
4. Vargas JY, Grudina C, Zurzolo C. The prion-like spreading of α-synuclein: From in vitro to in vivo models of Parkinson's disease. *Ageing Res Rev*. 2019;50:89–101. Available from: doi:10.1016/j.arr.2019.01.012.
5. Getting Diagnosed | Parkinson's Foundation [Internet]. www.parkinson.org. Available from: https://www.parkinson.org/understanding-parkinsons/getting-diagnosed.
6. Fernandes Gomes B, Farris CM, Ma Y, Concha-Marambio L, Lebovitz R, Nellgard B, et al. α-Synuclein seed amplification assay as a diagnostic tool for parkinsonian disorders. *Parkinsonism Relat Disord*. 2023;105807. Available from: 10.1016/j.parkreldis.2023.105807.
7. Galvin J, Lee VMY, Trojanowski JQ. Synucleinopathies: Clinical and pathological implications. *JAMA*. 2001. Available from: doi:10.1001/archneur.58.2.186.
8. Poll finds a quarter of people with Parkinson's are wrongly diagnosed [Internet]. Parkinson's UK. 2020. Available from: https://www.parkinsons.org.uk/news/poll-finds-quarter-people-parkinsons-are-wrongly-diagnosed.
9. Kurapova R, Chouliaras L, O'Brien JT. The promise of amplification assays for accurate early detection of α-synucleinopathies: A review. *Exp Gerontol*. 2022;165:111842. Available from: doi:10.1016/j.exger.2022.111842 10.1016/j.exger.2022.111842.
10. Calabresi P, Mechelli A, Natale G, Volpicelli-Daley L, Lazzaro GD, Ghiglieri V. Alpha-synuclein in Parkinson's disease and other synucleinopathies: from overt neurodegeneration back to early synaptic dysfunction. *Cell Death Dis*. 2023;14(3):1–16. Available from: doi: 10.1038/s41419-023-05672-9.
11. Siderowf A, Concha-Marambio L, Lafontant DE, Farris CM, Ma Y, Urenia PA, et al. Assessment of heterogeneity among participants in the Parkinson's Progression Markers Initiative cohort using α-synuclein seed amplification: a cross-sectional study. *Lancet Neurol*. 2023;22(5):407–17. Available from: doi: 10.1016/S1474-4422(23)00109-6.
12. Bräuer S, Rossi M, Sajapin J, Henle T, Gasser T, Parchi P, et al. Kinetic parameters of alpha-synuclein seed amplification assay. *Acta Neuropathol Commun*. 2023;11(1):162. Available from: doi:10.1186/s40478-023-01653-3.
13. Magalhães P, Lashuel HA. Opportunities and challenges of alpha-synuclein as a potential biomarker for Parkinson's disease and other synucleinopathies. *npj Parkinsons Dis*. 2022;8(1):1–26. Available from: doi: 10.1038/s41531-022-00357-0.



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